Conference Proceedings

16th Australian Wine Industry Technical Conference
Adelaide, South Australia 24–28 July 2016

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Winemakers' Federation of Australia

Outlook Conference

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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 16th conference, held in July 2016 in Adelaide, South Australia, attracted over 1,200 attendees. For the first time the program included the Winemakers' Federation of Australia's Outlook Conference, which brought the latest business and technical content together in one forum. Key topics explored included: industry outlook, terroir, vineyard health, wine flavour, authenticity, productivity and adapting to a changing climate. A total of 12 formal sessions were presented over four days, with 16 international and 42 local speakers. The main program was complemented by 38 workshops, a display of over 180 technical posters and an extensive trade exhibition.

The editors would like to thank Annette Freeman for her assistance in the preparation of these proceedings.

The Australian Wine Industry Technical Conference Inc.
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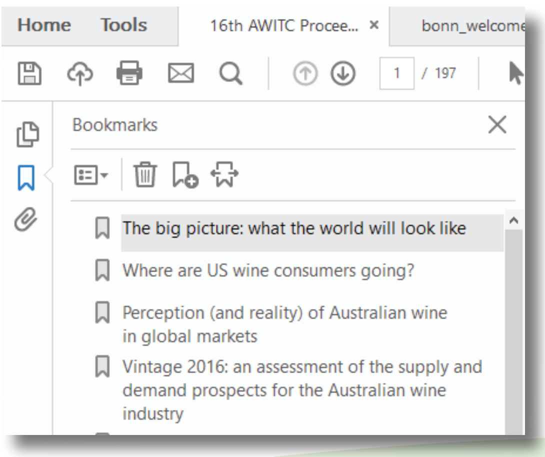

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edited by

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

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SESSION 8: Flavour and its contribution to unique wines

The big picture: what the world will look like

D. Rumbens

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Abstract

Australia has been undergoing a difficult economic transition in recent years, working through the aftermath of a significant commodity boom. This has had big impacts on the value of the Australian dollar, and while subsequent movements in interest rates have supported the transition, much of that benefit has translated into higher house prices, which also threatens to leave an overhang.

This session will explore the current and likely future economic environment for consumer spending in Australia, and for Australia's main wine export markets. Key areas that will be addressed include:

- *Following July's election, what is likely to be the capacity and willingness of the Australian consumer to lift their spending?*
- *How is China's economic transition playing out and what are the prospects and risks around further spending growth?*
- *The UK's status as a recent economic powerhouse is waning, and caution is currently taking hold amid the Brexit result.*
- *Zero interest rates delivered an economic resurgence in the US, but also pushed up the value of the US dollar which is proving challenging, while the country may enter its own period of caution in the lead up to November's Presidential election.*

No paper available, please view this presentation at http://bit.ly/16thRumbens.

Where are US wine consumers going?

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Abstract

The US wine market is now the world's largest, by volume and value, yet its per capita consumption is still well down the list, suggesting considerable upside opportunity. This presentation will cover the key factors affecting the US wine market, with a particular focus on several key industry structure, consumer and retail trends. Based on these, the session will offer some thoughts about maximising opportunities for Australian wine in the US.

No paper available, please view this presentation at http://bit.ly/16thBrager.

Perception (and reality) of Australian wine in global markets

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Abstract

Like the tide on a beach, or a sine wave, the image and perception of Australian wine has ebbed and flowed over time and through generations. Known for many years as the home of some of the finest fortified wines on the planet, Australia evolved to become the engine room of easy, sunny, full-flavoured wines to a new generation of consumers, especially in the US and UK, where demand was seemingly never-ending. Understandably, farmers and vignerons alike planted hectare upon hectare of vines to feed the insatiable demand. New regions emerged and irrigation became the life-blood of a booming industry.

Then other countries started to learn, and copy, the Australian way – inexpensive, easy to understand, branded wines with fun names created overnight to remind consumers of animals, hills, dales and rivers. It all became a bit, well, generic. One wine seemed infinitely substitutable with another, from almost anywhere. And oversupply meant prices, both for grapes and the bottled wine, plummeted. Emerging markets such as China failed to soak up the surpluses and Australia reached bedrock.

Or did it? All the while, determined growers and winemakers were aiming to remind consumers of what made Australian wines unique originally – a sense of place, regionality, refined and restrained styles and that unmistakable 'can do' attitude of the original exporters. Pride crept back in, and Australia began to demand the attention of the world's most discerning consumers.

No paper available, please view this presentation at http://bit.ly/16thJago.

Vintage 2016: an assessment of the supply and demand prospects for the Australian wine industry

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Webcasts of this presentation available at http://bit.ly/16thWeeks and http://bit.ly/16thBattaglene.

Introduction

While the 2016 crush report is cause for guarded optimism, it is important to look beyond the promising improvement in volume and value trends and consider the factors contributing to the result. There are some improvements in the value of wine-grapes, albeit in many cases from a low base, but real questions remain about the structural aspects of the imbalance between supply and demand. On the demand side we are seeing encouraging signs coupled with an improved international market economy.

Crush report

National production for 2016 is 1.81 million tonnes, up 6% on the previous season. Warm inland production, which accounts for most of the national crush, was effectively static, while cool and temperate regions increased significantly over the past year. It must be noted, however, that many of these cool and temperate regions recorded crop levels in the 2015 vintage that were well below the long-term average. Yields in 2016 were close to the long-term average.

Nationally, the average value of fruit was $526/t, a 14% increase from 2015. The growth in value is positive, however it is a broad national average; while national values serve as a useful trend indicator, their utility for regional wine businesses in decision-making and future planning is questionable. The real value for businesses can be found in more specific figures about supply and demand balance within value categories. There has been much hand-wringing about large crops and the national value of fruit and wine in the past, but in truth national averages are less relevant than the supply and demand balance in the various price categories of product. According to the Expert Report on the Profitability and Dynamics of the Australian Wine Industry (Centaurus Partners 2013), the relative proportions of total demand (export and domestic demand combined, less imports) of value categories A, B, C, D and E/F was 2.3%, 5.0%, 10.1%, 38.6% and 44.0% respectively. The massive variation across the value categories illustrates the folly of using national figures to accurately predict supply and demand balance in discrete value categories.

Volume

Warm inland production remained basically static overall – Riverland slightly up; Murray Valley and Riverina slightly down. Cool and temperate regions showed some significant increases in production levels in 2016 relative to 2015, for example:

- Padthaway up 77%
- Langhorne Creek up 54%
- Wrattonbully up 43%
- McLarenVale and Limestone Coast up 44%
- Barossa up 23%

Despite the increases in volume there has been uniform positive appraisal of the wine quality from the 2016 vintage, with many regions rating this recent vintage as 'outstanding'. The 2016 vintage was characterised by balanced vines and balanced crops that were able to ripen relatively free from excessive stress. This appears to have delivered good results in the final wine.

Value

The 2016 vintage saw welcome increases in average wine-grape price across most regions. It is also positive to see an increase in the proportion of fruit paid at the 'premium' price of greater than $1500 per tonne, predominantly for red varieties. Overall there was an increase in prices, including most of the warm inland regions, much of this off a low base. It is unclear whether these slightly higher prices reflect the positive influence of Free Trade Agreements (FTA), favourable exchange rates and effective marketing or a more favourable supply and demand balance arising from slightly lower vintage crush volumes in 2015. Indeed, it remains to be seen how much of the improvement in volume and value payments are the result of good management and how much is due to uncontrolled trends. If the result is driven by influences out of the control of growers and makers of wine, the celebration will likely be short lived.

What does this mean?

While these results are very positive, there is a question about the reliability of market signals in the industry, in particular the messages being sent to those growing fruit. The changes to the grower demographic in the Murray Darling and Swan Hill regions offer a case in point. Over the course of the past decade or so, Murray Valley Winegrowers has seen a decline in the number of members from approximately one thousand growers to less than 400 now (Murray Valley Winegrowers' Inc. 2016). The change to the regional vineyard area is not completely clear, as the region has table and dried fruit production that makes determination of the wine-producing vineyard area difficult. What is clear, however, is that production has not decreased in line with a 60% drop in grower numbers; production was 396 kt in 2004 and 368 kt in 2016, a decline of just 7%.

This result may be due to consolidation of individual growers into larger firms, but it is also likely due to remaining growers concentrating on maximising yield to offset the cost of production. Most production costs for vineyards are fixed, and where possible winegrowers may seek to amortise production costs across the greatest amount of yield. This reaction is also in response to a lack of incentives to grow wine-grapes that can be made into higher value wine or low confidence that higher quality fruit would attract commensurate payment. This reaction is a logical business response, and is not confined to the Murray Darling/Swan Hill region. The market signal provided by a low fruit price has resulted in increased national fruit production, not less.

This evidence challenges the common view about the need to remove vineyard area, or what might be an 'ideal' national vineyard area to achieve supply and demand balance. Reliable market signals; open, regular and transparent communication between those growing fruit, those making wine and those selling it; and an increase in demand and therefore wine value is more likely to achieve supply and demand balance than removal of vineyard area.

The macro-economic picture

Before the 23 June vote in the UK in favour of leaving the EU, economic data and financial market developments suggested that growth in most advanced economies was slow, with low potential growth and a gradual closing of output gaps. Prospects remained diverse across emerging market and developing economies, with some improvement for a few large emerging markets – in particular Brazil and Russia – pointing to a modest upward revision to 2017 global growth relative to April's forecast (International Monetary Fund 2016).

The outcome of the UK vote, known as 'Brexit', which surprised global financial markets, ushered in a more pessimistic global outlook for 2016–17 reflecting the expected macroeconomic consequences of a sizable increase in uncertainty, including on the political front. This uncertainty is projected to take a toll on confidence and investment, including through its repercussions on financial conditions and market sentiment more generally. The initial financial market reaction was severe but generally orderly.

As Brexit continues to unfold, growth forecasts for advanced European economies remain uncertain, with a relatively muted impact elsewhere, including in the US and China.

For Australia, Brexit has significant implications.

The UK is of great importance to global wine markets. Accounting for one third of all Australian wine exports, the UK is Australia's number one export destination by volume with 247 million litres of wine exported in 2015. The UK is the second largest import market by value globally and Australia is the second largest source of wine behind Italy by volume. Considering these facts, any changes in the UK market will be relevant to Australia's wine trade with the UK and the EU.

At this stage all we can do is list areas which will need to be assessed and monitored over the coming months:

1. Impact on demand of Australian wine: a weakening UK economy and drop in GDP may be reflected in reduced demand across a range of areas including wine.

2. Exchange rate: if the Sterling weakens, as some are predicting (International Monetary Fund 2016), against the Australian dollar, then our price competitiveness could be impacted. This impact will be dependent on the cross rates and their impact on our major competitors on the market.

3. Alcohol and health: the UK government has for a long time taken a stronger anti-alcohol position than the rest of Europe. For example, on 8 January of this year, the UK Chief Medical Officer proposed new alcohol guidelines (Department of Health 2016) that significantly reduced recommended levels for men, stating that there was 'no safe level' of alcohol consumption. The Conservative Party have also shown support for 'minimum pricing' in the past. There is a risk that, without the balancing aspect of EU regulation, the UK government may take a stronger populist anti-alcohol approach.

4. Trade agreements: following the exit from the EU, the UK will no longer be subject to the Common Customs Tariffs (CCT) and any tariffs will be established only after trade agreement negotiations. If no preferential agreement is negotiated with the EU after the exit, all imports will be treated equally, removing the benefit some wine-producing nations currently receive on exports to the EU.

More significantly, UK food law is now inextricably linked with EU food law. Nowhere is this more obvious than in wine regulation, where the British legislation gives force to European wine regulation. However, Australia currently has preferential access to the European market. In 1994, Australia signed the Agreement between Australia and the European Community on Trade in Wine (Agreement). The Agreement was the first wine agreement signed outside Europe and has treaty status. The Agreement harmonised winemaking practices as well as established protection for geographical indications and traditional expressions. Another immediate benefit was the reduction in analytical requirements for the European Import Certificate.

- The Agreement was renegotiated and signed in Brussels on 1 December 2008 and is a formal international agreement that regulates the trade in wine between Australia and the EU.
- The major benefits for Australian producers include:
- European recognition of Australian winemaking techniques
- Simplified arrangements for the approval of winemaking techniques that may be developed in the future
- Simplified labelling requirements for Australian wine sold in European markets. Protection within Europe of Australia's registered geographical indications (GIs)
- Simplified certification requirements for Australian bottled wines entering European customs.

Following Brexit, however, this Agreement may no longer apply and may need to be renegotiated. This has a number of important implications. First, the Agreement overrides EU regulations, giving Australian exporters the advantages outlined above. If the UK merely adopts European wine law as it currently exists on EU statute books, then these advantageous provisions will no longer apply. Second, many exporters send wine to the UK where it is then re-exported to other EU countries. There is also a lot of wine that it currently exported in bulk to the UK, then bottled and exported throughout Europe. These transactions currently fall under the Agreement and single market provisions of Europe. Brexit will require negotiations with both the EU and the UK to reduce transactional costs that may arise. This will become part of the EU-Australia FTA negotiations due to commence in 2017.

5. Intellectual property: trade mark owners have sought protection at an EU level rather than individual countries within the EU. Following Brexit, EU trademark law may no longer apply in the UK and businesses will need to reassess how they protect their marks in the UK and in the EU.

6. Imports and exports: currently the European Union customs and free movement principles ensure that most goods are traded and moved between states without tariffs, customs duties and customs declarations irrespective of origin of goods. It is not clear what changes may occur as Brexit proceeds.

Export demand for Australian wine

Wine Australia's Export Report shows that the value of Australian wine exports continued to experience strong growth in the 12 months to the end of June 2016 (Australian Grape and Wine Authority 2016). From July 2015 to June 2016, the value of exports grew by 11%, driven by bottled exports, particularly at higher price points. Bottled exports grew by 15% to $1.7 billion and the average value of bottled exports increased by 9% to $5.35 per litre, the highest since October 2003.

Table 1. Value and growth rate of exports above $10 per litre

Price segment (A$/litre)	Value	Added value	Growth rate
$10–14.99	$153,461,758	$24,903,246	19%
$15–19.99	$78,882,812	$19,440,823	33%
$20–29.99	$70,504,545	$12,084,718	21%
$30–49.99	$40,508,992	$11,655,989	40%
$50–99.99	$118,513,641	$32,806,135	38%
$100–199.99	$9,106,440	$429,503	5%
$200+	$28,427,693	$970,487	4%
Total above $10	**$499,405,882**	**$102,290,900**	**26%**

Higher priced wines contributed to almost half of the total value growth in the last 12 months, with exports priced at $10 FOB and over per litre up 26% to a record $499 million (Table 1).

According to Wine Australia 'Exports priced $10 FOB and over to the US grew by 16%, mainland China by 71%, the UK by 15%, Canada by 12%, and Hong Kong by 5%' (Australian Grape and Wine Authority 2016).

Exports by region

Northeast Asia continued to lead growth, with value increasing by $158 million (34%) to $618 million. Next in absolute growth was North America, growing by $46 million (8%) to $646 million. Growth slowed to Southeast Asia, up $7 million (5%) to $142 million.

Sustained growth in Northeast Asia

Australian exporters continue to see benefit in export markets with FTAs. Exports to mainland China grew by 50% to $419 million, despite a slowing economy. This exceptional growth was aided by the China–Australia FTA, and the growing Chinese middle class interest in wine.

When combined with Hong Kong ($124 million), China is the largest market for Australian wine exports.

Value to mainland China increased across the price segment spectrum, with the greatest growth once again in exports valued at over $10 per litre, up 71% to $169 million.

Exports to Japan increased by 4% to $45 million, while exports to South Korea were up 29% to $13 million.

US growth

The US remained Australia's number one destination for wine by value and exports grew by 8% to $449 million.

Conclusion

The 2016 crush report provides cause for cautious optimism. However, the wine community should not assume that good times will automatically follow without more hard work, and should also not avoid facing the fact that market signals must be improved along the wine supply chain.

A continued focus on building demand will lead to the ability to command greater value. This value must be maintained through production of the best possible fruit and selling the best possible wine at a range of profitable price points. In the absence of reliable market signals, and without the confidence to achieve a value premium, there will be a continued market signal that risks supply and demand imbalance.

The improvement in the 2016 vintage report is welcome, and the time is right for all in the wine community to work together to build demand and value to restore profitability for all in the wine supply chain.

Internationally, Brexit has clouded market prospects. However, growth in the major Asian markets and those of North America look strong. For Australia, there is more upside than downside in Brexit, and 2017 looks to be an exciting year.

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VinSites: insights from grape to glass

A. Clark

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Abstract

Andreas will introduce Wine Australia's new VinSites system that will provide comprehensive information about Australia's grape and wine supply and demand by region, by variety and by price point.

No paper available, please view this presentation at http://bit.ly/16thClark.

The future of retail-owned brands and exclusive brands

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Abstract

Rapidly evolving customer expectations and an intensely competitive environment means that traditional retail businesses will need to innovate more quickly if they are to remain relevant. Innovations are quickly copied and further developed by competitors and the advent of digital retail and its rapid acceptance by consumers means that barriers to entry have never been lower. The brands that feature on wine, beer, spirits and cider bottles and the people involved in producing them often invoke warm and positive feelings among consumers largely due to the romance and imagery used and through personal experiences at the cellar door. Yet, like nearly all forms of retailing, many brands in the marketplace are actually owned by the retailer or exclusive to their shelves. While this has been a feature of drinks retailing since the early inception of the Australian wine industry, it is timely to review their place in the current wine market and make some predictions on what the future may hold. Positive indicators suggest a true partnership approach developing between winemakers and retailers. While it is ultimately up to the customer to determine the success or otherwise of a wine, an active partnership approach in co-creating brands and delivering true innovation to the consumer usually ensures these wines are successful. This benefits all participants in the supply chain as they get closer to the customer.

Webcast of this presentation available at http://bit.ly/16thBaddock.

Introduction

Retailer exclusive brands (REB) have been a constant feature of grocery, general merchandise, and drinks retailing world-wide. Historically the market share of REB in Australia has been lower than that of comparable markets in the UK and Europe (Nielsen 2014).

Consolidation of both the retail and supply sectors has led to a restructure of the wider value chain, which has resulted in a fundamental change in traditional business models and relationships between retailers and producers. At the same time, the fragmentation of the wine industry at the production end has created an almost infinite capacity to innovate by creating new brands, investing in emerging varieties, and exploring developing markets in 'natural', organic and biodynamic wines.

Retailers pursue owned or exclusive brand strategies for a range of reasons. Primarily, the retailer sees REB as an opportunity to create customer loyalty by differentiating their range from competitors and protecting margins from the intense, price-driven competitive activity on major brands.

The ultimate arbiter of the success of these strategies is the customer.

A profitable future for retailers, brand owners and producers will depend on recognition by all sectors of the industry that we are no longer driving the bus. The consumer is well and truly in the driver's seat.

Suppliers and retailers need to find ways of working together to make the journey smooth and purposeful, rather than hanging onto the sides and trying to make sense of what they might currently perceive as a directionless customer-led journey. Our industry needs to seriously accelerate the transition from a production led to a consumer led model, and the retail sector of the industry, due to the presence of quality, timely consumer data, is best placed to lead this transition.

This new operating model, if we can recognise and exploit it, provides us with the opportunity to combine real-time consumer data and insights with the proven capacity of Australian wine producers to deliver outstanding wines across a spectrum of styles and price-points.

Australian drinks retailing is highly consolidated

This situation is, to some extent, a result of a market that has been highly regulated for much of its existence. In most Western and Asian countries consumers can purchase a bottle of wine in any convenience or grocery store.

In Australia, however, the country has operated under a strict licensing regime aside from a period of enlightenment during the late eighties and early nineties when the Nieuwenhuysen reforms in Victoria and National Competition Policy resulted in a brief relaxation of licensing regulations.

The opportunity for a regulatory framework that is aligned with the changing lifestyles of Australian wine consumers has been diminished, and the costs associated with obtaining and maintaining a traditional (i.e. bricks and mortar) liquor licence has been a significant barrier to entry for smaller players.

In 2013 the Winemakers' Federation of Australia (WFA) commissioned an *Expert Report* (Centaurus Partners 2013) that looked closely into wine sector profitability and found that domestic wine industry gross margin for the period of analysis grew by $66 million against a loss of $750million in export gross margin loss. This compares with flat or declining sales for beer and spirits over a corresponding time frame. It would appear that Australian retailer support of the Australian wine sector provided some help, but not enough to compensate for the massive loss of profitability in export markets.

Nevertheless, the Australian wine sector is an active voice in the debate on retailer consolidation with its peak body, the WFA, making 11 public submissions (www.wfa.org.au/information/submissions/) from 2014-to-date that have referenced 'retailer market power or consolidation' as having a deleterious impact on wine industry profitability.

The WFA *Expert Report* estimated the 'combined groups of Coles (Liquorland, 1st Choice, Vintage Cellars) and Woolworths (BWS, Dan Murphy's) liquor businesses distributed and sold up to 77% of all wine sold off premise up from circa 60% in 2007. This translates to about 70% of all domestic sales, on and off-premise'.

In contrast to this view is evidence that the opportunities for wine producers to engage directly with consumers, and the diversity of channels available to producers, is greater than it has ever been. All state and territory jurisdictions now have provision for online sales of wine and many have made the licensing of 'small bars' easier to obtain.

The Drinks Association maintains historical data on these changes to national licensing numbers (2016a) and banner/chain group size (2016b). Australian alcoholic beverage consumers have a significant array of shopping options with over 58,666 licences trading across the country that allow for the sale of wine to the public.

As a proportion of total liquor licence numbers the two large retailers hold 2,312 licences between them representing 3.94% of all liquor licences.

To delve a little deeper into 'packaged liquor outlets' through which the majority of Australian wine is sold, the numbers show there are 9,314 licences that are strictly defined as providing for packaged off-premise trade. As a percentage of packaged liquor outlets, the two large retailers represent 24.8%. However, the competitive market for packaged liquor is not restricted to just those who hold a packaged liquor licence. A truly accurate figure would need to incorporate all other forms of licences that have the capacity to sell packaged liquor and would include 'general' hotel licences (e.g. Bottlemart on–premise, PubMart, Hotel drive-throughs); club licences (e.g. ClubHost, Club Mart, Club Partners); and holders of a producer's licence that allow for cellar door and mail/internet order sales. This would further dilute the representation of the two main retailers as a percentage of the overall competitive packaged liquor landscape.

Large retailer chains undoubtedly have a number of competitive advantages. Their large volumes, disciplines and execution, single delivery point, marketing support and extensive logistics capabilities make dealing with a single retailer an attractive proposition for many producers. The competitive response from smaller retailers and independent groups has seen a range of strategies deployed. Many have sought to form buying groups or banner groups that collectively seek to scale up purchases to attract discounts or rebates, while others have pursued a bespoke or unique market offering.

These banner groups collectively form a sizeable chunk of the total packaged liquor market in Australia, and operators such as Liquor Barons in WA and the Goodstone Group in Tasmania prove that the best independent operators are perfectly capable of taking the fight to the chains.

As of 17 April 2016, there are 8,572 licences spread across 64 chains/banner groups (that have at least 50 stores as part of their group) as outlined in Table 1. There are a large number of multi-store/hotel owners or smaller buying groups that have less than 50 stores which have been excluded from this table (e.g. Red Bottle, Laundy Hotels, Porters, etc.).

It is clear that there are a significant number of participants in the retail market. Whether it is unique online-only offerings, boutique single category focused stores, or the independent sector and its supporting banner groups; all are involved in offering their customers exclusive brands and Stock Keeping Units (SKUs).

The challenge for wine companies (particularly the smaller ones) and their distributors is finding an efficient and profitable way of engaging and building relationships with a highly fragmented independent operator sector and servicing them through a low-cost logistics capability.

It isn't just the retail sector that has become highly fragmented. There has been a 115% increase in the number of winemakers following the introduction of the 'A New Tax System' in 2000.

To put this surge into perspective, in the fourteen years prior to the tax reform that accompanied A New Tax System (ANTS) an additional 500 producers entered the industry. In just four years post-ANTS there were an additional 600 wine producers pouring into an already crowded market at precisely the same time that the two decades of growth in our wine exports was tapering off and eventually declining.

The explosion in new wineries and the resulting competitive pressure throughout the value chain – including from businesses in other drinks categories – ensures that innovation and creativity will be the key to gaining new customers and maintaining their loyalty.

To distinguish their offer and reinforce their customer proposition in a highly competitive market, many retailers have looked to REB and partnered with wineries to various degrees of depth in order to deliver an appealing offer to their customers.

A brand's owner does not determine the brand's worth

The labelling of wine is governed by a complex overlay of industry and government regulations:

- The Australian Competition and Consumer Commission and State and Territory fair-trading regulators monitor misleading claims
- The Australian and New Zealand Food Standards Code sets out criteria for a beverage to be called a wine, alcohol level accuracy, and allergen information
- The Australian Grape and Wine Authority (Wine Australia) specifies rules for geographical indications and blending rules
- The Alcohol Beverages Advertising Code Responsible Alcohol Marketing Code establishes a voluntary code to prevent advertising that may appeal to minors, encourage excessive consumption, or suggest therapeutical benefits
- WFA administers the Wine Industry Display of Awards Code of Practice governing how trophies and medals can be displayed on labels.

Table 1. Licences held by chain/banner groups (minimum of 50 stores). Source: The Drinks Association (2016a, b)

Chain/Banner Group	Licence Numbers
1st Choice	96
ALDI	266
Bargains	134
Big Bargain Bottleshop	52
Bottlemart	474
Bottlemart Express	376
Bottlemart On-Premise	187
Bottlo-o Neighbourhood	362
BWS	1261
Cellarbrations	516
Club Mart	57
Club Partners	588
Country Wide Liquor	143
Dan Murphy's	205
Duncans	97
Foodworks	127
IGA Plus Liquor	435
Liquor @	233
Liquor Barons	57
Liquor Legends	232
Liquor Stax	364
Liquorland	668
Little Bottler	184
Local Liquor	172
Pubmart	65
Ritchies	51
Sip 'N Save (+SNS Cellars)	81
Super Cellars	230
Super Cellars Express	171
The Bottle-O	245
Thirsty Camel	364
Vintage Cellars	79

In addition to the above, industry leaders have called for specific additional regulations for brands that are owned directly by a retailer.

The WFA has made submissions calling on government to further regulate that all retailers should declare ownership of their brands on the wine label (Winemakers' Federation of Australia 2015):

> **Appropriate labelling for homebrands**
> WFAs consultations with industry has highlighted strong support for the labels of brands owned by retailers to be clearly marked as such to ensure consumers are aware of the origin of the wine. WFA recommends the Government require that all brands owned by retailers be clearly labelled to inform consumer purchasing decisions. WFA contends that additional regulation would improve the competitive process and enable consumers to make informed choices.

The entire industry, not just retailers, has a stake in ensuring that consumers are fully informed when making their purchasing decisions and if taken to its logical conclusion this proposal could result in a significant set of practical difficulties and unintended consequences:

- First of all, what is the definition of a homebrand? Is it a wine produced in a winery owned by a retailer? A wine where the brand IP is owned or used exclusively by a retailer? A wine that is produced exclusively for a retailer by another winery? A wine where the brand IP is owned by the winery but made available exclusively through one retail channel?
- What is the definition of a retailer? Will the requirement extend to single store owners? Online and digital businesses? Restaurants and hotels?
- Would this requirement extend to wines that are imported directly by retailers? And if it did not, would this encourage retailers to look to overseas producers to source exclusive brands?
- Would a winery based in the Barossa Valley be forced to expressly state that the wine in the bottle is not exclusively produced from Barossa Valley fruit?
- Would the Southern Highlands cellar door that sources wines in Griffith be made to call this out?
- Is the consumer fully informed when an ABV labelling tolerance of 1.5% is allowed?
- Would the requirement to disclose brand ownership be limited to retailers, or would it be extended to cover private equity companies, Japanese breweries, Chinese government owned enterprises and potentially doctors and lawyers who have entered the industry on a part time basis?
- Is the consumer fully informed when 15% of the wine in their bottle could come from a different variety, region or vintage to that which is stated on the label?

The answer to all of these questions is that winemakers (actual and virtual), growers, intermediaries, and retailers are all still beholden to the customer. The shopper makes a purchase decision on a range of factors: price, perceived value, taste, style, varietal, etc. Not all consumers are looking for a story behind the wine they buy, many are just interested in relaxing with a glass of something pleasant, but when they do the ones that resonate will always be about its place or the winemaker that crafted it, not on whether it was contract made for a winemaker, retailer, or a third-party.

Understanding the customer, therefore, becomes critical to ensure the vitality of a brand.

As the exclusive supplier to a diverse multi-channel retail business, Pinnacle Drinks is in a unique position to combine consumer insights with production and brand development capacity.

Pinnacle Drinks supports a broad range of beer, wine, and spirits into the Endeavour Drinks Group (formerly Woolworths Liquor Group) retail portfolio which comprises BWS, Dan Murphy's, Cellarmasters, Langtons, NZ Wine Society, winemarket.com.au, Australian Leisure and Hospitality, and Pudao.

Just like any brand owner, Pinnacle Drinks is incredibly proud of the stories behind the labels that make up their portfolio of brands thanks to the expert team of growers, winemakers, and facilities within its team.

It is also leading the retail and supplier industry with ownership transparency through the inclusion of its name and contact information on the label of all its retailer-owned brands. In addition, its full brand portfolio is listed on its own website – www.pinnacledrinks.com.au. Combined, these measures deliver a level of transparency that is not exhibited by many other industry participants.

The lines that define a grower, winemaker, distributor and retailer have become irrevocably blurred

While it is seen as natural for wineries to have cellar doors and operate a retail business, it is now equally true that many retailers own or lease vineyard assets and make or contract wine directly. The 'integrated player' description has been true of many participants across traditional retail, producers, growers, wholesalers and distributors for much of the history of the Australian wine industry.

For example:

- Four decades ago Manassen & Lucchitti, Claude Fay, Theo's, Dan Murphy's, and Farmer Brothers (to name just a few), all had large and successful exclusive label businesses built on wines that had been made under contract by others.
- It is a well-acknowledged fact that many growers are wine producers and traders who are even assisted in claiming the WET rebate as part of this process by their grower industry bodies (www.wgmb.net.au).
- Every cellar door and winery with an internet connection can be a retailer. In many wine regions it is common to see wineries, or groups of wineries, operate retail outlets in CBD or capital city locations to sell their wine – even in Chinese cities (Korporaal 2015; Spence 2016).
- Wineries are engaged in import-export business and act as distributors and wholesalers for brands not under their direct control or ownership.
- The last decade has been characterised by the emergence, worldwide, of virtual wineries and third-party promoters of 'Buyers-Own-Brands' (International Wine Exchange; Amphora Wine Group).
- At the time of its acquisition by Woolworths Limited, Cellarmasters Wines was the number one online wine retailer in the country, with the clear majority of its wine labels owned by Cellarmasters. Since launching Dan Murphy's online, this site has quickly established itself and overtaken Cellarmasters as the number one online wine site. Online is an area of significant movement and competition.

These examples show that there has been a fundamental fracturing of the 'traditional' lines and boundaries across the supply chain.

There is no doubt that Endeavour Drinks Group has been an innovator and has built an integrated supply chain model that is unique. It is not, however, the model itself that is unique but rather the scale on which it operates. One benefit of this adaptation is that there have been many partners who have also benefited from the changing market dynamics. A large component of the innovation and adaptation came as a result of open and transparent partnerships with 'traditional' producers and learning from each other.

But if the cyclical 'boom and bust' history that has defined the Australian wine industry is any guide, the only constant is change. With the dollar depreciating again, China on the cusp of a once-in-a-generation growth of its middle-class, and the appearance of some hardy green shoots in our traditional export markets, we may one day see a return to the days when grapegrowers would abandon a contracted delivery of grapes on the way to the winery as they took a phone call offering a higher price from another winery and when retailers – regardless of their size – would humbly wait cap-in-hand to have their allocation determined by the winery. Adaptation to such forces will mean securing supply firstly through strong partnerships and secondly through risk mitigation by direct ownership.

Both retailers and wineries mutually share the benefits and minimise the risks by creating exclusive brands

It is worth briefly explaining the role Pinnacle Drinks plays within the wider Endeavour Drinks Group's (EDG) exclusive brand strategy.

The first point to make is that these are not 'Woolworths-branded' wines. Woolworths is the retail trading name for Supermarkets and has in the past pursued a traditional UK-supermarket approach to its 'homebrand' offerings and labelled them with the name of the retailer. Endeavour Drinks Group, while part of the wider Woolworths Group of companies, is a separate business entity to the Supermarket Business Unit and is its own collection of retail brands.

EDG is interested in creating exclusivity, whether directly by Pinnacle using its own intellectual property or through brand partners.

Through Pinnacle, it is EDG's clear preference to partner first and buy or create a brand second.

This is reflected in the constitution of Pinnacle owned SKUs within the retail brands of Dan Murphy's and BWS being less than 10% of all liquor SKU's. Furthermore 66% of the brands in the Pinnacle range are owned by the winemaker, distiller or brewer and subject to long-term partnerships which will nurture and build the brands of the partner suppliers.

Retailers will only stock popular brands which deliver strong sales, which is why there is a great deal of emphasis in building and supporting brands and marketing them effectively.

EDG is interested in creating exclusivity of offering in its retail outlets in order to create customer loyalty by differentiating the range and protecting margins from intense, price-driven competitive activity on major brands. In reality, not everyone wishes to create a relationship with just one retailer. Pinnacle understands and respects this approach and works with many suppliers on single SKU exclusivity on the back of brands which are distributed market wide.

Pinnacle has operationalised the Cellarmasters and Dorrien Winery acquisition and the existing wider exclusive brand business. Its penetration of brands into BWS and Dan Murphy's is relatively modest in comparison to other retailers, both in Australia and internationally. As stated above, 66% of the brands in the Pinnacle range are owned by suppliers with less than 10% of the total SKU count being owned brands. Pinnacle is proud of these numbers and considers this as an example of supporting the industry's brand owners, winemakers and growers from almost every region across Australia and the world.

Retailers are aware they need to tell an authentic and traceable story if a brand is going to survive beyond an opportunistic sale. The future of exclusive brand business will not be so much about grabbing an opportunistic parcel of wine on offer and putting a label on it. It will be about wines that meet quality specifications and represent the brand being invested in so as to build consumer trust and confidence.

The wine made for Pinnacle at Dorrien, or through exclusive brand partners, is to expert winemaker specifications from regions across the country. These wines will find their way into brands managed by Pinnacle and will become part of an offering that can only be found at EDG retail brands.

In fact, whether it is Dorrien winemakers, or winemakers that make exclusive wine for EDG, these are award winning wines. Dorrien itself is now a five-star red-rated Halliday winery which is renowned for small batch, premium winemaking with 95% of its 11,000 tonne crush coming from 'premium regions'. And many of its winemakers are multi-award-winners and proud of what they do. They enjoy the freedom of working in a small batch, premium winery which mainly crushes from non-irrigated regions, supporting growers in pursuit of their living, and in many cases their love of making great wine. Their passion is repaid in kind by ensuring these wines make their way to the front of customer's eyes.

The growers, grapes, winemakers, and winemaking expertise and production of retail exclusive brands all contribute to the Australian wine sector, despite significant competition from low-cost overseas bulk wine-producing countries.

While absolutely respecting the right for winemakers to choose their supply chain constitution, the vast majority of wine producers who are in relationship with Pinnacle share the benefit of partnering with a retailer which has close to 1,500 retail outlets and a strong digital business that removes costs and creates efficiencies. For example, the EDG multi-million dollar investment in improving its logistics and transport capability has benefited supplier partners greatly. Whereas, previously, winemakers were required to distribute to every Dan Murphy's store across the country, now wine is delivered to a central location from which EDG manages the logistics.

The future will be about partnerships built on co-creation to innovate

In a highly competitive market with large substitutability between brands, retailers are acutely aware of how much a customer is willing to pay for a particular style, variety and brand of wine.

This creates natural tensions between retailers and suppliers and will to a certain extent create some strain on their relationship. The one thing all new recruits to retail learn is that the retailer is the buying agent and representative voice of consumers, and not the selling agent for the supplier. As their agent, the average consumer is expecting the buyer to get the best value for money. A supplier who understands this usually reaps the benefits of a strong partnership, including the respect that is gained when they feel secure enough to say 'no, we cannot do that'.

A new paradigm and operating model will likely distinguish profitable and sustainable wine participants from those clinging to historical business models.

As customers 'control the bus', it becomes business-critical for suppliers and retailers to understand and respond to their needs and wants.

Historically, the Australian wine sector has been slow to recognise that customers are now in control and will not simply drink a wine that a winemaker or 'expert' prefers. A case study has been the phenomenal success story that is New Zealand Sauvignon Blanc.

As a result, the area of vines in Marlborough grew from the vineyard area of Tasmania to more than all of Victoria combined.

While recognising the long lead times to bring a grape to acceptable levels of quality and volume, the ability of the Australian wine sector to act and respond to early trend information is worth exploring against the circumstance of what happened with NZ Sauvignon Blanc.

As retailers and producers tighten the bonds that bind them towards solving the customer's needs, they are hopefully in a stronger position to meet these changing needs.

Australian retailers have an absolute soft spot for Australian wine and sincerely want it to succeed. It's a global success story that we are all very proud to share a part in. The future, however, will be about how well the industry seeks to understand a domestic customer that continues to be promiscuous and explore international wines.

And to their credit, overseas producers are falling over themselves to partner with large Australian retailers and recognise the mutual benefits of working together in delivering on the customer's expectations. A short case study was initial advice provided by Dan Murphy's to a French winemaker to change from cork to screwcaps which took their sales from two containers a year up to fourteen (The Shout 2014).

What does 'co-create to innovate' mean and why focus on exclusivity

In the past, we witnessed two distinct roles for the retailer and the supplier: the retailer looked after the shop and the supplier looked after the brand. These traditional lines and roles have blurred over time and are evidenced by the growth around the world of retail offerings such as Aldi, where the consumer is fully aware that the brands sold in these outlets are 'fantasy brands'. We have also seen some bricks and mortar retailers in the UK grow their owned brands to 50% of their business.

A combination of brands the customer wants, which over deliver on value, and are exclusive to a great retail experience will create customer loyalty and advocacy. The supplier who is open to this creates a true partnership where sharing of forecasts, data, co-created product development, and sharing of the risks is common place.

The views of a young retail executive working in the Pinnacle team are both insightful and an indication of the changing landscape in front of the drinks industry:

> A brand is no longer what we tell consumers, it's what they tell us. And if customers are telling us they want exclusive label, then exclusive label belongs on the shelf as much as branded products do.

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The future for cellar door, food and art

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Abstract

Australian cellar doors are shedding their dusty heritage. Gone are the old shed and barrels. A future with food and art beckons. In the Old World, wineries trade on their past. Ancient castles and imposing estates lend an air of majesty and excellence. Aspirational buyers believe that quality and longevity is guaranteed. In Champagne, past exploits are now folklore. Corrupt trading practices saw Madame Clicquot give wine to bribe soldiers and bypass blockades. This led to sabrage (the practice of opening a bottle with a sabre) – something that is now a cellar door experience.

Today, Australia does have vineyards like Henschke's, with the world's oldest Shiraz vines, but that doesn't work for the rest of us. We compete in the New World with different economies, where tastings are hosted on linen draped tables, on spacious verandas with beautiful views. Quite often such economies have labour costs Australia can't compete with. If we can't deliver this experience, what can we do?

Australian cellar doors can create a new history by offering a new experience of wine. Partnering experiences with food offers depth and regional differentiation. We use light, landscape and Australian hospitality to engage. Restaurants are designed and bottles have labels. Art is important. Can it help target an intelligent traveller, ready for the new, with a high disposable income? Can art define a place and make an occasion special? Or does it distract from storytelling and experience? At MONA, we don't bother separating the ritual of wine drinking and art. And that's why, deep in the gallery, you'll find a bar.

No paper available, please view this presentation at http://bit.ly/16thWilsdon.

Future competition framework

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Abstract

The Competition Policy Review was the first 'root and branch' review of Australia's competition laws in over 20 years. Ian Harper chaired the review and will discuss key aspects of the final report that are relevant to the wine industry, including recommended changes to s46 of the Act (the so-called 'effects test') and proposed reviews of liquor licensing laws, retail trading hours and road pricing.

No paper available, please view this presentation at http://bit.ly/16thHarper.

Wrap-up of industry opportunity

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No paper available, please view this presentation at http://bit.ly/16thDAloisio.

Connection to country

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Abstract

As an Aboriginal person I have always held the belief that we are one people, one land with many stories:

- *One people – where culture doesn't divide us, but encourages us, to give, and take, to become one*
- *One land – a spiritual connection with country, we share this space with you, and you share it with us. When you walk the land, it becomes a part of you, and you become a part of it*
- *Many stories – have respect for the past stories, share knowledge of the stories we make today, and take the wisdom into building stories for tomorrow.*

'With enquiring ears, greater learning is gained.'

No paper available, please view this presentation at http://bit.ly/16thOBrien.

The importance of a sense of place in selling Australian wine

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Abstract

This topic involves fundamental issues with which the Australian wine community has been grappling for the past 15 years. Australian wine began a decline in image, sales momentum and average price point in all markets in 2001. The very successful establishment in the 1990s of Australia as the supplier of choice of well-made branded commodity wine was eroded by competition and oversupply which in turn undermined Australia's reputation as a supplier of fine wine. Recognition of Australia's fine wine opportunity in global markets is now slowly re-emerging. There has been contention about the importance of a 'sense of place' or 'terroir' in differentiating wine style and quality and its role in marketing and selling Australian wine. The definitions of 'fine wine' and 'terroir' have been endlessly debated in Australia to the point of obfuscation, detrimental to the development of strategies to identify and promote Australia's fine wine credentials. By looking at the current profiles and image of Australian fine wine in global markets and comparing them to those of Australia's fine wine competitors using the terroir stories of their regions and vineyards, we can gain some insights into the value of Australia promoting its regions and terroirs. Some important questions arise out of the acceptance of 'place' as a powerful force in influencing the style and quality of fine wine: What attributes of terroir should be researched and promoted? Does the success of Australian fine wine have positive implications for Australia's languishing branded commodity wines? Can Australia be the first choice supplier of fine wine to global markets?

Webcast of this presentation available at http://bit.ly/16thCroser.

Introduction

The Australian wine community's travails of the past decade are well documented and clearly visible in the export volume and value by market series, especially after the global financial crisis in 2008. The invasion of fine wine imports onto the previously unassailable domestic market further emphasised the fall from grace of Australian fine wine globally. There is evidence that Australia has an image problem as a credible fine wine supplier to the discriminating markets of the globe.

There has been much debate about the role of regionality and of the concept of terroir in the restoration of Australia's credibility as a supplier of fine wine.

With a growing emphasis on regional fine wines and their diverse terroirs and winemakers, there are recent consistent signs of recovery in nearly all markets as Australian fine wines at higher price points resume a growth trajectory (Wine Australia 2017a).

The $50 million (Wine Australia 2017b) questions for the Australian wine community and for Wine Australia are: How much emphasis should be placed on the regions and their terroirs in the promotion of Australian fine wine? What are the researchable questions that can support the authenticity and credibility of Australia's diverse terroirs?

The Australian fine wine malaise

In 2003 The Drinks Business identified a shift in UK press mentions about the (until then rampant) Australian wine category in favour of the traditional supplier France. The article heading was 'France leads fight back. The balance of press mentions is tipping away from Australia and back towards France.' (The Drinks Business 2003). The tone as well as the quantum of press mentions about Australian wine changed at that time with the words 'boring', 'standardised', and 'homogenised' prevalent and the consignment of Australia to the ultimate pigeonhole as a supplier of 'industrial wine'.

In 2004 Robert M. Parker Jr. made 12 messianic predictions about the global wine industry and in the final stanza of his final prediction stated, 'Australia has perfected industrial farming: No other country appears capable of producing an $8 wine as well as it does. However, too many of those wines are simple, fruity and somewhat soulless. Australia will need to improve its game and create accessible wines with more character and interest to compete in the world market 10 years from now' (Parker Jr. 2004a). Parker also famously declared the wines of the old vine Shiraz of the Barossa and McLaren Vale as the only fine wine styles at which Australia can excel and that our cool climate efforts with other noble varieties are 'imitations of the real stuff' (Parker Jr. 2004b).

These verdicts were levelled despite the fact that, of the 65 Geographical Indication regions in Australia, 24 are as cool or cooler than Bordeaux and that there were 2,000 small winemakers in Australia at that time—now 3,000—the majority being in those cool climate regions (Croser 2010).

By 2005 Australia was pigeonholed as a predominantly branded commodity wine supplier also producing some ripe warm climate Shiraz fine wine. The main market for the Shiraz fine wine was the USA and at the highest price point (>$10/litre free-on-board) the US market for Australian fine wine grew from $65 million in 2001 to $118 million in 2007, at the same time total Australian wine exports reached a peak of $3 billion. The USA was receiving 30.5% of the total value of Australian wine exports in 2007 and 32% of the highest price point fine wines.

In 2008 the GFC decimated luxury spending in the US and at the same time the fine wine market turned against ripe Australian Shiraz and in fact began shunning Shiraz from anywhere. Australian wine exports to the US dropped from $916 million in 2007 to $426 million in 2014 (−53%) and at the highest price point from $118 million in 2007 to $26 million in 2012 (−78%) (Centaurus Partners 2013).

The image of Australian wine in the US in particular, but also in all other main markets, was severely damaged and the demand curve for Australian wine exports shifted dramatically downwards. In 2016 the Australian wine community is still attempting to recover from these events and is looking for ways to enhance Australia's fine wine image and credentials.

Quality perception of Australian wine

Australia is a dominant exhibitor in international wine shows such as the Decanter Wine Awards and the International Wine and Spirit Competition. The success of Australian winemakers in these competitions, however, has not seemed to translate into an elevation of Australia's fine wine image. In wine shows, as in the regions in Australia, there is a big gap between performance and externally perceived quality.

Wine Australia commissioned Wine Intelligence to conduct a brand health tracking study for Australian wine among consumers in eight key markets including China, English-speaking Canada, USA, UK, Quebec, Japan, Singapore and Hong Kong.

This latest consumer study was completed in May 2016 and follows similar studies conducted in 2010, 2013 and 2015 (Wine Australia data unpublished).

In the UK, Australia's biggest market by volume, Australia's quality perception ranks behind France, New Zealand, South Africa, Italy and Chile. In the USA, Australia's quality perception ranks behind France, Italy, California, Spain, other USA and Chile. Only in China does Australia's quality perception nearly rank with France and Italy and is ahead of California and China.

Eleven associations were presented to the participant consumers for evaluation:

- Wines which offer good value for money
- Food friendly wines
- I would be happy to recommend wines from this country
- Has wine brands I recognise
- Offers a wide variety of wines
- I like the grape varieties produced in this country
- I am proud to serve wines from this country
- Has distinctive wine-producing regions
- Wines for a special occasion
- A long tradition of winemaking
- Expensive wines/fine wines

In all eight markets the perception of Australian wine is competitive, in the first six associations at 60% to 90% (100% being the best possible association) but in the last five Australia has poor perception (30% to 60%) as a supplier of wines for special occasions or expensive/fine wines from distinctive wine regions with a long tradition of winemaking.

The perception in the international marketplace does not reflect the reality of Australia's 200-year tradition of winemaking, its 65 diverse wine regions and 3,000 vignerons producing fine wines. In particular, the understanding of Australia's wine regions is very poor compared to those of France, Italy and Spain, which collectively dominate international fine wine commerce. The countries with a strong perception as suppliers of expensive/fine wine also enjoy a strong recognition of their distinctive wine-producing regions. The optimistic aspect of the Wine Intelligence findings is that the perception of Australian wine is improving in all markets that have been tracked since 2010.

Recently Professor Roberta Crouch of the University of Adelaide commented, 'Australia has image issues in USA and UK', following her interviews of trade and consumers in key and emerging markets. Her solution is 'to form the message, to occupy our unique premium position based on our unique attributes.' That must include our unique geographical circumstance on the globe or, in other words, our distinctive regions and terroirs (Reynolds 2016).

In Adelaide recently, Professor Liz Thach of Sonoma State University recommended that Australia revive its 'Regional Hero' strategy for the USA, 'A regional strategy makes more sense because you don't see other countries using a country strategy – they use region. For example, in Europe you don't see Brand France or Brand Italy, you see Burgundy or Tuscany. Even in the US you don't see Brand America for wine, you see Napa, Sonoma or the Finger Lakes. So, Australia needs to rethink Brand Australia and focus on promoting its distinctive and amazing regional wines' (Reynolds 2016).

There is growing recognition in the Australian wine community that we must overturn the pigeonhole stereotype of Australian branded commodity wine and replace it with a much more textured and nuanced story about our regions, their vignerons and the unique attributes of the terroirs in those regions which define our fine wines. This recognition has been manifested in Wine Australia's declared priority of 'Increasing demand and the premium paid for all Australian wine', using the strategy of 'Promoting Australian fine wine' (Wine Australia 2015).

Regions and terroirs

I do not wish to be diverted by yet another debate about the actual existence, importance or definition of terroir. I am going to crash into the jungle of terroir opinion by declaring terroir does exist and it is the most important determinant of wine style and quality. For clarity, my definition of terroir is confined to the geographical (environmental) inputs of the vineyard site, affecting in turn vine physiology, grape and wine composition and hence wine aroma, flavour, texture, quality and style.

I recognise the extended definition of terroir to include the potentially unique microbiota of site and the human and cultural influences on vineyard practices and site expression. I contend however that the core of the story of vineyard and wine differentiation will be revealed by focusing on the geographical inputs of site to define terroir and elucidating the subsequent physiological mechanisms that lead to reproducible and unique wine quality and style.

Ever deeper into the jungle, I further contend that the story created by terroir (aka site environment) is not merely important but is essential to the establishment of the authenticity and credibility of Australia and its regions as a global fine wine supplier; that is, the selling of Australian fine wine.

Two well-qualified guides accompany me on my journey deeper into the jungle of terroir opinion. The first and foremost guide is Dr John Gladstones, my hero and the author of the inspired and inspiring *Wine, Terroir and Climate Change*. In the first chapter of that book he sets out a definition of terroir, which is consistent with the one I have announced:

> Here I use the term *(terroir)* in what I believe is its original and correct sense, as set out by French writers such as Laville (1990). That is simply the vine's whole natural environment, the combination of climate, topography, geology and soil that bear on its growth and the characteristics of its grapes and wine. (Gladstones 2011)

My second guide into the terroir jungle is Mark A. Matthews, the Professor of Viticulture at the University of California, Davis and the author of *Terroir and Other Myths of Winegrowing*, an unlikely guide on a mission to discover the holy grail of terroir (Matthews 2016). Any vigneron trying to unravel the apparent mysteries of their vineyard should read this book as it provides some valuable insights into vine response to environment and challenges conventional thinking, although I would argue he has used selected and sometimes inappropriate examples of conventional thinking.

Professor Matthews has spent a lot of words trying to persuade me to abort the mission, that terroir is a confused and unnecessary concept and should not be used. He condemns terroir as a geo/agropolitical construct to protect the established order of fine wine producers and allow them to extract a rent. He traces the use of the word terroir from its simple age-old use meaning soil to the pejorative 19th century description of wine with a pronounced unpleasant taste as suffering from 'gout de terroir'.

Professor Matthews contends that the word terroir only achieved its current status and meaning as a positive attribute of fine wine after the famous 1976 Judgement of Paris. Finally, Professor Matthews' definition of terroir, begrudgingly given, is the same as mine and the same as Dr John Gladstones', when Matthews writes:

> When terroir is used in the context of viticulture, it is most effective as a synonym for *environment*, in which case using the term *environment* would be clearer and more accurate.

He then announces the challenge that is central to Australia's research community in support of proving Australia's unique attributes as a fine wine supplier:

> The challenge for the viticultural side of winegrowing is to learn which parts of the environment impact vine growth and development sufficiently to result in significantly different fruit, and to exploit that knowledge to identify the best sites and practices for selected wine models.

Having defined terroir and the mission to elucidate it, Professor Matthews commits a faux pas by delivering the coup de grace to his argument against using the French term terroir when he argues 'Terroir reflects a wine business perspective that is manifested on wine labels. Today, *terroir* is primarily a marketing term'. Well hello!

He goes on 'The use of *terroir* has expanded to cheese, coffee, and other products because of its success in selling products, just as it expanded from Champagne and Burgundy to the wine world at large.'

If you need any stronger endorsement of the importance of the use of the word terroir and viticultural understanding of the concept of terroir for the Australian fine wine community, he gives it in two graphs which demonstrate the rise and rise of terroir in 'Google Ngram analysis of occurrences of 'terroir' relative to 'wine' in digitised books published since 1900' and 'the frequency of 'terroir' and 'wine' appearing together in research papers in the CAB abstracts database'.

Terroir is indeed lingua franca for the world of fine wine and its gatekeepers and knowledgeable consumers. Fait accompli.

In a recent ironic development, some defenders of the faith of the primacy of French vineyards and culture are proposing abandoning the use of the word terroir, which the rest of the world is debasing, and adopting a much more localised word, 'climat', to describe the uniqueness of their vineyards.

In *Wine, Terroir and Climate Change*, Dr John Gladstones gives real and hypothetical logic to the interaction of the vine with its environment and the physiological responses that lead to flavours, aromas, tannins, colour and texture in wine. He describes the primacy of climate and particularly temperature as the basis of choice of variety and the effect on grape and wine composition. He carefully integrates what we know about the many elements of the viticultural environment with the physiological mechanisms of the vine's response and their effect on grape composition and thence on wine style and quality. Gladstones reviews and emphasises the environmental stimulation of the root formation of plant hormones in the physiological chain that leads to ripe grapes. In this Gladstones takes the concept of terroir and how it works into much greater detail than Matthews. Both books are essential reading for committed vignerons.

I hope I have made the case for the use of the word terroir in the marketing of Australian fine wine and the need for an understanding of triggers and physiological mechanisms that underpin wine quality and style to give real meaning to the word.

I would like to understand whether there is an Australian macro terroir based on the unique boundary of our viticultural regions on the benign climatic 30 to 40 degree latitudinal range with the Southern Pacific, the Great Southern and the Indian Oceans. Most other wine countries span a large longitudinal range with much greater climatic variability. Australia is the flattest, windiest, driest continental surface with the oldest surface geology and the most weathered soils. I would like to know how this might affect an Australian macro terroir.

On the meso scale, terroir manifests as the heat summation of the region and its daily range, the rainfall quantum and timing, the wind factor, the sunshine hours and intensity by wavelength and the relative humidity. These have a profound influence on the choice of variety, the vineyard management practices and the final quality and style of the wine. The influence of the vineyard slope and longitude-driven angle of sunlight incidence, day length and its rate of change, and the true continentality are all meso terroir parameters that beg better understanding.

If there is complexity in the atmospheric environment of terroir, then that multiplies when we consider the edaphic environment, that below the earth's surface. The soil, its biota and organic and mineral nutrition, the amount of light it reflects, its physical structure (silt, clay, sand and rock), its water and heat transmission and holding capacity and boundary with the geology (lithosphere) all have profound effects on the nutrition and hormonal control of the phenology of the vine and on ultimate grape and wine composition.

Then we have the micro terroir, the climate around the leaves and the bunch of grapes, which we can influence through management practices. So much to understand and it is different in every region and vineyard.

All of us who have worked in regions and vineyards understand that regions are best suited to a given suite of varieties. In the words of Dr A.C. Kelly in his 1867 treatise, *Wine Growing in Australia:*

> In the great diversity of soil and climate to be found in Australia, there is little doubt that every variety cultivated in Europe would somewhere find a suitable location in which to develop its most valued qualities. (Kelly 1867)

All of us understand that within a region not all vineyards are created equal. Within a region there are superior sites that are more expensive as real estate, the grapes are worth more and the price of the wine made is higher because the wine is better. Comparing Shiraz from the Upper Tintara vineyard on the ironstone ridge of northern McLaren Vale to Shiraz from Blewitt Springs or comparing Cabernet from Coonawarra, Wrattonbully and Margaret River, the consistent and real differences of wine style and quality are recognised by the fine wine community. What drives these differences? That is the story that will allow us to grow better grapes, make better wines and give us authentic and better stories to tell the consumers. In the words of Professor Matthews:

> Today more than ever, the traditional explanations for wine quality and the stories that accompany the wines we drink are cherished almost as much as the wines themselves. (Matthews 2016)

And finally, in the words of my good friend, Andrew Jefford (pers. comm. 2006):

> Wine is one of the loveliest and most intricate of nature's gifts to us, since its creation is unlocked by human interaction and it enables us to taste the landscapes and seasons of the natural world with extraordinary precision. To drink wine is to drink nature. That is why most of us love wine, it is also a kind of love for the world itself, for being alive and being here.

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Unique Australian wine offerings beyond the single vineyard

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Abstract

Australian winemakers have enormous freedom to express themselves and their viticultural and oenological situation. Whether that situation is based on vineyard, region, variety, house style or history, Australian winemakers are unfettered by appellations and onerous rules. The resulting wines and experiences are therefore unique and may be enduring or continually evolving or both. Central to this is a culture of inquisitiveness, exploration, optimism, self-belief and unapologetic honesty. A culture that celebrates innovation and minimises barriers to entry will always yield unique wine offerings.

Webcast of this presentation available at http://bit.ly/16thDonald.

Introduction

As Australian winemakers we have enormous freedom to express ourselves and create the best possible from our own viticultural and oenological situation. The wines that we offer to the market may be the result of our philosophy, a brilliant idea, courage, a requirement of our business, interpretation of a plot of country, somewhere in between or a mixture of these. Appellations and onerous rules do not hinder the winemakers' craft in Australia.

I believe whatever we all do individually or collectively, we are a work in progress; respecting and appreciating our history but not letting it interfere with our push forward to understand our country and our climate and to make delicious wines that suit our food and our lifestyle and to successfully service our markets.

For this paper I have been asked to give some thought to unique Australian wine offerings beyond the single vineyard.

So, what defines single vineyard?

My preferred definition comes from a 2012 Lisa Perrotti-Brown article about Henschke Hill of Grace (Perrotti-Brown 2012). She asks, 'Is there a limit on how big it can be? Is there an implied uniformity of terroir and vine in these words, and to what extent is that even possible?'. She argues that 'when taken to its ultimate extreme, the words 'single vineyard' should conjure images of miniscule parcels of near mono-geological turfs that have long been married to a single varietal soul-mate'. But whatever the size of the vineyard or the number of distinct blocks of varying soils, vine ages and varieties, Perrotti-Brown asserts that a wine should only be classified as a 'single vineyard' wine if it represents a 'thoughtfully delineated example of elevated quality that stands apart from that which surrounding vineyards can achieve and expresses something singular'.

All winemakers make single vineyard wines each year as part of the vintage and creative process. Why or how do some of these wines make it to the wine public in a bottle as single vineyard wines? Single vineyard for single vineyard sake is not good enough – the wines must be different enough to make it worthwhile. The wine produced must be singular and enduring – produced each vintage where quality expectations of the winemaker are met and followed and anticipated by wine consumers.

Single vineyard offerings are well placed to lift both image and price points but more consideration needs to be given to criteria for single vineyard label claims to ensure integrity. The discussion, debate and investigation of individual site and terroir must continue but it should not obscure other considerations or appreciation or other presentations of Australian wine.

So, additional to single vineyard wines, what else does Australian wine offer? Here is a list of six items: it is, by no means, exhaustive.

1. South Eastern Australia – a uniquely Australian GI

Let's go large! Like the classification of life from species to kingdom, let's go from individual vineyard to South Eastern Australia!

Varietal wines being imported into the EU must be labelled with an officially recognised region. The Australian Geographical Indication 'South Eastern Australia' was entered in the Register of Protected Names on May 1, 1996 and was created to meet EU regulations for offerings of varietally labelled wines that were blended from wines from multiple regions or states.

The heartland of value wines, the wines of South Eastern Australia have a reputation for being well made, bright fruited, consistent, accessible, varietally labelled, vintage specific and with volume to take to the world. Effectively, wine from South Eastern Australia is 'wine from everywhere and nowhere that is from somewhere' giving it its own identity.

This is a uniquely Australian advantage we have, admittedly by responding to EU requirements. However, given recent changes to EU laws about labelling of wines from Europe, Europe is taking a similar approach and our advantage might be less prominent in the future.

Jancis Robinson in a 2014 article discusses the 2010 evolution in labelling of wines from Europe; the EU has decided that a major shake-up in the structure of its wine market is needed to make it more competitive with New World wines (Robinson 2014). In France, for example, the bottom category of wine, Vin de Table, has been replaced by PGI – protected geographical indication – to remove the word 'table' and its connotations of low quality; France has chosen to use Vin de France. These wines are those that do not meet the criteria stipulated by *Appellation d'Origine Contrôlée* or *Vin de Pays* appellation laws. This might be because the vineyards are outside the delimited production areas or because the grape varieties or vinification techniques do not conform to the rules of the local appellations. This is important because producers are now permitted to put grape varieties and vintage years on a Vin de France wine, making this category much more interesting for the producer and the consumer. The Vin de France regulations are more liberal as well and contributions to the governing body are far less.

The point here is that the uniquely Australian response to EU laws 'wine from everywhere and nowhere that is from somewhere' has been adopted by the EU! I think it is reasonable to make the observation that while France is moving to options for origin labelling with less regionality so her wines can be more competitive with New World wines, Australia is deep into her own journey for greater understanding of subregionality and site.

2. Regionality and multi-regional blending

Trial and error, experimentation and time have yielded tried and true regional/varietal combinations that are uniquely Australian.

To make the case for Australia, nowhere else in the world can deliver Frankland River Riesling, Hunter Valley Semillon, Yarra Valley Chardonnay, Tasmanian Pinot Noir, Barossa Shiraz, Coonawarra Cabernet, Margaret River Cabernet, Rutherglen Muscat and so on. So, regionality can be explained as the ability of a region to produce wines, or wine from specific varieties, of a distinctive and recognisable style. A very clear message – wine from somewhere with a unique identity.

Matching the variety to the climate and the turf is an important part of regionality but regionality also covers the region's history, heritage and reputation. The full experience of enjoying a region's flagship wine varietal with the regional dish or local produce in a picturesque corner of the valley or the vale cannot be underestimated. Add the company and occasion and wine in context becomes wine in your context, your experience.

Enter freedom. The tried and true combinations are not appellated, there are no rules about what can be planted and how to make it. If you want to make a rosé with 108 varieties, go right ahead! If you want to plant a variety new to a given region or an emerging varietal new to Australia, go for it! We may just learn that it produces better wine than the tried and true variety for that region.

Multi-regional blending is also a unique Australian wine offering where house style or the idea of a wine in the winemaker's head drives winemaking philosophy.

Well-known multi-regional blenders are Penfolds and I quote from their 'Rewards of Patience Fifth Edition':

> The concept of multi-regional and vineyard blending, a feature of Penfolds house style, exemplifies the 'all round' wine style. Without the constraints of single vineyard, winemakers choose the best possible fruit, showcasing the outstanding characteristics of each vineyard. The idea gathered pace during the 1960s as a result of the success of Bin 389 and experimental cross-regional blends such as Penfolds Bin 60A. This method of fruit selection also contributed to a consistency of style.

Of course, throughout the 1940s, 1950s and 1960s there was a roll call of winemakers who undertook multi-regional blending: Maurice O'Shea, Colin Preece, Roger Warren, Colin Haselgrove and others as well as Max Schubert from Penfolds.

Whatever the genesis of multi-regional blending and by whom, there is a sense of the relentless pursuit of the very best fruit to achieve the style in mind; taking the strengths and typicity of the variety in two different regions and successfully blending, creating synergy, creating a stronger or better wine.

Other producers who undertake multi-regional blending, for example, are Hardy's Wines with their HRB range and Brokenwood with their HBA Shiraz (a Hunter/McLaren Vale blend), both acknowledging Australian winemaking history.

3. The great Australian red blend

Cabernet Shiraz, Shiraz Cabernet – the dominant varietal depending on your point of view, your history or your blending results. But it is the quintessential Australian red blend.

Whoever blended these varieties first, it was presented in the late 1800s as claret. The blend rose to prominence again in the 1950s as a blended varietal wine, the aforementioned winemakers playing their part. There is no doubting that the blending of Cabernet Sauvignon and Shiraz brings something extra to each variety and therefore the resultant wine.

Some of Australia's top-flight reds are a blend of these two varieties, for example, Penfolds Bin 389, Yalumba Signature, Wynns V&A Lane and Wolf Blass Black Label.

In Australia, in terms of regional selection, often the two varieties are taken from different regions to create extra levels of ripeness and layers of complexity – Coonawarra Cabernet and Barossa Shiraz being a classic example.

Matthew Jukes and Tyson Stelzer have collaborated to celebrate the great Australian red blend by way of a competition now in its 10th year (http://thegreataustralianred.com/); in their words:

> The Shiraz Cabernet blend is an Australian institution. This country championed it, refined it and still does it better than anyone else on the planet. It's our only unique, definitive red. This is Australia's national treasure of the red wine world, and it deserves to be recognised and celebrated as Australia's greatest red wine.

4. Premium Australian sparkling wine

Australia has a long history of sparkling wine production, with sparkling wine production beginning in the Hunter Valley in the 1840s.

In the 1890s Hans Irvine set up facilities for making sparkling wine in the Great Western region of Victoria.

In the 1890s the first Sparkling Burgundies were produced, a unique Australian contribution to the world of wine, with the quintessential Sparkling Burgundy style developed by Colin Preece in the 1930s and 1940s. Sparkling Shiraz is another quintessential and uniquely Australian product, which is little known outside Australia. Seppelt today continues to honour the style with its Show Sparkling Shiraz; only released in exceptional vintages with significant bottle age, it is a testament to the region's fruit style and quality and the winemakers' craft.

Only in the 1980s did Australia produce the first Traditional Method sparkling wine using the classic varieties Pinot Noir, Pinot Meunier and Chardonnay.

The emergence of Tasmania as a sparkling-focused wine region in the 1990s and 2000s has rapidly elevated our collective expectations of sparkling quality in Australia. Australia's coolest climates are now recognised as producing sparkling wines that challenge the notions of the supremacy of Champagne and carve out a unique identity in doing so. Our premium sparkling wines utilise a 300-year-old French technique, to capture raw and ancient landscapes and astounding climates unfettered by regulation.

Ed Carr, in a 2014 interview with Nick Stock, stated: 'The proof is in the wines really, but when we looked at cooler climates it was the more southerly higher latitude wines that had the most supple tannin, elegant structure and greater longevity'. He maintains that sites on the mainland of Australia that achieve cooler conditions by means of altitude rather than latitude produce richer, more fruity and more angular sparkling wines as a general expression of their terroir (Stock 2014).

So, in the theme of a unique Australian wine offering, what have we learnt from sparkling? It is again the art of blending, the strength of house style and the impact of our climate and our terroir; again this notion of blending to achieve a desired style but the regionality and terroir provide first class raw material.

Discussing premium Australian sparkling with Natalie Fryar she made the following point: 'Australian sparkling wines challenge the perception of Australian wine as warm climate and full bodied; they offer restraint and elegance, coupled with defined fruit expression'.

5. Fortified winemaking heritage

Australia has a long history of fortified wine production – another unique offering we can be proud of that sometimes can be overlooked or underappreciated. Fortified wines were the dominant wine style made and consumed in Australia until the mid-20th century.

With a changing demographic, changing tastes and new winemaking techniques, fortifieds became dominated by table and sparkling wines and the production and maturation of the fortified wine styles decreased. However, with the torch being carried by Rutherglen winemaking families and wineries in the Barossa Valley, Swan Valley, South Australia's Riverland and elsewhere, Australia's unique fortified wine treasures can be discovered and appreciated.

Visualise tin roofs, hot summers, cobwebs, 'angel's share', dusty floorboards – the preserve of time and place, of many summers and many winters, fortifieds are the most patient of wines styles.

Rutherglen in north-eastern Victoria is home to Brown Muscat. The Muscat wines produced here are truly a national treasure; virtually fortified juice at a Baumé of anywhere from 17 to 24°, these wines are borne from fruit concentration and balance, time, maturation and patience.

One of the world's great sweet wine styles, the best examples of Grand Muscat are deep and complex but at the same time display freshness and some rose petal attributes; the best examples of Rare Muscat are incredible – deep, dark, complex, concentrated raisins and fruitcake.

Another unique fortified wine offering from Australia is the Seppeltsfield 100 Year Para Tawny. Not only the wine itself but the story of its genesis and the fact the idea of it has been respected by subsequent generations. The result is that Seppeltsfield has the longest unbroken line of year-dated tawnies in barrel in the world. In 1878 to celebrate the completion of the first wine cellar on the Seppeltsfield property, Benno Seppelt selected a puncheon of his best tawny from that vintage and decreed it was to be matured for 100 years before release. That indeed happened and in 1978 the first 100-year-old tawny was released. This tradition has endured through varied ownership of Seppeltsfield and wine has been laid down for every succeeding vintage and matured as pending 100-year-old. This tradition is also evolving; with the refurbishment of Seppeltsfield and the addition of a destination restaurant, consumers are discovering and rediscovering fortified wine styles; the ultimate being the ability to taste your birth year in Seppeltsfield's centennial cellar.

The fortified wine styles are excellent examples of not only unique Australian wine offers but of optimism and tenacity. Which is an appropriate segue to the final item on my list of unique Australian wine offerings.

6. The Australian condition and culture

Ourselves. If we are an industry of grapegrowers and winemakers who wish to make wines that speak of place then surely we must have a sense of self.

What is that sense of self? That we are Australian and live on this soil. That we are explorative, brave, optimistic, enthusiastic, possess self-belief and unapologetic honesty; that we celebrate innovation and that anyone can have a go!

In terms of our winemaking we are not prescribed by history and we are not prescriptive by nature. We have no centuries-old wine traditions determining all our grapegrowing and winemaking activities. Australians seek out the world and bring ideas back to be interpreted in our own context – from James Busby to Maurice O'Shea and Max Schubert to viticulturists and winemakers today.

The Australian wine community is inquisitive and responsive, relishing innovation and research – consider the reputation of our educational institutions and the Australian Wine Research Institute.

Being inquisitive and responsive:

- drives the push into cooler climates, responding to changing weather conditions and the search for different quality, clearer and more focused expressions of grape varieties
- drives the push to explore varieties new to Australia, responding to changing weather conditions and the search for different and interesting varieties to work with, to match with our food, our climate and to push winemaking experience and continue instinctive and thoughtful winemaking
- drives the push to develop new practices to manage the impacts of drought and heat.

While European style regulation is not the end game, the amount of work across the country on subregionality is extremely exciting. Australia has worked on the grape varieties – the 'what is it' and now across the country we are really drilling into the 'where is it from' in terms of soil, geology and weather patterns at a subregional level. Projects such as the Barossa Grounds project, McLaren Vale's Scarce Earth project, the Clare Valley Rocks project, Western Australia's mapping of their six districts and the Hunter Valley collaborating with the University of Sydney to study their soils, just to name a few.

We are diverse and authentic; look at the ways we collaborate to showcase our wines, our wine stories, our wine experiences – First Families of Wines, the Landmark Tastings, Women in Wine Awards, and Rootstock as examples.

Searching for a third party comment on the Australian disposition, I found this by Oz Clarke in his *Oz Clarke Australian Wine Companion* (2004):

> But as the second half of the twentieth century hurried in it became clear that what Australia did have was personalities determined to impose their wills and ways upon wine. People, who were gritty, determined, focused, passionate, imaginative and opinionated. Shy, retiring and conciliating? No. Never. What would that have achieved...sometimes the only motto worth knowing is 'you gotta have a go'. And have a go they did.

Closing remarks

In closing, Australian wine history has given us solid examples of unique Australian wine offerings.

But what of the future? The arrowhead for unique Australian wine offerings of the future is protecting the natural resources of this continent. And it is happening now. There is much work being done around the country to protect our ability to express our country and taste our landscapes through winemaking in the face of environmental challenges.

Consider the commitment and years of work by the Chalmers family on alternative varietals, availability and improvement of vine stocks and sustainable vineyard practices (http://chalmerswine.com.au/about.aspx). Consider the producers dotted around the country committed to biodynamic, organic and/or sustainable principles to sustain soil health – Cullen Wines, Yalumba, Botobolar, Delatite, Battle of Bosworth to name just a few and the achievements of programs such as Sustainable Australia Winegrowing.

Overall, I believe the next decades will bring further refinement – continuation of our understanding of our places and climate, the ongoing strive for quality wines that speak of the site, the fruit and the winemaking philosophy and minimising viticultural and winemaking inputs. Combined with new ways of communicating our craft and sharing our authentic wine stories with consumers globally, we have the key elements at hand to grow our reputation and our market share. Continued enthusiasm, the celebration of innovation and minimal barriers to entry will ensure Australian winemakers continue to yield unique wine offerings.

So, with unique Australian wine offerings in mind – those listed here, those not listed here and those we haven't thought of yet, I leave you with a quote from Max Schubert:

> …we must not be afraid to put into effect the strength of our own convictions, continue to use our imagination in winemaking generally and be prepared to experiment in order to gain something extra, different and unique in the world of wine.

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Terroir in the Old and New World – what sensory is telling us

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Abstract

Although geological diversity has been described for many vineyard sites, there is little scientific knowledge on how specific vineyard soil and climate conditions translate into the sensory properties of the wines. In order to establish direct relationships between terroir and sensory properties, many transitions have to be taken into account: the translation of site-specific factors into grape and then wine composition, followed by the sensory perception of a wide array of wine consumers. Nevertheless, there is a common perception among wine experts that certain soil types such as granite, slate or limestone are able to shape the wines in a consistent way and even allow consumers to pick up common sensory patterns of bedrock types.

Due to the long historic focus on a few or even a single variety in different regions of Europe, there is a wealth of knowledge on how varieties such as Pinot Noir, Chardonnay or Riesling react to specific terroirs. However, to think that boundaries of appellations have been solely established based on specific geology aspects or even common sensory patterns neglects the strong economic driving forces also at play. In contrast, the New World is free of these historic restrictions and when the first American Viticultural Areas were established in the late 1980s, it was requested that their wines should be differentiated by sensory means.

Trying to establish and implement a sensory classification based on different bedrock types, we identified three crucial steps:

- *to evaluate wines made in a consistent winemaking process from different sites and detect common and differentiating sensory patterns*
- *to convince winemakers that they need to agree regarding these sensory patterns, in order to help consumers to recognise them and to believe that terroir really matters beyond marketing*
- *to relate in a scientific approach sensory properties and wine composition with specific chemical and physical soil characteristics and climate aspects in order to assist winemakers to enhance and clarify the sensory perception of terroir.*

Webcast of this presentation available at http://bit.ly/16thFischer.

A definition of terroir

The notion of terroir derives from the Old French terms *tieroer* and *tieroir*, which themselves stem from the Vulgar Latin term *terratorium*, a variation of the Latin *territorium* which translates into domain, district, territory (Woodhouse 1987; Rey et al. 1998). Terroir is a gallicism without a synonym in any other language and today is incorporated in many languages.

The complexity of the notion of terroir in its original language, French, and the resulting mistaken translations (Vaudour 2002) may contribute to the large number of differing interpretations that can be found with regard to wine. For several authors, terroir comprises all natural environmental factors of a site, which can be classified into soil, topography, and climate, including their interactions (Dubos 1984; Audier 1993; Falcetti 1994; Robinson 1994; Riou et al. 1995). The specific combination of these factors provides each site with a characteristic terroir. The distinctive sensory properties of wine, being impacted by the above interactions, are at the centre of the terroir concept. They should be perceivable across vintages and ideally independent of viticultural or oenological practices.

Apart from the interactions of soil, topography and climate, many authors also consider sociocultural, socio-economic and historic aspects to have an impact on terroir as well (Mesnier 1996; Salette 1996; Salette et al. 1998; Garrier et al. 2001; Moran 2001; Vaudour 2002; Morlat 1998; Turner and Creasy 2003; van Leeuwen et al. 2004; Deloire et al. 2005; van Leeuwen and Seguin 2006; Jones et al. 2012; Tomasi et al. 2013). For example, van Leeuwen and Seguin (2006) argue that vineyards would not exist per se without human activity. Historically, vineyards emerged on sites with low agricultural productivity, such as steep hillsides, or shallow, stony soils, whereas the more fertile soils were reserved for farming and grazing (van Leeuwen and Seguin 2006).

Viticultural practices and oenological procedures are regarded as a means for exploiting the full potential of vineyards, and are therefore also often included in the range of terroir components (Salette et al. 1998; Deloire et al. 2005). Others are convinced that 'unfortunately, the "discovery" of terroir in the popular press was not preceded by scientific discoveries of soil-derived flavors, or other validations of putative characteristic flavors from a more broadly defined terroir.' (Matthews 2015).

Finally, if any differentiation of wines according to terroir aspects is to be honest and sustainable, going beyond pure marketing considerations, they have to translate into sensory differences, which ideally should be consistent and recognisable by consumers and experts.

In conclusion, the terroir expression of a wine should be defined as the perceptible sensory and chemical dimension of the interactions between the grapevines, the geological and pedological factors of a given site, the site's topography, and the site-specific climatic conditions (Bauer et al. 2011). Although anthropogenic elements are excluded from this definition, terroir cannot be regarded in isolation from human activity, as viticultural and oenological practices can enhance or mask the terroir expression among wines.

A historic view on terroir in Europe

Naming regional sites and wine heritage date back to ancient sources in the Bible and records from Egypt, Greek and Roman times differentiated wine qualities based on regionality (McGovern 2003). The concept of terroir was introduced by the Cistercian monks in the 12th century in Burgundy, who observed that grapes grown in different locations indeed changed the sensory properties of the resulting wines.

The wine legislature in Europe was and still is a strong driving factor for the terroir concept of the Old World. Viticultural regions and vineyard sites are precisely defined, starting with the classification of borders of the Chianti Appellation in 1716, Tokaji in 1730 and the Douro in 1756. Going beyond definition of borders, the French started with the legislation of the Appellation d'Origine Contrôlée (AOC) in 1905 to build a legal framework (Trubek 2008). Today, Protected Designations of Origin (PDO) such as the Barolo Denominazione di Origine Controllata e Garantita (DOCG) in Italy are distinguished from the larger protected geographical indication (PGI) such as the Dolomiti Indicazione Geografica Tipica (IGT) comprising among others the Denominazione di Origine Controllata (DOC) of Alto Adige and Trento.

Due to a long history of winemaking and experience of the viticultural methods that suit the local vineyard conditions, the PDOs in France, Italy and Spain not only define geographic boundaries but specify as well the choice of grape varieties, trellising systems and maximum yields per hectare. This strict policy can be beneficial in communicating typicality and uniqueness of a region, such as allowing only a single Sangiovese clone to be planted in the small Brunello di Montalcino DOCG in Tuscany or devoting the Mosel Valley mainly to Riesling. In other regions however it may act as an obstruction limiting the necessary adaptation to ongoing change in consumer preferences, as it is the case in the Beaujolais AOC, where wines can be made only from Gamay, although many vineyards would be suitable for more renowned and esteemed varieties such as Pinot Noir from the northern neighbour Côtes du Nuits and Syrah from the southern neighbour Côtes du Rhône. However, in recent times France successfully eliminated the obvious restrictions of its PGI system, which allowed mentioning only the region such as Vin de Pays de la Loire but not the variety Sauvignon Blanc. Today Vin de France wines are eligible to label grape varieties which has fostered the current success of these wines in export markets.

Some European PDOs require the wine to pass a sensory assessment before wines may be sold. This task is accomplished by interprofessional organisations, where experts from trade and wine producers evaluate the wines in terms of geographic and varietal typicality with the intention to shape the sensory expression of the PDOs. However, this important quality management tool has been recently watered down to a mere examination regarding absence of off-flavours and meeting minimum quality standards.

Arguments for terroir initiatives in the New World

In contrast, the New World winemaking areas such as Australia were free of the Old World historical limitations partially because nobody knew which variety and trellising system best matched the climatic and soil conditions of the newly developed areas. This flexibility allowed fast progress in establishing high wine quality and facilitated economic success in many export markets such as the UK or the USA. However, wine experts soon recognised that the next step needed a more refined reference to the geographic heritage, as Brian Croser expressed:

> In fact, it is Australia's penetration of global markets with highly successful commodity brands that should create the incentive for distributors to exploit the well developed, but still evolving, Australian regional wine treasure trove. … the restless search for excellence in some of our very best terroirs. (Croser 2004)

In 2013, Brian Walsh went further by stating at the 15th AWITC:

> The traditional differentiators in wine have been variety, place of origin and style. The most difficult to copy is place of origin and that continues to provide the best opportunity for value creation through differentiation. Generally, but not exclusively, the highest value wines in the world are single vineyard wines, followed by wines of finite appellation. It is strongly recommended, to build on what is unique in their environment and celebrate its differentness. Ultimately, one's place and intellect will be the value creator. (Walsh 2014)

As a consequence, in the 1980s the first American Viticultural Areas (AVAs) were established and, in the beginning, the process of recognition requested a formal sensory evaluation to prove that wine from different AVAs could be differentiated by sensory means (Shimoda et al. 1993). However, currently this is not required to formally establish AVAs. In Australia, the Barossa Grounds working group and the Scarce Earth project of McLaren Vale, explored in great detail the local differences in geology, climate aspects and physical properties such as elevation or aspect. This is used to communicate the natural diversity of the regions but at the same time to advise viticulturists and winemakers to find similar lots to be blended to exhibit matching terroir effects.

Sensory impact of terroir

Obviously, the factors that contribute to terroir represent physically immovable properties which are unique to local winegrowing sites (and possibly regions) that cannot be found and applied elsewhere, unlike grape varieties, winemaking technologies and yeast strains. Thus, terroir serves as an excellent unique selling proposition, safeguarding local wine producers from the market power of global wine merchants. A growing number of consumers show a particular interest in how different terroirs translate into individual sensory properties; they want to know how to acquire expertise in recognising and distinguishing different terroirs based on the sensory appearance of the wines. Moving terroir beyond a clever marketing idea, it is crucial to deliver sound scientific proof that terroir really matters at the sensory level, which can be perceived and recognised by consumers.

Although geological diversity has been described for many vineyard sites, there is not much scientific knowledge about how the specific soil or climatic conditions translate into specific sensory differences among vineyard sites.

As is true for many analytical challenges, a successful sensory investigation requires proper methodologies. Lacking access to appropriate sensory tools, many authors have failed to differentiate terroirs by applying hedonic approaches and using poorly defined and highly subjective 'quality' measures. Or they follow ill-designed objectives such as trying to identify sites of outstanding or ordinary quality potential. In 1984, Ann Noble applied descriptive sensory analysis using well-defined sensory standards and highly trained judges to discriminate by sensory means the Bordeaux parishes based on 1976 commercial wines, revealing a great deal of overlap (Noble et al. 1984).

Descriptive analysis of Alsatian Gewürztraminer wines from five pedologically and climatically different vineyards showed distinctive differences in the sensory quality of the wines according to site of origin, varying from neutral character to full varietal expression. Multiple factor analysis on sensory ratings, and on the levels of terpenes and phenols, separated the samples from each vineyard plot (Dirninger et al. 1998). Over several vintages, a relationship between the sensory properties of Cabernet Franc wines from the Saumur, Bourgueil and Chinon appellations was observed (Morlat et al. 1984; Asselin et al. 1992).

Sensory ratings of Sangiovese and Cabernet Sauvignon wines provided a differentiation among different vineyards of the Italian Tuscan Bolgheri appellation across three vintages (Bogoni and Mela 1996). Studying 20 commercial Riesling wines from two vintages and different wine estates in six Rheingau regions, the statistical methods ANOVA and PCA on sensory data revealed that both vintage and the individual wine estate had a stronger impact on the wines' sensory properties than region of origin (Fischer et al. 1999).

In the New World, Guinard and Cliff (1987) examined 28 commercial Pinot Noir wines from the Californian regions of Carneros, Napa and Sonoma, and revealed significant differences in 13 out of 14 sensory attributes among Pinot Noirs from the newly established cool Carneros versus the warmer Napa and Sonoma appellations. While Heymann and Noble (1989) successfully discriminated Cabernet Sauvignons from four Californian regions (Napa, Sonoma, Southern and Lake), Noble and Shannon (1987) did not find any distinctive regional characterisation of 24 Californian Zinfandel wines from the 1980 and 1981 vintages. Similarly, a PCA of the sensory ratings of 20 commercial 1986 Cabernet Sauvignon wines from six districts in Napa Valley also failed to cluster the wines by origin (Shimoda et al. 1993).

Between 1988 and 1992, Reynolds et al. (1996) observed a consistent site effect on pH, titratable acidity, terpene levels and sensory attributes of Gewürztraminer wines from three vineyard sites in the Okanagan Valley of British Columbia. Hakimi Rezaei and Reynolds (2010) found significant sensory differences between Cabernet Franc wines from 10 Niagara Peninsula sub-appellations during the 2005 and 2006 vintages as well as among 41 commercial Bordeaux-style wines from three Niagara Peninsula sub-appellations (Kontkanen et al. 2005). Repeatedly, sites in great proximity to Lake Ontario and the Niagara River yielded wines rich in vegetative aromas and flavours. In contrast, wines from sites located further away from large water bodies were characterised by high intensities of 'red fruit', 'black cherry' and 'blackcurrant' aromas and flavours. A similar study with 24 commercial Chardonnay wines from the previously mentioned Niagara Peninsula sub-appellations failed to classify the samples by origin (Schlosser et al. 2005).

Research on sensory properties of 2003 Sauvignon Blanc wines from six New Zealand winegrowing areas revealed significant regional differences. While the wines from Hawke's Bay had a distinctive 'mineral flinty' character, those from Marlborough had high intensity ratings in 'sweet', 'sweaty', 'passionfruit' and 'capsicum'. The Sauvignon Blanc wines from Wairarapa showed intensive 'cat urine'/'boxwood' characteristics (Lund et al. 2009).

Investigating 30 Australian Cabernet Sauvignon wines from 10 geographic indications (GI), Robinson et al. (2012) were able to discriminate GIs from one another by sensory means and enhanced statistical differences were observed when geographical distances increased. Discriminating factors were 'green bell pepper', 'canned vegetables', 'minty', 'earthy' and 'smoky' notes as well as 'astringency'.

Most of the authors who failed to discriminate wines by sensory means from different terroirs justify this outcome by lack of control and standardisation of applied viticultural and oenological measures, such as yield, ripeness, yeast strain, use of malolactic fermentation or oak ageing, which is most likely when commercial wines were investigated. Other critics of the terroir concept mention the high spatial diversity of soils within a region and even vineyards and advocate limiting statements of terroir to small-scale vineyard lots (Bramley 2012).

Outcome of a five-year sensory case study with German Riesling

To overcome this crucial limitation of most studies with commercial wines, we selected in the first year 12 vineyards in one region and in the following four years 25 vineyards overall with distinct terroir in four Riesling appellations of Germany (Mosel, Nahe, Rheinhessen and Pfalz). Grapes were vinified in parallel by a standardised process applied to all 24 lots at the experimental winery of the Institute of Viticulture and Oenology in Neustadt and individually at the participating wine estates, using the same Riesling Heiligenstein yeast strain (Lallemand) as in Neustadt. Statistical analysis of the sensory results, which were obtained eight months after harvest for each vintage, revealed that across four to five vintages, 24 sites varied significantly in seven sensory attributes including aroma notes and three criteria to describe the complex perception of acidity (Table 1). Grouping the vineyard sites in seven parental rock types, such as limestone or slate, diminished the number of significant terms to two: sour taste and smoky odour. Limiting the geographic heritage by studying only 12 sites within the Pfalz appellation across five consecutive vintages, increased the number of significant attributes due to varia-

Table 1. Analysis of variance of sensory intensities as varied by seven parental rock types and two vinification regimes at 24 vineyard sites across four/five vintages (n=178 Wines).

source of variance	Vineyard site	Bedrock	Vinification
degrees of freedom	23	6	1
Odour			
Mineral	1.2	0.5	0.0
Citrus/Grapefruit	1.6	1.7	1.9
Rhubarb	1.1	1.2	**19.7 *****
Apple	1.1	1.4	**13.2 *****
Peach/Apricot	1.5	1.5	**35.3 *****
Mango/Passionfruit	1.4	1.5	**38.2 *****
Honey/Caramel	**1.8 ***	1.8	**20.2 *****
Cantaloupe	**2.3 ****	1.0	**16.1 *****
Smoky	**2.1 ****	**3.3 ****	0.2
Floral	1.4	0.8	**15.8 *****
Green grass	0.8	0.7	2.5
Boxtree	0.9	1.1	1.9
Yeast/Brioche	1.1	1.0	1.5
Colour			
Yellow colour	1.3	1.1	**16.3 *****
Taste			
Sweet	**2.2 ****	1.6	**5.1 ***
Sour	**2.8 *****	**3.2 ****	0.8
Harsh acidity	**2.5 *****	2.2	0.9
Hard mouth-feel	**2.1 ****	1.1	2.2
Bitter	1.1	1.5	0.0

Table 2. Analysis of variance of sensory intensities as varied by six parental rock types by both vinification regimes, standardised vinification alone and individual winemaking in estates across five vintages of the Pfalz region (n=105 Wines).

source of variance	Vineyard site	Bedrock	Vinification
degrees of freedom	23	6	1
Odour			
Mineral	0.2	0.3	0.4
Citrus/Grapefruit	1.4	1.4	0.9
Rhubarb	**2.6 ***	1.0	2.3
Apple	0.9	1.0	1.1
Peach/Apricot	2.3	1.1	**3.6 ****
Mango/Passionfruit	2.2	0.9	**3.9 ****
Honey/Caramel	2.6	0.9	**3.5 ****
Cantaloupe	2.2	0.8	**4.9 ****
Smoky	**4.1 ****	**3.4 ***	1.4
Floral	1.4	1.2	**5.0 ****
Green grass	0.2	0.4	0.7
Boxtree	1.0	0.9	0.6
Yeast/Brioche	1.0	0.9	0.8
Colour			
Yellow colour	1.9	0.7	**2.6 ***
Taste			
Sweet	**10.9 *****	**6.5 *****	**5.4 *****
Sour	**9.2 *****	**5.2 *****	**4.4 ****
Harsh acidity	**8.9 *****	**5.5 *****	**4.0 ****
Hard mouth-feel	**5.9 *****	**4.4 ****	**2.6 ***
Bitter	2.1	1.0	**1.1**

tion in bedrock type to six among the wines made by standardised vinification and seven among wines made by wine estates (Table 2). Obviously, using grapes from vineyards located at a distance of up to 200 km introduced a stronger impact of climatic variation than limiting sample collection to the Pfalz appellation and a maximum distance of 50 km and more topographical similarity.

For the large data set, the largest variation was due to individual versus the standardised vinification yielding differences in seven aroma attributes, but only one taste attribute, sweetness. Although all wines were classified dry (below 9 g/L residual sugar), variation in sweetness is strongly governed by style considerations (Mosel) and less by terroir, as it is the case for acidity-related perception, which is influenced by the concentration and composition of acids, but also soil-derived buffering cations. One has to bear in mind that the EU law does not allow any acidification of juice or wine in Germany and at the same time none of the wines underwent any de-acidification, which would have altered acid and cation composition.

Examining PCA plots of sensory properties of 105 Riesling wines from five vintages, six bedrock types (basalt, sandstone, limestone I, limestone II, reddish slate and grey slate) and both standardised and individual vinification within the Pfalz appellation, a meaningful differentiation was obtained (Figure 1). Of course, some overlap occurred, which is not surprising, since climatic variation among 12 sites and five vintages accounted for extra sensory variability, as well as the different viticultural and oenological measures individually applied to sites and wines. Still, the pictures give a unique insight about the true sensory variation between the parental rocks, which the consumer faces when the wines are labelled according to them.

Much less overlap was obtained in Figure 2 when the heritage of the wines was limited to the smaller region of the Südpfalz stretching 40 km in the north-south and 15 km in the west-east direction. Furthermore, only wines made by the wine estates were included. While wines grown on limestone, slate and sandstone did not show any sensory overlap during four vintages, the very shallow soil on top of the reddish slate parental rock varied considerably and was somewhat congruent with the wines grown on sandstone, but not with slate and limestone.

Implementation of a stronger terroir footprint

Dealing with terroir as a comprehensible sensory determinant of wines leads to a conflict regarding the self-perception or self-realisation of winemakers and how they view their impact on wine style. Two acclaimed terroir winemakers in Germany, Hansjörg Rebholz from the Pfalz appellation and Reinhard Löwenstein from the Mosel, neatly describe their different views on terroir. Rebholz argues that:

> Riesling allows me to exemplify the terroir impact so that the provenance of a wine can be smelled and tasted. Although everything that happens around the soil will vary from year to year, the specific character of a vineyard site should be implicitly perceivable across the years. It's all about recognisability.'

In contrast, Löwenstein states that:

> Terroir describes the comprehensive and complex system vineyard. Terroir wines belong to the world of culture. Their interaction with wine consumers cannot be comprehended scientifically nor evaluated objectively. They are subtle and full of finesse, not predictable, variable, challenging, provocative, questioning everything.' (Fischer and Svoboda 2005).

Rebholz adopts a pure and non-invasive approach, comparable to taking a photograph of the vineyard site; he tries to avoid any masking of the terroir expression by winemaking. In contrast, Löwenstein acts like an impressionist and the winemaker creates a piece of art called terroir.

How to solve this conflict? Winemakers have to decide if they want to express themselves by making the most individual iconic wine or if they want to exemplify the impact of terroir, by maximising the transfer of grape constituents formed in the vineyard into the juice and fermented wine, but minimising any oenological impact which could mask the subtle terroir signal.

German Riesling producers seem to resolve this conflict by combining site-specific properties of the grapes with individual winemaking statements. Use of oak, spontaneous fermentation by wild yeast or long yeast contact may be adopted to increase the

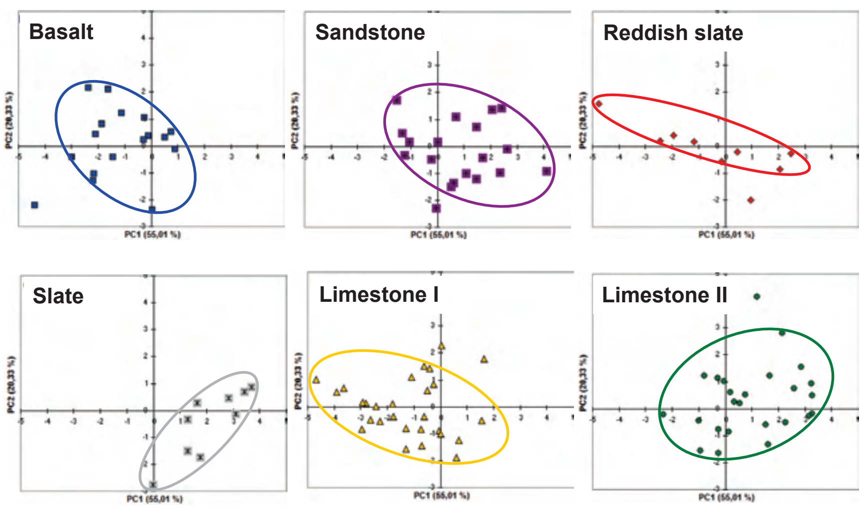

Figure 1. PCA of significant sensory attributes of Riesling wines obtained from six parental rock types, five vintages and both experimental vinification and individual winemaking (105 wines, Pfalz region only).

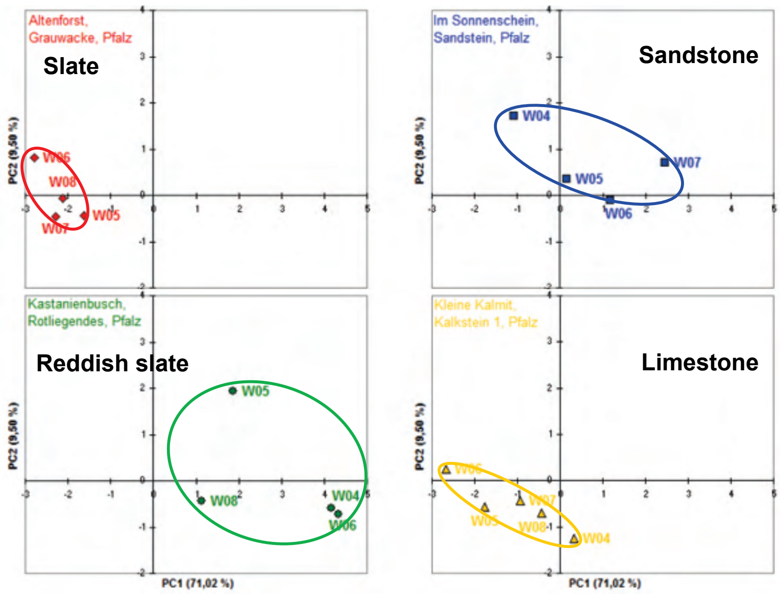

Figure 2. PCA of significant sensory attributes of Riesling wines obtained from four parental rock types, four/five vintages and individual winemaking by the wine estates (17 wines, Südpfalz region only).

complexity of the wines. So, for these iconic wines, the terroir aspect is one, but not the solely dominating aspect of the sensory expression. The showy front label highlights the vineyard site and sometimes does not even mention the variety, similar to Burgundy. The success of these wines can be demonstrated by the hefty price increase seen during the last 15 years. For the five most expensive dry Riesling wines from the Pfalz published in the Gault-Millau wine guide, the average price increased from €10 to €48 per bottle.

In the next lower quality tier, designation of the vineyard sites is ignored in favour of naming the dominating parental rock type of the site such as granite, shell limestone or greyish slate. Given that consumers will taste numerous Riesling wines from greyish slate or Pinot Blancs from shell limestone, it is of utmost importance that winemakers agree on the stylistic expression of each parental rock type and promote similar sensory properties. Thus, consumers are able to perceive the sensory bracket among those wines coming from the same rock type and concurrently pick up recognisable deviations among wines originating from different parental rocks.

Nobody claims that this is an easy endeavour, but we accomplished the same task for varietal expression. For most wines, consumers are able to differentiate between Riesling, Chardonnay and Sauvignon Blanc or Pinot Noir and Shiraz. Similar to the degree of terroir impact, the iconic wines may focus more on complexity than on an easy identification of the variety, partially due to blending and other measures.

In his recent book, *Terroir and Other Myths of Winegrowing*, Mark Matthews made the provocative statement 'that grapevines have next to no interaction with rocks' (Matthews 2015). This is in line with the statement of Maltman (2008, p. 1):

> The notion of being able to taste the vineyard geology in the wine—a goût de terroir—is a romantic notion that makes good journalistic copy and is manifestly a powerful marketing tactic, but it is wholly anecdotal and in any literal way is scientifically impossible.

Looking more closely at Maltman's opinion, he merely questions that the bedrock itself (or minerals) can be tasted and described by attributes such as 'slaty', 'earthy' or 'minerality'. Therefore, why have we focused on the parental rocks and made the first step to unveil the secrets or myths of terroir?

First of all, many wine experts and ambitious wine consumers share the general experience that at least certain bedrocks indeed modify sensory properties and can be recognised. For example, when we tasted Shiraz from the three areas dominated by granite (Northern Rhône, France; Swartland, South Africa; and Grampians, Australia) during a two-day symposium devoted to 'Soil and Wine', the participating sommeliers, winemakers and trade experts could perceive similarities regarding a pronounced and lean acidity, due to a lack of potassium or calcium cations in the granite-derived soils and wine. The lower pH also modifies the colour and tannin perception.

With the exception of alluvial and sedimented soils, the parental rock contributes by weathering to a varying degree to the soil composition, especially soil nutrients and its physical characters. The more shallow and stony the soils, the more distinct the impact and there are numerous studies documenting soils' effects on wine composition (Imre et al. 2012; Burns 2012).

Secondly, as Rob Bramley and others have pointed out, spatial variation of soils can be tremendous, even within a single vineyard. However, parental rocks vary much less and can be the dominating factor of whole appellations such as the slate in the Mosel or limestone in Burgundy (Bramley 2012).

But, what may be most important of all, consumers do relate and connect much more to the terms granite or sandstone than to the more technical and complicated terms used for soil description. It is always amazing how much consumers are fascinated by the fact that through a sophisticated winemaking process the heritage of a wine can still be smelled and tasted.

Trying to establish and implement a sensory classification based on different parental rock types or other factors characterising terroirs in a comprehensible way, we identified three crucial steps:

- First, to evaluate the wines made in a consistent winemaking process from different sites and detect common and differentiating sensory patterns.
- Second, to convince winemakers that they need to agree regarding these sensory patterns, in order to help consumers to recognise them and to believe that terroir really matters beyond marketing.
- Third, to relate in a scientific approach, sensory properties and wine composition with specific chemical and physical soil characteristics and climate aspects in order to assist winemakers to enhance and clarify the sensory perception of terroir. This process can benefit a great deal by remote soil mapping techniques, to assist not only in defining homogenous areas, but also to assist mechanical harvesters to accomplish a selective harvest according to terroir factors.

Conclusion

The concept of terroir aims not to distinguish between superior and inferior sites for grapegrowing or wine production. Its main objective is to identify and name a major contributor of sensory diversity among wines. Consumers are attracted by this direct link between ancient geology and the cultural complexity of wine. Increasingly well-designed experiments show that different terroirs modify sensory properties of wine in a way that can be consistent across vintages and the impact of a multitude of winemakers, if they apply non-invasive winemaking by avoiding strong oenological statements. On the path to establish a terroir-driven wine portfolio which goes beyond marketing, it is important that consumers can recognise different terroirs or parental rocks by sensory traits. To accomplish this task, the winemaking community has to agree upon sensory profiles of the different terroirs, enabling scientists to study the underlying principles and to develop ways to make the terroir expression in wines more consistent, expressive and distinct.

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Do we make too much of terroir?

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Abstract

Terroir is a concept that lies at the heart of fine wine. The notion that a wine can express characteristics that in some way capture the place where the grapes were grown is central to most of the classic Old World wines which to this day define how winegrowers worldwide operate. But it is also a controversial topic. Some of the mechanisms claimed for the way that place is translated into wine are scientifically questionable. Notions such as minerality are hotly debated. In this paper I will explore the notion of terroir, and question some of the ideas surrounding it. How do soils influence wine quality? Is there a human component to terroir? Do all fine wines have to express place? Does the obsession with terroir hold back the wine industry? I will argue that terroir is a brilliant concept, but also that - yes - we do make too much of terroir.

Webcast of this presentation available at http://bit.ly/16thGoode.

Introduction

Terroir, the French term that means different things to different people, is one of the most hotly debated topics in the world of wine. It's in danger of becoming the next cork/screwcap discussion: one that creates lots of heat, very little light, and which risks becoming incredibly boring and repetitive. But it's an important topic, because the idea of terroir lies at the core of fine wine.

Terroir and other myths of winegrowing is the provocative title of a recent book by Mark Matthews, Professor of Viticulture at the University of California Davis (Matthews 2015). Matthews argues that the scientific literature doesn't support the popular notions of terroir as held dear by many winegrowers, particularly in the classic European wine regions. In Australia, much less is made of the importance of soils, with differences in wines from various sites mainly being ascribed to climatic influences. Many wine scientists seem frustrated by the way that the wine trade as a whole holds onto notions that they believe aren't scientifically proven, and in some cases aren't scientifically plausible.

Here, I'm going to look at whether or not we make too much of terroir, and whether the wine scientists have a point. I'm coming at this topic from two perspectives. First of all, as a lapsed scientist (I have a PhD and spent 15 years as a scientific editor before branching out as a freelance wine journalist). Secondly, as a wine lover who travels the world of wine. This year alone I have visited New Zealand, Australia, Portugal (four times), Spain (twice), Beaujolais, Bordeaux (twice), Provence, Champagne, South Africa (three times), Canada (three times: BC, Ontario, Nova Scotia), California (twice), Chile, Argentina and Oregon. When you visit cellars and vineyards, spend time tasting wine, and listen to experienced winegrowers, you learn. Having both perspectives is important if we're to make sense of this concept. The science matters, but it needs to be placed in context. And we need to know the gaps in our scientific knowledge: things we are unable to frame into experimental questions, or just haven't researched.

An aside: It's hard to have an intelligent and interesting discussion of terroir if we restrict ourselves to the published scientific literature. This is because of the difficulty and expense of doing these sorts of studies, which means that there are very few of real interest. For this reason, this paper will not be based solely on the published literature review, but will also discuss some of the concepts based on anecdotal observation and third-party testimony from practitioners.

Defining terroir

First, let's define terroir. This is necessary because it means different things to different people.

The first definition is the simple one. It is the sum of the vineyard environment. A terroir is an actual vineyard site, with its climate, aspect and soils.

Then there's a second definition. Terroir is this specific site as it is expressed in the wine. The vineyard site alters the way the vine grows and this alters the physical properties of the grapes. The site influences factors such as flavour precursors in the grapes, the thickness of the skins, the sugar and acid content of the grapes, the way they ripen ('phenolic' versus 'sugar' ripeness). It also, to an extent, determines which grape varieties are grown.

There is a microbial aspect to terroir. Wine is a microbial product, and grape juice doesn't possess terroir. If you were to taste grape juices from several sites it is unlikely that you'd pick those site characteristics up, even though it might be possible in the final wine. The characters that each site contributes to a wine are realised through fermentation, as the yeasts convert flavour precursors into the actual flavour molecules, and the yeasts actually synthesise flavour molecules themselves. Each vineyard harbours microbial populations which can influence the fermentation (when the must isn't inoculated). Some studies have shown that the microbes that carry out these wild ferments have come from the vineyard and are quite local. Even if cultured yeasts and bacteria are used, the vineyard site affects the must (for example, its nutrient status) which will affect microbial performance, and thus the flavour of the wine. Next generation sequencing technologies have allowed scientists to study the vineyard microbiome, and there are currently several studies underway. The results so far have been intriguing, and do indeed suggest a microbial contribution to terroir.

Terroir is evident in differences between wines. This is a subtle but important further definition. If you taste two wines made the same way from different sites, and they taste different, then what you taste – the difference – is terroir, largely. Terroir only matters if you can taste it. This is the proof of concept.

If we accept these three definitions, there is no doubt that terroir exists. What of the myth of terroir, as Mark Matthews claims it to be? He's referring there to the notion that characteristics of the soils are being transmitted to the grapes and thus flavouring the wine.

This is scientifically indefensible. With a few notable exceptions (such as eucalyptus flavours in wine that come from cineole - eucalyptus oil - finding its way onto grapes and thus directly flavouring the wine), the taste of wine is a result of chemicals synthesised by the vine from water, carbon dioxide, oxygen and mineral ions. None of these taste of anything, with the possible exception of mineral ions at high enough concentrations (some mineral waters have flavour). So in this sense, Matthews is right. But there are very few people who'd have this as their primary definition of terroir.

Considering the concept of terroir

There are other things we need to consider. Terroir is fragile. Yields that are too high or too low can cause it to be lost. Picking at the wrong time – too early or (more commonly) too late – can cause it to be lost. The result is wines of style rather than wines of place.

There aren't many great terroirs. Terroir is the limiting factor in the quality of the wine a winegrower can make. Look at Burgundy: it's the poster child of terroir. Here, top wines sell for hundreds of times the price of the cheapest wines. If terroir was a myth, then someone could get one of those lesser terroirs, lavish attention on it, and work skilfully in the cellar and produce something great. After all, there would be a massive financial incentive to do this. Smart people have tried it and it doesn't work. The quality of your terroir creates a quality ceiling that's impossible to break through. Great terroirs are rare.

The example of Burgundy emphasises the importance of soils. There are consistent differences between sites year after year. If this were just climate, then it wouldn't be so. Vines never see climate, which is an average of the season's weather over many years. They see the weather of that year. This changes from year to year, more than the climate changes from one site in Burgundy to a neighbouring site.

Do all wines have to be terroir wines? What about multi-region blends? What about most Champagne? My first response would be, no. Clearly there exists a market for ripe, rich, oaky red wines that could have come from anywhere – the so-called 'international' wines. That's fine. I don't like them, but some people do. I would argue, though, that it's such a shame if these wines are made on special, unique terroirs that are capable of yielding interesting wines that speak of their place. It's almost a moral issue.

Terroir is best expressed by grape varieties grown at the margins of where they can successfully ripen. Grape varieties seem to interpret a place in more interesting and more distinctive ways when the later stages of ripening take place in cooler conditions. In warmer climates where grapes are being picked during summer conditions, it's harder to produce a wine of place and the result is frequently a wine of style.

Too much is made of terroir, however, if the human element is left out of it. For a site to be a terroir it must be interpreted. And it takes a skilled winegrower to interpret a terroir.

An analogy would be to see a terroir as a musical score, or a chord sheet (depending on the sort of music you have a preference for). The winegrower is either playing that score, or is covering that song. Without picking up an instrument (or selecting a range of instruments) and then playing and/or singing, the score or chord sheet is not music. There is a great deal of skill needed to interpret that score or chord sheet and produce something that people want to listen to. There is also room for interpretation. So it is with a winegrower and a site. They must choose their instrument and play it well, to produce something that is true to the music but which also is an interesting, intelligent, personal take on the score or chord sheet.

A few years ago a number of Riesling producers in New Zealand were each given a portion of grapes from the same vineyard and asked to interpret this particular terroir, in this particular vintage. The result was a dozen different wines that showed some resemblance, but which were individual interpretations of a single site.

Terroir only exists when a vineyard site is interpreted through the winemaking process. To this end, the human aspect is critical. The winegrower can make an intelligent or less intelligent interpretation of the site. A skilled winegrower can make a good wine that speaks of its vineyard origins, and another skilled winegrower can do the same; both will be different wines, but will share something in common. There is certainly a cultural aspect to terroir.

I remember speaking with a famous New Zealand producer, Felton Road in Central Otago, who make a range of block series Pinot Noir wines, and then use the rest of their barrels to make a subregional Pinot Noir, Bannockburn. Owner Nigel Greening explained his approach. 'We try not to making any blending decisions, and we also try not to taste the wines with a view to adjusting or making blending decisions. Essentially, Block 3 and Block 5 are those blocks, with the exception of young vines from these blocks which get blended into Bannockburn, our 'village' wine. When it comes to Cornish Point and Calvert, about 30–40% will go to a single-vineyard bottling, and the balance goes to Bannockburn. So we have a dilemma: we have to choose which 30–40% to use. There will typically be eight lots from each of the vineyards, and we will taste them about three times, blind. We score them not for their quality as a wine but for their expression of site. The wines that get the highest scores for Calvertness, depending on how big the lots are, we will take sufficient wine down that scoresheet and then draw the line. The Calvertiest ones become Calvert, and those that show the least Calverntess go into Bannockburn. The same applies to Cornish Point. Calvert is the more elegant, tighter, more linear wine. Cornish Point is voluptuous, perfumed. This is naturally what this vineyard does and we want to show that expression of site as clearly as we can.' The human factor is important here in interpreting these vineyards: the winegrower has a sense of what constitutes the best interpretation of site.

What about natural/low intervention winemaking? Is this the truest interpretation of terroir? To add nothing in winemaking (save for a bit of SO_2 at bottling) requires great skill (and/or a lot of luck) if the result is to be a good terroir wine. Many natural wines are wines of style rather than wines of place: they taste more natural than they taste of the place they come from. But many good natural wines, expressive of variety and place, are made.

Soils matter for wine. This is clear to anyone who visits a region with different soil types, such as Alsace, and tastes the same variety (Riesling) from different soils. The difference is marked, where the sites are interesting and the right varieties are planted. Of course, geology and pedology are complex, and wine people often simplify them. But in some way the soils are influencing the flavour of the wine. Increasingly, serious wine producers in the New World are talking about soils, when a few years ago they were more interested in matching variety with climate. On a recent trip to Chile and Argentina I saw literally dozens of soil pits, and even tasted trial wines made from the same vineyard but from sections with specific soil types. The differences were fascinating.

Parental bedrock type, soil depth, irrigation/rainfall, soil management techniques (organic/conventional/composting/herbicide/working the soil/no till) all seem to make a difference. As an example, take the difference between sandy soils, versus limestone soils, versus clay soils, versus grantitic soils. There are certain flavour characteristics of wines from these soils, all other things being equal. This is increasingly being talked about by wine professionals, although so far there have been very few studies examining it. One study, though, stands out. It's by the group headed up by Ulrich Fischer at Neusdtadt, Germany, and shows that similar soil types produce similar flavours in wines made from the Riesling grape variety (Bauer et al. 2011).

The famous study of Gerard Seguin (Seguin 1986) in Bordeaux indicated that the major influence of the soil is in moderating the water supply to the vine, rather than any contribution from soil chemistry. However, this is on a particular type of alluvial soil. Increasingly, evidence (much of it empirical, admittedly) points to the importance of the actual soil composition.

Terroir is also cultural. Within a region, there is a lot of spread of information and ideas. Similar techniques are often applied in the vineyard and the winery. This sharing of information and group thinking can certainly contribute to giving the wines of a particular region shared flavour characteristics. This is especially true in regions with a long history. The ways vineyards are managed is often uniform in these regions, and the harvest date is often officially set.

So, do we make too much of terroir? Yes, it is a term that is overused, that is used imprecisely, and is used without definition. An intrinsic part of terroir is the human element, and we don't make enough of this. Great wines of place are made by skilled winegrowers, rarely by accident. They certainly don't make themselves. We need more intelligent, informed discussion of this practice, rather than simply the parroting of received wisdom. We could also do with more and better studies of terroir, looking at interesting patches of ground and asking really intelligent questions.

But then again, terroir is such a pivotal, organising concept in the world of fine wine that it's almost impossible to make too much of it. Even the creation of the world's great blended wines, such as top Champagnes and Vintage Ports, requires a great deal of understanding of terroir, because it is terroir wines that are blended.

Why terroir is so important

Fundamentally, terroir is a vital concept for wine, because linking a wine to a place is the future for the wine industry. Without this link, wine is just another alcoholic beverage. And for an individual producer, or a region, or a country, the linking of wine to place means that no one can compete with that wine. If a wine is simply a varietal wine, or a wine of style, someone else can do it, and probably cheaper. This price competition in a world of oversupply leads to one outcome: a race to the bottom of the market, leaving wine production unprofitable. The only sustainable solution for wine producers is to make wines that in some mysterious way have a connection with place. The beautiful experiments of Ulrich Fischer and his team from Neustadt in Germany (Bauer et al. 2011) show quite clearly that, if the winemaking allows it, specific soils produce Riesling wines with statistically different flavour profiles. People can taste the place. This is the way forwards for fine wine.

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Understanding the components of terroir

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Abstract

For a term that is so ubiquitous in the wine world, it may come as a surprise to many that there is no single universally accepted definition of 'terroir'. For example, does it refer to just the original 'natural environment' of the vineyard or does it also extend to human influences such as choice of grape variety and alteration of the landscape before planting? Should it extend to the management of the vineyard and winemaking practices? The various definitions of terroir, the history of its use in a wine context and the spatial extent to which it may be applied will be discussed. This paper will attempt to describe terroir in terms of its many components with a strong focus on climate (at all levels), soil (both physical and chemical characteristics), geology and geomorphology (aspect, slope, elevation and so on). This reductionist approach is appropriate if one wishes to understand the mechanisms of terroir—how the components influence the ecophysiology of the grapevine and consequently fruit composition. Most importantly, the interaction of these components is paramount in terroir expression and for this reason it is futile to attempt to attribute terroir differences in terms of a single component such as geology.

Webcast of this presentation available at http://bit.ly/16thPDry.

A brief history of terroir

> Terroir. A highly fashionable term which has as many shades as a chameleon locked into a mirror box (Mueller and Sumner 2006)

In one French-English science and technical dictionary (DeVries 1976), *(le) terroir* is translated as 'soil, ground, earth'. It is derived from *la terre*: earth, soil, land or territory. Until the 20th century, in the context of wine in France, terroir was used in a pejorative sense. For example, the following are translated examples from dictionaries of 17th to 19th century (cited in Matthews 2015): 'Terroir is similar to soil and a taste of terroir is considered to be a disagreeable quality arising from that soil' (Furetière 1690) and 'The repulsive taste of terroir' (Chaptal et al. 1801).

Matthews (2015) argues that rise of the use of terroir by French wine producers from the late 19th century can be attributed to two periods when the economic viability of the French wine industry was threatened. Firstly, the reconstruction post-phylloxera led to the creation of the appellation control system to regulate production and reduce fraud. There was also a need to remind the rest of the world that French wine was back in business and that its wines were unique—a regional terroir explanation was used for their distinctive wines. This led to the concept of terroir wines controlled by appellation laws enforced by government. Controlling the area for the wine label gives the impression that the physical boundary is the cause behind the fineness and the rarity of the wine. But in reality the boundaries of the territories are set by economic and cultural agenda more than aspects of the environment that are known to cause wine flavours (Mueller and Sumner 2006). Coincidentally, from about the beginning of the 20th century, the use of terroir changed from a negative descriptor (a soil-derived off-flavour of wine) to a positive one. For example, the following is a translation from a 1970 French dictionary:

> Terroir. This word which designates the land the soil has when it is about wine a particular meaning in the expressive taste of terroir. This one characterises a distinctive taste particularly almost indescribable that all the wines have when they come from certain types of soils (Debuigne 1970)

The second event when the economic viability of the French wine industry was threatened (according to Matthews 2015) was the fallout as a result of the so-called Judgement of Paris in 1976 when non-French wines were deemed to be better than French by French wine experts—also known as 'the vinous shot heard around the world'. This caused the French wine industry to build a defence against the real or imagined New World threat to the Old World wine hierarchy. The French producers had to say that although New World producers could grow French grape varieties, they were not able to replicate the unique environment (i.e. the terroir) of their vineyard sites.

Also in the second half of the 20th century, terroir became a loanword in English and there was a surge in its use in the English language popular press, particularly promulgated by British and American wine writers, many of whom had close links with the international wine trade, and may have had a vested interest in the promotion and marketing of French wines. As a result of close contact with French wine producers these writers were influenced by the passion (and good sales talk) of those producers who extolled the virtues of the unique terroir of their vineyards which they claimed accounted for the distinctive taste of their wines: 'The unmistakeable *gout de terroir*, the taste of the soil that distinguishes the great wines of the Côte d'Or' (Prial 1979).

Of course, many of those French producers were also adamant that it was only French wines that expressed their terroir: 'I drink foreign wines. Very good wines are produced … but they lack terroir, and terroir is what makes everything.' (Denise Capbern Gasqueton, owner of Château Calon-Ségur, Saint-Estèphe, cited in Gergaud and Ginsburgh 2005)

It also became fashionable for New World wine producers seeking to emulate the French style to mimic the French by invoking terroir. This particularly applied to impressionable young winemakers from the New World on their pilgrimages to Burgundy and elsewhere. But until relatively recently, *gout de terroir* still had negative connotations, for example: 'Earthy taste; it denotes a peculiar flavour imparted by certain soils' (Lichine 1967).

There was a relatively dramatic increase in use of 'terroir' in combination with 'wine' in both English language books and scientific and quasi-scientific publications from the early 1980s (Matthews 2015). In the English literature there was still a strong emphasis on soil effects. Even today, though it is common to see terroir described as inclusive of environmental factors, for many wine 'experts', soil remains predominant with reluctant acquiescence to non-soil factors in wine flavour (Matthews 2015). The first English language book to use 'terroir' in its title was published in 1998: the author, James Wilson, was a geologist (Wilson 1998). The viticultural scientists were the last to start using 'terroir', and even then largely as a synonym for 'environment'; perhaps because there was no discovery in the vineyard that led to a revelation regarding the role of terroir in wine production. The term terroir is rarely used in plant science journals and there are no scholarly works invoking terroir of plants other than grapes. It is not just because we are dealing with a secondary product, namely wine, in this case. There are not, to my knowledge, any works on terroir of cider apples, or terroir of barley for beer or whisky. Furthermore, references to terroir are exceedingly rare in science journal papers: a search by Matthews (2015) of 'grape + wine + terroir' found no more than 1% of papers with this combination.

Definitions of terroir

> Terroir when transferred to English language has been given a bewildering array of meanings depending on the user's perspective. (Gladstones 2011)

As far as the components of terroir are concerned, it depends on which definition of terroir is used - and there are many of them. This entire paper could be devoted to a discussion of this topic alone. In summary, definitions include references or attributions to one or more of the following causal 'components' or 'factors':

- Natural environment: climate, soil, geomorphology (landscape), geology
- Human influence: variety, choice of site, vineyard layout, vineyard management practices
- Winemaking practices
- Microbiology (of both natural environment and winery)

Definitions may emphasise just one of the above or a combination, for example:

> A terroir is a unique and delimited geographical area for which there is a collective knowledge of the interaction between the physical and biological environment and applied viticultural practices (International Office of Viticulture and Wine OIV 2010)

> An interactive ecosystem, in a given place, including the climate, soil and the vine (rootstock and cultivar) (Seguin 1988)

> The vine's whole natural environment, the combination of climate, topography, geology and soils that bear on its growth and characteristics of its grapes and wines. Local yeasts and other microflora may also play a part. All these factors interact with each other and the management in the vineyard and winery to shape the wine (Gladstones 2011)

However, other definitions are much less expansive. For example, in the popular press, discussions of the term terroir commonly have a strong emphasis on soil effects alone. Surprisingly there are even relatively recent oenology texts with this same narrow view, such as: 'Terroir, a term coined by the French, refers to the influence of non-climatic environmental factors (soil, topography) on wine composition and quality' (Ribereau-Gayon 2000). Some acknowledge the human influence/intervention, whereas others do not, for example: 'A terroir therefore is defined as a complex of natural environmental factors that cannot be easily modified by the producer' (LaVille 1990, cited in Matthews 2015).

But even those who are reluctant to include human influence in their definition may concede that soil modifications such as drainage, terracing, or progressive fertility changes related to soil management become semi-permanent features of their sites and can be broadly considered to be parts of their terroirs—likewise, human-induced climatic change (discussed later). van Leeuwen and Seguin (2006) go even further and argue that the history of the socio-economic environment is important in understanding terroir—influencing why a given vineyard has emerged in a given site and why it has prospered.

So where does this leave us? There needs to be some flexibility of definition depending on site variability and commercial purpose. Also, the definition will depend of the scale of the territory. Some popular references talk about terroir as if it is a property of the wine itself—but as Gladstones (2011) and others have reminded us, good wine reflects the terroir of its origin.

Human factors

> There are few examples of famous wine-growing areas developing in inhospitable and remote areas, far from centres of consumption (van Leeuwen and Seguin 2006)

In their 2006 review, van Leeuwen and Seguin confined their discussion of human factors to grape variety, and the socio-economic factors that have determined vineyard location. They did not include the design of vineyards at the time of planting which become permanent features and which may have a profound influence on wine characters, for example row orientation and training system—perhaps because the latter is largely homogeneous within the boundaries of many French (and other) appellations. In the author's view this is a serious omission. Vineyard management has a profound effect on microclimate, particularly at the bunch level. Research over the past two decades has demonstrated that it is not just the degree of bunch exposure that impacts on fruit composition but also the timing and duration—for example, recent work on the use of defoliation at flowering for yield control and reduction of bunch compactness has revealed the importance of the pre-veraison period for determination of fruit composition, not just the post-veraison period (Poni 2014). There is also the effect of training system, soil cover and row orientation (Keller 2008; Iland et al. 2011).

Variety

The traditional view of terroir is that it is the place not the variety that determines the wine. The variety's (or varieties') influence is often underrated in the terroir discussion, particularly when comparing the different appellations of Europe where often the varieties differ between regions. For example, compare two red wines, one from Cotes de Nuits and one from Medoc. If we conclude that each expresses its terroir, aren't we just concluding that Pinot Noir tastes different to a Cabernet Sauvignon blend? Variety often overrides any differences in climate or soils: for example, certain varieties can produce high acid or well coloured wines irrespective of the site. In Australia where we grow the same dozen or so varieties in every region this may not apply. People chose the varieties for particular sites based on their ability to ripen successfully and to produce wine of desired end use rating. van Leeuwen and Seguin (2006) argue that for optimal wine quality, the variety should ripen its grapes at the end of the growing season so that ripening takes place during the coolest possible period, thus developing optimal composition.

Changing the landscape

Without the use of terracing, steep slope viticulture along the rivers of Germany, the Rhone, the Douro and so on would be near impossible. Likewise, vineyards could not be planted in most of the Medoc until it was drained from the 17th century.

Site selection

Vineyards emerge in locations where the socioeconomic conditions are favourable for wine production. Many vineyards arose in the vicinity of a concentration of consumers or near a navigable river or near a harbour because of the difficulty of transporting a liquid beverage.

Natural environment

> Invoking terroir in grapevine biology is largely gratuitous because at best it is just a synonym for environment (Matthews 2015)

For the factors of the natural environment: climate, soil, geomorphology (landscape), geology, each will be discussed separately. However, most importantly, the interaction of these elements is paramount in terroir expression and for this reason it is futile to attempt to attribute terroir differences in terms of a single element such as geology. As to which of these is most important, it depends on the scale.

Climate

'Macroclimate' is also called regional climate. 'Mesoclimate' is the climate of a site as influenced by its particular location, altitude and topography. The scale is tens of metres up to kilometres depending on the uniformity of the topography. Hence it is also called topoclimate. Apart from altitude, the main determinant of mesoclimate is local air drainage, particularly on still cloudless nights (Gladstones 2011). 'Microclimate' is the term used for the smallest scales, less than tens of metres, for example local conditions behind windbreaks or terraces or between vine rows or within a vine canopy. Unlike macro- and mesoclimates which are fully determined by initial site selection, vine microclimate is largely dependent on vine management, and inherent soil factors and the way the soil is managed including irrigation-regulated soil water status. Soil temperature is part of the vine's microclimate. It is useful to our understanding of terroir to consider it on a macro-, meso- or micro-scale. Key climatic factors are listed below:

- **Temperature:** Temperature is central to all aspects of viticulture. It largely controls phenological development.
- **Radiation:** Radiation is important because it interacts with temperature to regulate dry matter production and potential yield, but it does not directly influence phenology. The crucial role of both temperature and radiation in the bunch zone on fruit composition and wine character is now well understood (Iland et al. 2011). There is ample literature on the effects of bunch microclimate on berry ripening and composition (Poni 2014): for example, anthocyanin synthesis and degradation are both very sensitive to bunch temperature (Mori et al. 2007). For white wine varieties, prolonged exposure of bunches to high light and temperature results in excessive loss or degradation of aroma, reduced wine freshness, increased overripe character, decreased acidity and increased pH, and decreased microbial stability (Marais et al. 1999).
- **Rainfall:** Water supply to the grapevine, whether from rainfall alone or rainfall in combination with irrigation, impacts on vine balance and wine quality. Which rainfall pattern produces the optimal conditions under non-irrigated conditions? It is not possible to be absolutely prescriptive but in general, a combination of the following is optimal: a) sufficient winter/spring rainfall to fill the soil reservoir without waterlogging; b) low rainfall between fruit set and veraison so as to induce moderate water stress and cause slowing down and eventual cessation of shoot growth, producing an open canopy and limiting leaf area so that transpiration later in the season will be reduced when water supplies are low; c) from veraison to harvest, low enough rainfall so as to induce mild water stress and reduce risk of disease but not so low that leaf function is impaired; d) sufficient rainfall post-harvest to maintain leaves in good condition and promote reserves.
- **Atmospheric humidity:** The role of atmospheric humidity and its importance for wine quality is appreciated more now than in the past (Gladstones 2011). Low humidity plus high temperature not only increases the risk of stress but also lowers the grapevine's water use efficiency—that is, more water is required per unit of dry matter produced. Furthermore, low humidity plus high temperature accelerates berry accumulation of sugar (and probably also potassium)—this is one of the reasons why grapes grown in warm to hot and sunny climates typically reach high sugar levels before phenolic and flavour ripeness (Gladstones 2011). The good wine quality of many coastal regions in Australia can be attributed in part to the increased humidity during summer/early autumn afternoons caused by sea breezes.

Topography

Topography affects mainly macro- and mesoclimate. Key topographic factors are listed below:

- **Altitude (elevation):** Most of the world's great wine-producing regions are close to sea level. Few are greater than 500 m, many are less than 300 m. Increased elevation leads to higher light intensity and UV. A low site and high latitude gives better wine than a high site at low latitude (Gladstones 2011).
- **Proximity to large water bodies:** This affects both macro- and mesoclimate. Data from continuously-recording meteorological stations have shown us that some regions classified as 'hot' (based on mean daily maximum temperatures) are cooler in the afternoon than predicted as a result of sea breezes that arise soon after the maximum temperature has been reached. Large water bodies are not only important as a source of sea or lake breezes. They also have a role in raising night temperatures in cool climates, and in afternoon cooling and humidifying in hot climates. In the latter case these benefits are relatively greatest in regions that are well inland and are hot and dry because high evaporation rates maximize air cooling and humidifying under the local convections created (Gladstones 2011).
- **Aspect and slope:** Unless it is a very cool climate where south-facing (northern hemisphere) or north-facing (southern hemisphere) aspects are required for maximum radiation interception and reliable ripening, an east-facing aspect is generally best because a) excessive rain and cloud are mostly associated with weather systems from the west; and b) there is earlier exposure to morning sun when ambient temperature is at its lowest and most limiting, thereby promoting temperature equability (Gladstones 2011). The exception to this rule is on west coastal areas where a westerly aspect catches the afternoon sea breezes.

Geology

> The simple fact is that grapevines have next to no interaction with rocks (Matthews 2015)

Why has there been so much emphasis on soil and geology in the past? From a linguistic perspective, the word 'terroir' stems from 'earth' or 'land', so it is understandable that, at a conceptual level, an understanding of terroir may be sought from characterising certain geologies, soils and specific minerals present in individual vineyards. However, the role of geology has often been misinterpreted (van Leeuwen and Seguin 2006). In Chablis, the best wines are said to come from vineyards on Kimmeridgian limestone and the lesser wines from Portlandian limestone. Kimmeridgian vineyards tend to be on south-facing sunny slopes whereas the Portlandian are on exposed and windy plateaux. Clearly mesoclimate is playing a significant role in this case.

Geologists look at rocks and the minerals locked up therein and try to infer something about the mineral status of the soil and the plant – and even the taste of the wine from grapes grown in the soils above those rocks. This mantra has been taken up with great enthusiasm by some wine writers. Flavours in wine are assumed to come directly from rocks and attributed to specific minerals.

> Schist – the geological equivalent of Viennetta icecream ... comes in green, brown and black, all stuffed full of tasty minerals (Jefford 2013)

> But unlike clay, (the) Kurrajong (Formation) is composed of big chunks of rock stuff, each of which has a flavour (White 2013)

Soils with surface rock elicit allusions of minerality:

> But what separates Beechworth from so many other regions, …, is minerality. It's there, you can see it, with minerals glistening in the sun—slate and shale and great boulders of granite…While some disagree, you can taste minerality and it's there in the wines (Port 2012)

Minerality is commonly used as a taste descriptor for wine, implying that individual minerals can be transported from soil to the berry where they confer a sensory impact on wine. However, a recent French study has demonstrated that 'minerality' is an ill-defined concept from a sensory perspective (Ballester et al. 2013). It is not just rocks that elicit such allusions. So do volcanic soils:

> Nerello [Mascalese] can offer a finesse and structure…. with the minerality that only the volcano can offer (in reference to the black volcanic soils of Mt Etna) (Capalbo 2016)

What are the facts? Soils are not just broken up bits of rock beneath them. Some soils are derived largely from weathering of rocks beneath but many factors determine the composition and nature of those soils: climate (usually considered to be the primary factor), organisms, parental material (rocks), topography and time. Soil can have inputs from elsewhere due to wind or water and may not be derived at all from rocks beneath, for example many alluvial soils. None of the nitrogen or organic matter in soil comes from rocks. Minerals derived from rocks play a small role in soil's impact on plants except where mineralogy makes farming difficult, such as excessive levels of boron, aluminium, sodium or deficiencies of hard to correct micronutrients such as iron. Roots grow in soil, not in rocks, and the soil physical attributes are almost always important to root growth and water supply, regardless of the mineral basis.

That berry components such as colour and flavour are synthesised in the berries and not transported to berries from soil or even leaves has been known for decades (Iland et al. 2011) but conveniently ignored by the geologically obsessed. So, readers can be grateful to a professor of geology, Alex Maltman, who has clarified the geological 'picture' (Maltman 2014):

- Geological minerals are complex chemical compounds that are mainly tasteless
- Vine roots can only take up ions in solution
- Membranes only allow certain ions to be taken up
- Even then not all ions taken up by roots will end up in fruit
- Soil minerals get from the roots to the berry in either xylem or phloem (Iland et al. 2011)
- Before ripening, this occurs mostly via xylem from roots to all plant organs; during ripening, there is almost no xylem transport to the berry so minerals have to go to leaves first then to the berry via phloem
- Different ratios of mineral nutrients reach skins, seeds and pulp. So even if a particular mineral ion ends up in the fruit, that mineral ion (tasteless) has to become complexed with an organic aromatic compound for the apparent flavour of the mineral to be expressed
- The inorganic chemical profile of the grape berry bears only distant and indirect relationship with vineyard geochemistry
- This disconnect is magnified during vinification. Fermentation can remove mineral nutrients such as copper and zinc, while adding others, for example aluminium, calcium and iron. Fining removes further metals and adds others if bentonite is used. Stabilisation and ageing can add copper, iron and more whereas others are removed along with tartrate precipitation

Therefore, the proportions of mineral ions in wine bear little relationship to the geological minerals in the vineyard (Maltman 2014). Even berry nutrient status is not as closely linked to vine nutrient status as one might expect (Bramley et al. 2011).

Soil physical properties

> Terroir-driven wines can be produced with finely-tuned deficit irrigation in low rainfall regions (van Leeuwen and Seguin 2006)

Soil can play a major role in terroir expression, particularly at the meso- and microclimatic level. It has been known since the 18th century that soils producing fine wines are highly variable. It is well accepted that within the same mesoclimate, different soils can produce wines of different quality. But at the same time, the same soils in different but neighbouring mesoclimates can produce quite different wines with the same variety; for example the more elevated and thus cooler Hautes-Côtes of Burgundy include soils that are just as good as those of the Côte d'Or but the better wines are produced in the latter because ripening is more reliable (van Leeuwen and Seguin 2006). The human influence in connection with soil may be significant: soils of the best sites (for wine) in Bordeaux are richer in organic matter, potassium and phosphorus than lesser sites. But the best sites are not intrinsically richer but rather have been enriched by fertilisation (a higher price for wine allows more intervention) (van Leeuwen and Seguin 2006).

To summarise, in this context, the physical properties of a soil appear to be much more important than the chemical properties. The former include water holding capacity, root penetration ability, rootzone temperature, susceptibility to waterlogging and effect on above-ground microclimate. This paper will focus on four key aspects of soil physical properties:

- **Water availability:** Grapevine water status depends on climate (rainfall and evapotranspiration), soil (water holding capacity) and canopy (leaf area and its arrangement in space). It has been known for some time that vine water uptake conditions are a key factor in understanding the effect of terroir on grape/wine composition and quality (van Leeuwen and Seguin 2006; Tomasi et al. 2015). Seguin and colleagues in France showed that grape quality potential is related to a regular but moderate supply of water (Seguin 1986). Soils of superior terroirs are well drained, have a good supply of subsoil moisture that supplies the needs of vines through the summer and autumn and a topsoil that dries out sufficiently to induce cessation of shoot growth, ideally by veraison (van Leeuwen et al. 2004). Diverse soil types can provide this optimal soil moisture regime depending on the interaction with prevailing rainfall and evaporation patterns. The irrigation strategy known as partial rootzone drying (PRD) can potentially mimic these ideal water relations (Dry and Loveys 1998; Loveys et al. 2001).

  High grape quality for red wine production is obtained when a limiting factor reduces vine (shoot) vigour. In most terroirs known for their high quality performance there is a mild water deficit either because the climate is dry (high evapotranspiration and/or moderate to low rainfall) or because soil water holding capacity is low (shallow or high stone content) (van Leeuwen and Seguin 2006). But it is not just soil water holding capacity as determined by depth and texture that may be important. Tomasi et al. (2015) have shown that the capacity of the vine to extract that water (a function of root density and distribution in the soil) is also important.

  Water status also affects berry flavonoid components. This may be an indirect effect related to bunch exposure or a direct effect. For example, Castellarin et al. (2007a, b) demonstrated changes in gene expression of the flavonoid pathway under water deficit conditions. Such changes in flavonoid composition translate into altered wine composition, with deficit irrigation shown to increase colour, total anthocyanins and total phenolics (Chalmers et al. 2010). There is evidence that continued supply of hormones such as abscisic acid is important for ripening and flavour development.

- **Rootzone temperature:** Well-drained soils, rocky soils and slopes facing the sun (or all three) have strong and deep soil warming. This is a feature of a good terroirs irrespective of macroclimate (Gladstones 2011). Soil temperature is part of the vine's microclimate in two ways: a) heat re-radiated from soil contributes to the immediate above-ground thermal regime most significantly at night and during the day under intermittent cloud cover; b) it has a direct impact on vine roots—both temperature and water status regulate production of hormones such as cytokinins and abcisic acid in root tips and thus control of growth and fruit composition.
- **Soil effect on above-ground microclimate:** Soils affect the above-ground microclimate through absorbance and reflection of radiation by the soil surface and later re-radiation of long-wave heat. Soils with surface rock are particularly good at doing this. The benefits of such soils for viticulture giving good wine have been recognised for centuries. Furthermore, rocky soils give protection from erosion, making mesoclimatically-desirable slopes available for planting.
- **Soil colour:** Light soils reflect a high proportion of radiation up into the canopy. Dark soils absorb most radiation and re-radiate much of it later when ambient temperatures are low. This is particularly important in cool climates. For further information on this topic, refer to Gladstones 2011.

Soil chemistry

> Plant physiologists are not interested in where a soil mineral comes from but whether it is available to grapevines and how it will contribute to its growth and fruit composition (Matthews 2105)

Is there a relationship between soil nutrient status and wine aroma and flavour? There have been many studies but correlations between wine quality and soil content of any nutritive element (with exception of nitrogen and salt) are few. Of all mineral nutrients, nitrogen has greatest effect on growth, yield and fruit composition. There are both direct effects (reprogramming of gene expression) and indirect effects of soil nitrogen on fruit composition and wine quality. In the latter case, excess nitrogen leads to increased vegetative growth, denser canopies and more bunch zone shading with consequent effects for fruit composition, such as increased methoxypyrazine concentration in response to nitrogen fertilisation. Also, the nitrogen composition of fruit affects yeast metabolism (fermentation rate and production of flavour-active compounds) (Keller 2008; Iland et al. 2011). In general, red wine quality is negatively correlated with vine nitrogen, particularly when water is not limiting. For white wine, moderate soil nitrogen is best for quality: low nitrogen leads to decreased aromatic precursors and increased tannin, high nitrogen increases risk of Botrytis bunch rot (van Leeuwen et al. 2004).

This is no evidence for a direct effect of soil potassium on wine quality except that potassium deficiency may impair sugar accumulation. Factors such as rootstock type, irrigation and canopy management are much more influential on the potassium composition of fruit than the potassium status of soil (Keller 2008; Iland et al. 2011).

One of the few references to a link between a soil micronutrient and wine sensory character is found in Bramley et al. (2011): they reported an apparent association between a 'red confection' sensory attribute and soil extractable iron. The authors concluded that this does not mean that there is a direct effect: iron could be a surrogate for soil drainage status or aeration. But because ferrous and ferric ions are enzyme co-factors in plants it is possible that differing uptake of iron may affect production of compounds that confer flavour. It is possible that trace amounts of soil minerals may affect fruit composition via their influence on gene expression.

As we know, rootstock type has a major influence on the mineral nutrient composition of the scion variety. Rootstock use in most regions of Europe has changed significantly over the past 150 years, but the reputation of those vineyards has not.

Impact of global warming on terroir

> 'It is no joke to consider that Champagne producers may one day jump the English Channel in pursuit of their runaway terroir' (Veseth 2011)

The whole notion of terroir is built on the idea that through decades of trial and error it is possible to understand the relationship between the vine's environment and the wine. But global climatic change threatens to fundamentally alter this relationship. Different varieties perform best under particular climatic conditions, and some varieties, such as Pinot Noir, are very specific in terms of their requirements for fine wine production. Increased temperature will change the terroir × variety interaction (Sadras and Moran 2012) and the interaction of temperature and vine physiology, for example, Shiraz water deficit leads to more phenolics but this may not hold under elevated temperature. Increased temperature will also lead to changes in flavour profile.

Conclusions

It is difficult to find a definition of terroir that satisfies everyone from viticulturists to wine marketers. Perhaps it is a pointless exercise. The definition will depend on scale because it is the scale that determines which components are most important. In reality, perceived differences between wines attributed to terroir is generally when wines are compared at the macro- or meso-scale.

Human factors are important at all scales of terroir as they interact with the natural environment. The role of variety, both scion and rootstock, is often underrated in the terroir discussion. If comparing two regions with the same variety, particularly in the Old World, one needs to be mindful that the genetic differences may be significant even though it is the same variety. This is why clones from different regions may have very different wine characters. And as for the potential impact of epigenetics on terroir – the surface has only just been scratched.

If the wines are from different regions, then the major driver is climate. If wines are from different sites within the same region, again it is mainly climate, modified by topography and soil. If wines are from different parts of the same site, or even within the same vineyard, then it is mainly soil physical properties having both a direct effect on vine metabolism and an indirect effect via microclimate. Soil chemistry, particularly nitrogen status, has both direct and indirect effects. The evidence suggests that geology has a minor and indirect role at all scales.

The term terroir is mainly used by people with more interest and expertise in wines than in grapevines. For some, terroir is not a desirable term because of its strong linguistic connection to the soil and because it does not appropriately convey the notion that climate at all levels is the major driver of terroir. However, the global wine community has probably gone too far down the road of international acceptance of terroir to find a more suitable alternative.

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Vineyard variability and terroir – making sense of a sense of place

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Abstract

Much is written about terroir. In Australia at least, most of this is written by wine writers and sellers in pursuit of a market advantage derived from the notion that our terroir is unique. Alas, most of the writing has little, if any, basis in science and relies predominantly on notions of regional or subregional terroir and the idea that single vineyard wines are especially evocative of their 'sense of place'. This paper uses three recent examples from research into vineyard variability, coupled with an analysis of soil variation at regional scale, to argue that rather than a focus on regional and subregional terroir, our real opportunity for uniqueness may lie in our ability to understand the impacts of biophysical variation on the attributes of grapes and wine at the within-vineyard scale. Such understanding of 'place' should promote the implementation of decisions in the vineyard which enable us to grow the grapes that we need in order to make the wines that we want to make. It should also promote a more informed commentary on our 'unique' Australian terroirs, providing stories that wine writers, marketers and scientists can all share.

Webcast of this presentation available at http://bit.ly/16thBramley.

Introduction

Much is written about terroir. In Australia at least, most of this appears in the popular press and is written by wine writers and marketers in pursuit of an advantage derived from the notion of the uniqueness of a wine's terroir; the title of this session and of others in this conference suggest that they are not alone! Alas, most of the writing has little, if any, basis in science. Whilst much of it seeks to evoke a wine's 'sense of place', it does so with little recourse to real understanding of the various factors which, from a grapegrowing and winemaking perspective, make that place what it is. Thus, 'single vineyard wines' are deemed virtuous and evocative of their regional terroir, even though, as will be discussed below, both the single vineyard and wider region might be quite variable, and the location of the vineyard boundaries might be little more than an accident of fate. It is also a fact that what is arguably Australia's finest wine has a provenance that is both multi-vineyard and multi-region.

In their 2015–2020 Strategic Plan, Wine Australia (2015) state that to 'increase demand and the premium paid for all Australian wine, we will focus our efforts on our very best wines, those fine wines of exceptional quality and finesse that reflect their provenance and terroir and will most quickly elevate the image and reputation of the wines we produce.' Whilst this statement is reflective of the notion of marketers and wine writers that terroir and provenance (and conjuring stories about them) are good, and conversely infers that wines without a story are perhaps not so good (!), the Wine Australia (2015) plan also expresses the need to 'continually invest… to better understand and express our unique terroirs…'. It is not the intention of this paper to dissect the definition of terroir, or to examine in detail the various factors which contribute to it (soil, landscape, geology, climate, traditions in viticulture and winemaking and other social factors). We know, for example, that regional differences in terroir expression (e.g. 'Shiraz from the Grampians is different to that from the Barossa Valley') can be readily attributed to regional differences in climate (Smart and Dry 1980; Gladstones 1992). Rather, with a particular focus on soil and topography, this paper offers some suggestions as to where investment in understanding terroir might be expended. It also seeks to highlight how the Australian (and international) wine sector might benefit from a greater appreciation on the part of marketers, wine writers and others, of what we already know (and do not know) about terroir.

Soils, scale and block boundaries

Figure 1 shows a map of the soils in a small section (5.2 km^2) of the McLaren Vale winegrowing region. By comparison, the total McLaren

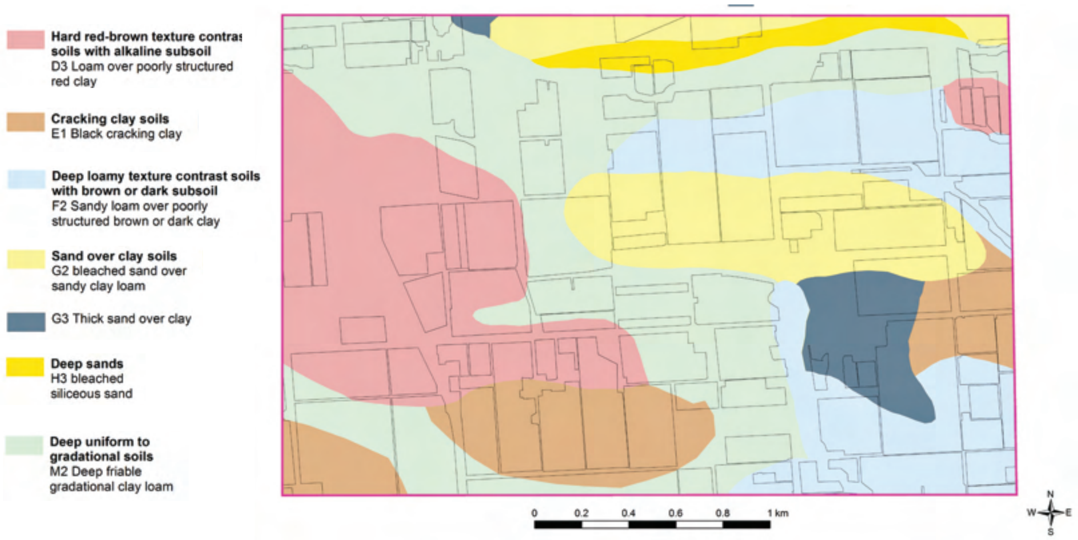

Figure 1. Soil map (1:50,000) of a 5.2 km^2 section of the McLaren Vale winegrowing region. This map was produced using the soil data of DEWNR (2016) and vineyard block boundaries supplied by Vinehealth Australia (formerly the Phylloxera and Grape Industry Board of South Australia). Legend categories are from Hall et al. (2009).

Vale geographical indication is 431 km^2 whilst the total vineyard area is approximately 75 km^2. The map shown in Figure 1 derives from the extensive efforts of soil scientists working in the South Australian State Land and Soil Mapping Program (SMP) over the last 30 years, and is supported by the description of over 28,000 soil profiles statewide (Hall et al. 2009). It is important to note that this map was produced at a scale of 1:50,000, reflecting the reconnaissance purpose of the survey which underpinned it. It is also important to highlight that soil maps generated through the SMP, like the surveys which preceded it (e.g. Northcote et al. 1960–68), are generally presented in terms of 'soil landscape units' (SLUs), which reflect geology and/or landform (collectively referred to as 'land type') in addition to Soil Group or subgroup (i.e. soil type). The SMP identified over 15,000 SLUs, and 61 subgroup soils aggregated into 15 Soil Groups (Hall et al. 2009). In Figure 1, subgroup soils are shown; the only difference between this map and one classified on the basis of SLUs is that the 'sand over clay soils' separate into the two subgroup soils (G2 and G3) shown (Dept. Environment, Water and Natural Resources (DEWNR) 2016).

With respect to the soil component of terroir, Figure 1 highlights a number of problems with both its regional and single-vineyard connotations. First, even though the SMP has delivered the best regional soil resource information available in Australia in terms of scale (most other Australian reconnaissance soil survey data are only available at 1:100,000 or 1:250,000), it has recognised the 'fuzzy' nature of soil mapping (McBratney and De Gruijter 1991), along with the short-range nature of soil variation (McBratney and Pringle 1999). Thus, it is explicit that, as mapped, the soil subgroup classes are not pure (Hall et al. 2009). Whereas Figure 1 indicates that the area shown contains seven mapped soil types, in fact the situation is more complex. Thus, Table 1 indicates the degree of membership of the mapped soil types which, in total, include 11 of the soil subgroups identified by the SMP, not just the seven as mapped. Overall therefore, the message from Figure 1 and Table 1 is that in McLaren Vale, soil variation is complex. Vineyards on the central western side of Figure 1 are shown as having soils belonging to the D3 subgroup (loam over poorly structured red clay), which means that there is only a 35% chance that these soils are actually D3 (Table 1). Similarly, there is a 30% chance that at any location in these vineyards, the soil type may be G4 (sand over poorly structured clay). The D3 (formed in fine-textured alluvium) and G4 (formed over 'Blanchetown clay') soil types have different water holding capacities and so differ in their plant water availability – a soil property which several authors have identified as being important to terroir expression in wines (Seguin 1986; van Leeuwen et al. 2004; Bodin and Morlat 2006; Morlat and Bodin 2006; van Leeuwen and Seguin 2006; Ramos et al. 2015). Likewise, the contrast between the M2 soils (deep friable gradational clay loam) and those of the E1 (black cracking clays) or G2 (bleached sand over sandy clay loam) subgroups might be expected to have a major impact on both terroir and vineyard management, especially with respect to irrigation. As Figure 1 indicates, these different soils can occur quite close to each other.

Table 1. Proportionate membership (%) of soil subgroups in the mapped soil subgroup classes shown in Figure 1[A]

Soil subgroup contributing to mapped units	Soil type (subgroup) as mapped in Figure 1						
	D3	E1	F2	G2	G3	H3	M2
D2 Loam over red clay	20						
D3 Loam over poorly structured red clay	35	10					
E1 Black cracking clay		55					
F1 Loam over brown or dark clay			20				20
F2 Sandy loam over poorly structured brown or dark clay	15	15	80		20		
G2 Bleached sand over sandy clay loam				65		20	
G3 Thick sand over clay				15	80	15	
G4 Sand over poorly structured clay	30						
H3 Bleached siliceous sand				20		65	
M1 Deep sandy loam							35
M2 Deep friable gradational clay loam		20					45

[A]Data of DEWNR (2016)

What then, does Figure 1 say about the soil component of terroir, or our understanding of it – either in the 5.2 km^2 area shown, or in the 75 km^2 under vine in the McLaren Vale region more broadly? Even when mapped at 1:50,000, McLaren Vale soils are clearly markedly variable, something that is also highlighted by the regional soil analysis reported by Ouzman et al. (2016). However, and even ignoring the variable composition of an individual soil mapping unit (Table 1), some of the vineyards in Figure 1 are seemingly underlain by a single soil type, whilst others may contain two or three (often contrasting) soil types. Of course, a part of the problem here is that Figure 1 reflects a soil survey that was conducted for the purposes of natural resource reconnaissance, land-use suitability assessment and land planning; it was not intended as a basis for management of crop production which would require a map at much finer scale. Thus, for example, Senarath et al. (2010) showed how a similar soil survey conducted at 1:25,000 (i.e. four times as intensive as that in Figure 1) was an inappropriate basis for management of soil drainage, whilst the work of Manderson and Palmer (2006) suggests that for soil survey information to be consistent with the scale at which many viticultural decisions might be made (a few ha or less), it would be required at scales of 1:10,000, 1:5,000 or better (i.e. 25 or 100 times as intensive as Figure 1). Examples of regional soil survey at such high resolution are rare in Australia, with that of Wood et al. (2003) in the Herbert River sugarcane growing region being the only one that this author is aware of. Whilst Vaudour (2002) advocated the use of soil maps at a scale of 1:25,000 to assist in the definition of 'functional terroir units', Van Leeuwen and Seguin (2006) stated that 'it is generally not possible to equate a soil map of a given region with a map of quality potential for vine-growing'; Figure 1 and Table 1 tend to lend weight to this notion.

The mismatch between the scale at which information is available and the scale at which it might be used – whether for management of winegrowing or consideration of terroir - together with the complexity of soil variation, raises questions as to whether and how each of the vineyards in Figure 1 are deemed reflective of the terroir of the McLaren Vale region? Quite clearly, there are likely to be some important differences between them in terms of the soil component of their terroir. Doubtless, a similar question could be raised in regard to within-region climate variation if a sufficient number of local weather stations, and a digital elevation model (e.g. Bramley 2007; Bramley and Williams 2007; Figures 2–4) were available to support such an analysis. More importantly, the present focus in McLaren Vale on geology as a driver of terroir is also dependent on a 1:50,000 map (see https://mclarenvale.info/wine/the-geology/), although it is unclear why this might be preferred to the soil map available at similar scale.

Which soil properties might be important to wine?

A further problem in interpreting soil maps such as Figure 1 in a winegrowing context is the fact that, in spite of the long history of wine production worldwide and much research on soil-vine interactions, our understanding of the impact of specific soil properties on vine performance or the sensory properties of grapes and wine is seriously lacking. White (2003) has noted that 'scant attention' has been paid to soil and its complex interaction with wine-grapes in the New World, and whilst much research has been done in Old World wine-producing countries, almost all of this has occurred in non-irrigated production systems and has examined soil property-wine interactions at regional scale (c.f. Figure 1) using relatively small numbers of samples (e.g. Bodin and Morlat 2006; Morlat and Bodin 2006; Pereira et al. 2006; De Andrés-de Prado et al. 2007; Ramos et al. 2015; van Leeuwen et al. 2004). Because this work was done in dryland viticulture, it is hardly surprising that soil hydraulic properties are those that have been highlighted (e.g. Seguin 1986). Soil hydraulic properties are important to terroir because they play an important role in determining plant water availability (e.g. van Leeuwen et al. 2009). However, with the possible exception of soil salinity (Lanyon et al. 2004), quantitative relationships describing the effects of soil properties on either grape production, grape composition or the sensory attributes and composition of wine are almost impossible to find. As a consequence, recommendations as to the desirable values for various vineyard soil properties are seemingly based much more on expert opinion than robust quantitative experimental research (Oliver et al. 2013; Riches et al. 2013; White 2015). Against this background, it is no surprise that the potential importance of soil chemical properties has been largely ignored, although this ought not to justify them being dismissed as unimportant (Deloire et al. 2005). However, Australia is home to some of the world's oldest geologies and most extensively weathered, and so nutrient-depleted soils (McKenzie et al. 2004). As a consequence, much important work on the essential nature of plant nutrients, especially micronutrients, has been done in Australia (Loneragan 1997 and references therein), with the physiology of plant mineral nutrition now well understood (Marschner 2012). Progress in understanding the importance of plant nutrition to the genetic regulation of key biochemical pathways is also being made (e.g. Hirai et al. 2004; Bielecka et al. 2015). It is therefore surprising that, in Australia, we have largely ignored the possibility that these factors may contribute to the uniqueness of our terroirs. More recent research is also suggesting a role for soil microbiology in terroir expression (Burns et al. 2015; Zarraonaindia et al. 2015). Key questions that could usefully be addressed so as to advance understanding of the soil component of terroir include: does variation in specific soil properties, including those associated with fertility, nutrient availability and microbiology, have a (functional) impact on grape and wine composition? If so, at what scales (between-region, within-region, within-vineyard) are these effects expressed? With respect to which aspects of grape and wine composition are they expressed? How do these effects interact with climate? Are they consistent over time, especially in the case of any soil microbiological effects (Gupta et al. 2011)? Are these effects consistent across varieties?

This last question is an important one given the present focus in some parts of Australia on subregionalisation. For example, it would be useful to know whether any subregional discrimination based on the sensory attributes of Shiraz wines noted through either the 'Scarce Earth' project being run in McLaren Vale (https://mclarenvale.info/scarce-earth/), or the 'Barossa Grounds' project being conducted in the Barossa Valley (http://www.barossa.com/wine/barossa-grounds), were also seen with respect to Grenache, Chardonnay, Cabernet Sauvignon or any other variety. If they are, then arguments in favour of such subregionalisation and our understanding of terroir might be greatly facilitated; if they are not, the problem arguably becomes more complex than it already is. Of course, the same question about variety effects could be addressed in pursuit of understanding any differences *between* McLaren Vale and Barossa wines.

Overall therefore, consideration of the soil component of terroir at regional scale is highly problematic because there is much variation and we are far from clear as to which soil properties affect the sensory attributes of wine. Thus, unless there are gross differences in soil type and properties (or geology) between regions or subregions, the attribution of terroir expression at regional/subregional scale to soil (or geological) effects may be difficult to substantiate.

A way forward through digital technologies and a focus on vineyard variability

Recent research clearly shows that vineyards are highly variable at the within-vineyard scale in respect of vine vigour, grape yield, grape composition and vineyard soils; Tisseyre et al. (2007) and Bramley (2010) provide reviews of much of the early work in this area. A common thread in both this, and more recent, vineyard variability research (Taylor et al. 2010; Bramley et al. 2011a, c; Tardaguila et al. 2011; Scarlett et al. 2014; Bramley et al. 2017), is that the within-vineyard variation in vines, grapes and wine is driven by variation in the land (soil, topography) underlying the vineyard. As a consequence, it has been suggested (Bramley 2007; Bramley and Hamilton 2007) that at least some of the elements of terroir should be considered manageable. Overall, the vineyard variability research makes very clear that, in spite of the difficulty of assessing the importance of soil to regional terroir (see above), at the within-vineyard scale, it might be more straightforward and so provide some important learnings.

Figure 2 summarises the basis for the delineation of zones in an 8.2 ha section of a vineyard near Mildura in the Murray Valley (Bramley et al. 2011a); the zones were delineated with a view to selective harvesting (Bramley et al. 2011b). The data layers used to derive the zones included a high resolution survey of bulk electrical soil conductivity (EC_a) using an electromagnetic 'EM38' sensor, along with yield maps, derived from a yield monitor fitted to the harvester, and remotely sensed imagery of vine vigour (PCD), in both cases collected over a four-year period. Proffitt et al. (2006) describe the use of EM38 soil sensing, yield mapping and PCD imagery in Precision Viticulture.

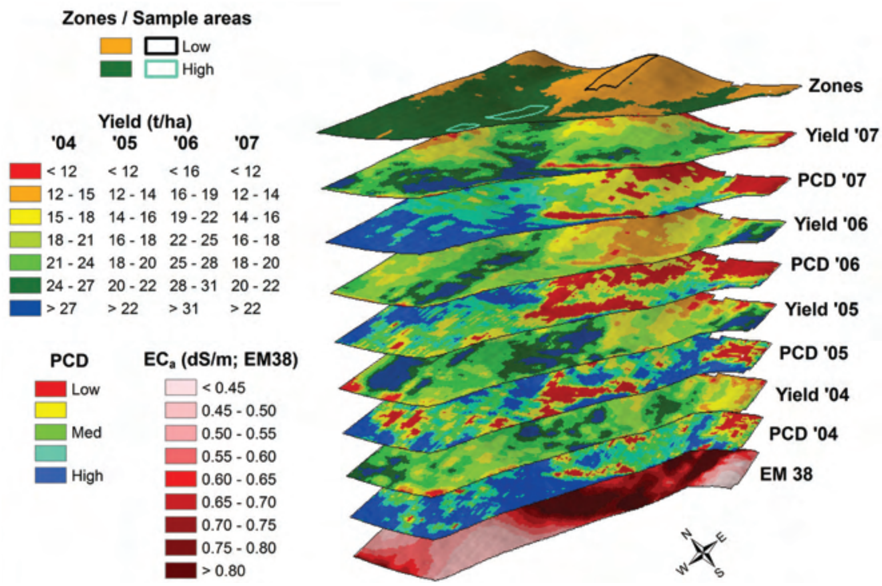

Figure 2. Variation in topography, bulk electrical soil conductivity (EC_a), vine vigour as assessed using remotely sensed imagery (PCD) and yield in an 8.2 ha section of a block of Cabernet Sauvignon in the Murray Valley. The data were used to identify zones (top map layer) in which sampling areas were identified for small-lot winemaking. Data sourced from Bramley et al. (2011).

Sensory analysis of small-lot (Bramley et al. 2011a) and commercial scale (Bramley et al. 2011b) wines produced from fruit harvested from these zones showed the wines from the two zones to differ markedly – despite the management of the entire block, and the winemaking protocols used, being uniform. In addition to sensory analysis, the small-lot wines were also analysed for volatile compounds. The soils in the two zones were also analysed for a range of attributes, as were samples of grapes at harvest in each of the three years, and a number of measures of vine performance (bunch number, berry weight, etc.) were also made annually. The sensory analysis showed that wines derived from the characteristically higher vigour, higher yielding part of the block, where deep sandy topsoils overlay imperfectly drained heavier clay subsoils, were typical of 'premium' bottled Murray Valley Cabernet Sauvignon. In contrast, the wines from the lower vigour, lower yielding 'hill', where the soils are much shallower and sandier, were more highly regarded by the sensory panel and showed more appealing fruity characters than those from the higher yielding zone which were described as having green attributes and 'meaty' aromas. It was on this basis that selective harvesting and product streaming were pursued in this, and a nearby block (Bramley et al. 2011b).

Analysis of volatile compounds in the headspace of the wines enabled the discriminating sensory attributes to be associated with certain chemicals or groups of chemicals. Importantly, this study produced evidence of links between soil, grape, vine and wine attributes. Relationships were explored between sensory and wine chemistry attributes on the one hand, and soil, grape and vine attributes on the other. Whilst the statistics involved must be regarded as circumspect due to inadequate replication of wines, many apparently significant relationships were identified. Soil extractable iron and manganese and grape berry phenolics predominated amongst soil and grape attributes, with the aroma sensory attributes 'red confection', 'fresh berry' and 'floral', and the wine compounds 2-nonanone and ethyl decanoate prominent amongst the sensory and chemical attributes. Importantly, the analysis conducted over 3 consecutive seasons, emphasised the differences between the two zones; that is, their terroir is different.

Figure 3 shows the results of some other recent research (Bramley et al. 2017) in which within-vineyard variation in the concentration of rotundone was analysed. Rotundone is the compound that is responsible for the 'pepperiness' of some Shiraz wines (Siebert et al. 2008; Jeffery et al. 2009), an attribute that is generally considered desirable. Knowing that Shiraz from the Grampians region tends to have higher concentrations of rotundone than Shiraz from other regions (Jeffery et al. 2009), it was of interest to Grampians producers to know whether this pepperiness was spatially variable at the within-vineyard scale and therefore whether strategies such as selective harvesting could potentially be used to manage the pepperiness of wines. In the season which culminated with vintage 2012, the concentration of rotundone in berry samples collected from 177 geo-referenced 'target vines' was analysed (Scarlett et al. 2014). Analysis of the resulting map of berry rotundone concentration, in conjunction with maps of soil and topographic (slope, aspect) variation, suggested both a soil effect on the concentration of this grape-derived flavour and aroma compound, and especially of a major impact arising from topographic variation (Scarlett et al. 2014) – most likely due to the effect of aspect on variation in ambient temperature and/or the amount of incident solar radiation (Zhang et al. 2015). Those parts of the block orientated furthest from north tended to have the highest berry rotundone concentrations.

Given the results of Scarlett et al. (2014), it was of interest to know whether the patterns of rotundone variation were stable in time; stability in these patterns would lend weight to the inferred role of soil and topographic variation in driving the rotundone variation, and also to the opportunity for selective harvesting as a tool to manipulate wine pepperiness. Thus, Figure 3 provides an update to the Scarlett et al. (2014) results by including rotundone maps from the 2013 and 2015 vintages when the same 177 target vines were sampled and analysed for their berry rotundone concentration. Vintage 2012 proved to be a 'high' rotundone year with a mean concentration of 399 ng/kg. In marked contrast, 2013 was a 'low' rotundone year (mean of 10 ng/kg), as was 2014 when, as a consequence, the full sample set required for map interpolation was not collected. Vintage 2015 was intermediate between these. As Bramley et al. (2017) have demonstrated, the patterns of spatial variation in each of the three mapped vintages is the same, in spite of there being a 40-fold difference between the 'low' and 'high' rotundone years (Figure 3). Like the vineyard shown in Figure 2, the vineyard shown in Figure 3 is under conventional uniform management, yet as Figure 3 illustrates, the 'pepperiness' of the grapes grown in it is markedly spatially and temporally variable, with the spatial variation related to variation in the land underlying the vineyard – which is why the pattern is stable over time. In other words, this vineyard's terroir is markedly spatially variable. Also of interest is the fact that, whereas the variation in fruit and wine composition in the Murray Valley example (Figure 2) was closely related to variation in vine vigour in addition to soil and topographic effects, in the vineyard shown in Figure 3, there was no indication that variation in vine vigour had any influence on variation in berry rotundone concentration.

Figure 3 also suggests that a strategy such as selective harvesting would enable a winemaker to exert some control over the pepperiness of final wines, especially if some pre-vintage grape analysis provided a signal as to the likely mean berry rotundone concentration at harvest. Thus, in a 'low' rotundone year, selective harvesting would be highly

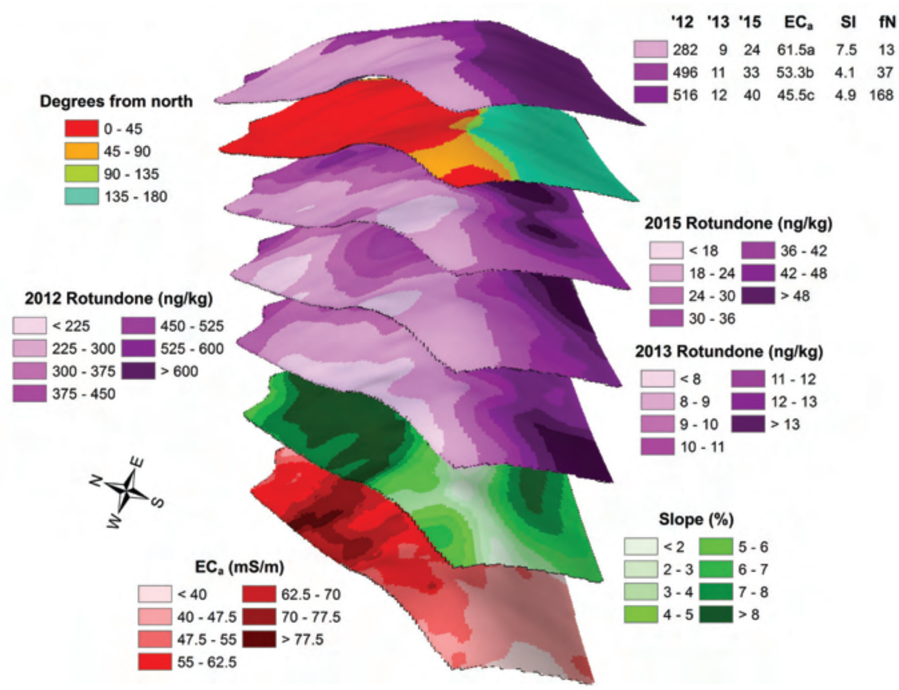

Figure 3. Interactions between soil (EC$_a$), slope (Sl), aspect, expressed as degrees from north (fN), and berry rotundone concentration over 3 seasons in a 6.1 ha vineyard in the Grampians region of Victoria. Note that the position of the north arrow is approximate only. Data sourced from Bramley et al. (2016).

unlikely to deliver a benefit. In contrast, in a 'high' year, the opportunity to manipulate the pepperiness of wines through a combination of selective harvesting and wine blending could be highly commercially significant. The same could be said of a 'medium' rotundone year when keeping low rotundone fruit separate from the remainder may enable production of a wine of desired pepperiness which it would not be possible to produce if the block were harvested as a single parcel. It is not suggested here that the goal is necessarily to maximise pepperiness, but rather to optimise it to the desired wine style. But what does such a strategy say about the terroir of such wines? The fact that the maps of berry rotundone from contrasting seasons (Figure 3) show an identical spatial structure indicates that the spatial variation in this 'terroir effect' is constant, albeit subject to seasonal variation in the magnitude of its expression. Of course, the expression of this terroir effect in the final wines is also subject to manipulation by the winemaker.

Clearly, with further carefully targeted research, great potential exists for truly understanding the soil component of terroir at the fine, within-vineyard scales illustrated by Figures 2 and 3 and using knowledge acquired through such research to improve the management of winegrowing. This knowledge might enable the efficiency of grape production to be optimised through targeted differential management of irrigation (Sanchez et al. 2014), nutrients, other soil amendments and the vine canopy. It would also promote an ability for wine style to be managed to a greater extent in the vineyard rather than through interventions in the winery, for improved matching of variety to site, and for wines to be produced which express desired elements of terroir.

The apparent mismatch between such ideas of vineyard-scale management opportunity and the information provided by regional scale reconnaissance mapping raises a question as to whether it might be possible to also gain useful information at a scale intermediate between the vineyard (Figures 2 and 3) and that of the small region shown in Figure 1. Figure 4 suggests that the answer may be 'yes', although this too, raises a question about the regional concept of terroir. It shows a 61.8 ha section of a property in the Eden Valley in which 38.6 ha are under vine (i.e. excluding headlands, unplanted areas) in blocks planted to Shiraz, Gewürztraminer and Riesling. The vineyard is steeply sloping in many sections and the south-east facing slope planted to Shiraz to the west and north-west of the property has a range of elevation of approximately 100 m. As in the previous examples (Figures 2, 3), Bramley and Williams (2007) used a survey-grade GPS whilst conducting the high resolution (EM38) soil electrical conductivity survey as the basis for producing the elevation model of the site. This was then used in conjunction with locally available climate data and estimates of leaf area development (Bindi et al. 1997), for modelling variation in temperature and solar irradiation across the site using SRAD (Wilson and Gallant 2000). Note that SRAD was only run for the period between budburst and harvest - denoted as 'season' in Figure 4.

As can be seen in Figure 4, the marked variation in elevation leads to similarly marked variation in modelled season degree days (base of 10°C). On the steeply sloping south-east facing slope planted to Shiraz, the approximately 100 m range in elevation leads to a difference of around 100 degree days between the top and bottom of the slope. Assuming a mean daily temperature in the October-March period of 16.6°C (http://www.bom.gov.au/climate/averages/tables/cw_023763.shtml), it is estimated that this difference equates to around 6 days in terms of likely harvest date. One might wonder which of the Shiraz blocks on this slope most closely reflects the terroir of the Eden Valley?

Perhaps counter-intuitively, the best Riesling wines to come from the vineyard shown in Figure 4 derive from the blocks at the south-eastern edge of the property, which is the warmest part of the property. If, hypothetically, market opportunity were to suggest that the Shiraz blocks should be re-planted to Riesling, then the difference in season degree days between the coolest and warmest sections of the property equates more closely to around 11 days in terms of likely harvest date, assuming that harvest takes place at the same level of ripeness in all blocks. Trought and Bramley (2011) have similarly drawn attention to the temporal component of within-vineyard terroir expression. At the time of the Bramley and Williams (2007) study, the fruit from the Riesling blocks went into as many as five different products with retail prices that ranged from around $18 to $35. Two of these were essentially quality-based variants of the same wine style; a third was made in a quite different style. Which of them most closely evokes the terroir of Eden Valley could presumably be debated at length!

Single vineyard wines

In addition to the issues discussed above in relation to soil variation at a regional scale, an additional problem which is evident from Figure 1, relates to block boundaries. It is clearly seen that almost all of these are straight lines and have an orientation that is approximately north-south and east-west. It is also obvious that the location of block boundaries bears no relation to the mapped soil variation, and with the exception of a few boundaries which run along creeks or drainage lines, there is seemingly no relationship between the positioning of the boundaries and natural biophysical variation. This is especially evident when the area shown in Figure 1 is viewed using a satellite image (not shown), such as is available from 'Google Earth'. In other words, the location of block boundaries – which one might think rather important to terroir, especially in the case of 'single vineyard' wines – is little more than an accident of fate, conditioned by the location of roads, the affordability of land, the availability of planting material, etc. One might therefore wonder why it is that single vineyard wines are often deemed by wine writers and marketers to be especially evocative of their local terroir? It is accepted that the sort of complex soil variation discussed in relation to Figure 1, and which is indicated in terms of ECa in Figures 2 and 3, may in fact contribute to the expression of terroir in a single vineyard wine. But if that is so, then given the arbitrary location of block boundaries from

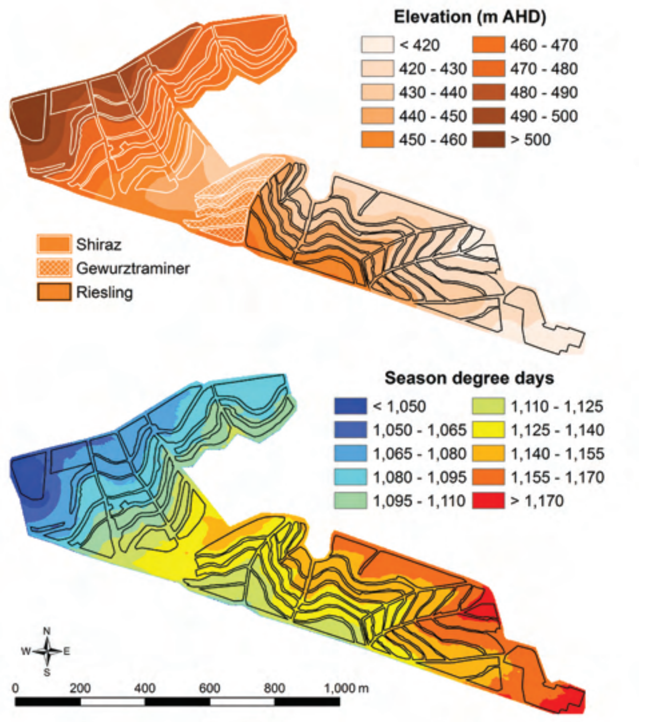

Figure 4. Variation in elevation and modelled season degree days in a 61.8 ha vineyard property from the Eden Valley. Data sourced from Bramley and Williams (2007).

the perspective of biophysical variation, the wide range in vineyard sizes, and the fact that biophysical variation is clearly spatially structured (i.e. not arbitrary), it is very difficult to see how two single vineyard wines in the same region or subregion could be deemed equally evocative of a regional terroir. The idea that single vineyard wines have some special virtue therefore seems rather poorly founded and difficult to justify – especially given the multi-vineyard, multi-region provenance of many highly regarded wines. As it happens, the wine which is produced from the fruit grown in the vineyard shown in Figure 3 is a 'single vineyard' wine; no fruit from other vineyard blocks contributes to the wine produced from this block. However, not all of the fruit grown in the vineyard shown in Figure 3 is used to produce that 'single vineyard' wine. Rather, a very careful selective harvesting process, supported by a similarly careful sensory assessment of fruit pre-harvest, is used to ensure that the winemaker ends up with precisely the fruit that he wants so as to produce the desired/intended wine. Such a practice, which is presumably not unique, also casts doubt on what is meant by 'single vineyard wine'. Whatever it does mean, a consequence of the harvest strategy used in this block is surely that, strictly speaking, the resulting wine only evokes a part of the vineyard's terroir and not all of it! Whilst neither marketers nor consumers are likely to be too bothered by this, it is certainly worthy of consideration in trying to understand terroir.

Concluding comments – the uniqueness of Australian wine

It seems to this author that the Australian wine sector is beating itself up unnecessarily over the question of the uniqueness of its wines and their terroir. It ought to be axiomatic that all terroir is unique – for the simple reason that no two places are the same. However, as is argued above, a key issue in making sense of a 'sense of place', is the scale at which we consider 'place'. Neither the varietal mix nor the climatic range in which grapes can be grown differ all that much amongst wine-producing countries. Therefore, at national and regional scales, the uniqueness of Australian wines probably lies in the age of our winegrowing landscapes, which are much older in geological time than most others worldwide. So on the one hand, we can simply state that Australian wines are unique because they are made in Australia (by Australians) using grapes grown in Australia; to the extent that wines from different regions in Australia express a distinct regional terroir, it is almost certainly largely climate-driven. Nevertheless, with access to geographical information systems and georeferenced block boundaries (c.f. Figure 1), it would not be difficult to produce regional scale maps of attributes such as fruit grade, fruit price, wine price or sensory attributes as a means of discerning the merits of subregionalisation – a strategy which is perhaps more sensible than starting with a soil or geology map since it focuses on terroir expression rather than the possible drivers of that expression. Similarly, with access to digital elevation data (www.ga.gov.au/scientific-topics/national-location-information/digital-elevation-data), an analysis analogous to Figure 4 could readily been done at a regional scale. Of course, if different areas are then deemed different in respect of important attributes, the reasons for these differences can be explored subsequently. However, our real opportunity for uniqueness may lie in our ability to understand the impacts of biophysical variation (soil, topography, climate) at much finer scales, and to use this knowledge to implement decisions in the vineyard which enable us to grow the grapes that we need in order to make the wines that we want to make.

As Brian Walsh states in the 'Introduction' to the Wine Australia Strategic Plan (Wine Australia 2015), Australia's 'natural endowment of diverse, unique and superior terroirs, combined with our skilled and innovative people, means that we have the capacity to be recognised as the best in the world'. Thus, rather than relying on stories conjured by wine writers and marketers to evoke our 'sense of place', it might be to our greater overall advantage to invest, as Wine Australia (2015) suggest, 'to better understand' our terroirs, and thereby ensure that claims about Australian terroir are founded on robust science and understanding. Such science may also make a major contribution to both the desirability of our wines and the skill used in producing them. For these reasons, they may be recognised as 'the best in the world.'

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Microbial diversity at work in vineyards and wine

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Abstract

Many environmental variables impact on the health and productivity of a grapevine, and on qualities of the fruit produced by a vine, such as colour, flavour or sugar content. Similarly, many variables in a winery impact on vinification, thereby affecting wine style. One of the most complex variables at work in both of these settings is microbial diversity, but until recently this was difficult to investigate. Thanks to technological advances in DNA sequencing, however, a detailed picture is now emerging of the microbial ecology of these environs. DNA sequencing technologies enable an approach known as metagenomics to be employed to determine the microbiota (i.e. microbial composition) of a range of environments including soil, plant surfaces, and liquids. Unlike traditional microbiological approaches where microbes had first to be isolated and then subjected to identification tests, metagenomics relies on isolating DNA from environments, sequencing this and using the data generated to characterise the microbiota. The relative affordability and accessibility of this approach and the volume of data generated is revolutionising the field of microbial ecology. Questions that are being addressed using metagenomics in a wine research context include: Are there regional differences in the microbiota of vineyards (including soils and vines)? And are there regional differences in microbiota of wine fermentations? While still at an early stage, patterns are emerging and what is being discovered is tantalising.

No paper available, please view this presentation at http://bit.ly/16thChambers.

How can we enhance the uniqueness of Australian wine?

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Abstract

To be the only one of its kind, unlike anything else, is the annual pursuit of many grapegrowers and winemakers. They make a continuing effort to deliver a unique snapshot of a place in time.

Australia has long been noted by scientists and historians for its unique flora and fauna, 40,000+ years of human history and the oldest geology and soils on earth.

Our winemaking community until recently relied on the ease with which we can ripen fruit, innovate, adapt and learn from our travels. The size of our continent in comparison to all of Europe highlights the diversity of regions we have growing grapes and making wine, with further complexity from the impacts of migration of different cultures and practices.

As we seek to survive in a competitive environment we have sought to validate our stories and sites. Wine regions have been exploring what is unique about their geology, soils, climate, biodiversity and impact on the environment, providing us with knowledge of the past to help us better adapt to the future.

This presentation will discuss a range of geology and soil projects conducted across Australia, as well as work done on biodiversity in vineyards and natural ferments, and research on the environmental footprint of our industry.

No paper available, please view this presentation at http://bit.ly/16thBell.

The global adaptation challenge for viticulture

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Abstract

Climate change is the primary challenge for the future wine industry worldwide. Both the direct consequences (e.g. temperature, precipitation, CO_2 concentration) and indirect consequences (e.g. resource management, energy efficiency, sustainability in production, consumer acceptance) of a changing climate will affect all facets of the wine industry. The predicted developments in climate are region-specific and adaptation can only be successful if regional characteristics with their diverse technical, environmental, economic and social implications are considered. Beyond some obvious adaptation strategies there are many more basic challenges below the surface. One of the key concerns for many regions is the availability of water and how increasing temperature will drive the evaporative demand of the atmosphere. For this, individual regions need to be analysed to quantify possible associated risks. Linked to the question of water availability/demand are many underlying adaptation challenges and open questions:

- *Are 'conventional' adaptation strategies (i.e. irrigation) compatible with the requirements for sustainable solutions in all cases and for all regions?*
- *How can we deal with the 'pendulum' of heavy precipitation and water scarcity in many regions within short time periods and can we devise intelligent solutions for 'dynamic resource management systems' for these situations?*
- *What are the consequences of climate change for our soils? How will it affect our viticultural systems/terroir in the future and how can we respond?*
- *Can we improve the resilience of our classical approach to viticulture?*

This paper will address differences in regional water relations of grapegrowing areas in different parts of the world as a basis to address the points listed above.

Webcast of this presentation available at http://bit.ly/16thSchultz.

Introduction

Climate change effects on the terrestrial water cycle show regional differentiated patterns. While temperature is increasing in many world grapegrowing regions (Jones et al. 2005; Schultz and Jones 2010; Webb et al. 2012; Hannah et al. 2013; Tóth and Végvári 2016), precipitation patterns can vastly differ between regions and can show substantial temporal variations (between and within years) (IPCC 2014). From rising temperatures it is mostly assumed that water holding capacity of the atmosphere will increase in the future as a function of the Clausius-Clapeyron law (Krysanova et al. 2008) which predicts an increase in the saturation vapour pressure of the atmosphere of 6–7% per degree Celsius. As a consequence, a simultaneous increase in potential evapotranspiration (evaporation of water from the soil and transpiration of water from plants, ETp) is assumed in many cases, which would alter soil and plant water relations. However, the same underlying principles also predict an increase in precipitation by 1–2% per degree Celsius warming (Farquhar and Roderick 2007). Additionally, model predictions for many regions forecast altered precipitation patterns and thus in combination with the possibility of increased ETp, farmers around the world fear an increase in the likelihood of water deficit and the availability of water for irrigation.

However, the large spatial and temporal variability in precipitation patterns between regions preclude generalisations in predicted consequences with respect to soil and plant water status development. In particular, the temporal variability may mask longer-term trends in the development of ETp and consequently soil and plant water status (van Leeuwen et al. 2010). Additionally, the focus on the development within a growing season (spring-summer) in many studies may miss decisive effects occurring during the 'off-season' (winter-early spring) but having substantial carry-over effects into the season.

Evaporation is driven by changes in temperature, humidity, solar radiation and wind speed and contrary to expectations due to climatic change, there have been reports on a reduction in evaporative demand worldwide (Farquhar and Roderick 2007). In many cases this has been related to a decrease in solar radiation observed for many areas on Earth including winegrowing regions in Europe ('global dimming') (Wild et al. 2005; Hofmann and Schultz 2010) during the last century. However, ETp in some areas has continuously increased which suggests that changes in the aerodynamic component must have more than offset the decrease in radiation over that part of the observed time span (Schultz and Hofmann 2016). For some regions in Germany, wind speed and vapour pressure deficit (VPD) have increased in the past and contributed to changes in evapotranspiration (Bormann 2011) but this is not in agreement with a worldwide observed decrease in wind speed and pan evaporation (Farquhar and Roderick 2007; McVicar et al. 2012).

These conflicting observations depending on climate classification, country or region, make it necessary to analyse grapegrowing regions with respect to developments in ETp and precipitation patterns in more detail in order to make predictions with respect to an increased risk in terms of water shortage. There is a general lack of studies analysing the past development in ETp and precipitation for different winegrowing regions across the planet in order to answer the question whether the threat of sustained drought will increase. When ETp was set to increase in a future climate scenario, substantial reductions in pre-dawn leaf water potential resulted when a dynamic physiological grapevine water model was used (Lebon et al. 2003) to estimate water consumption (Schultz and Lebon 2005). However, the large spatial and temporal variability in precipitation patterns between regions preclude generalisations in predicted consequences with respect to soil and plant water status development.

Water-limited worlds versus energy-limited worlds

Those parts of the earth where evaporative demand exceeds supply (rainfall), like much of Australia, are very different from those parts of the world where rainfall exceeds evaporative demand, for example, many German or French grapegrowing areas. In the latter areas there is runoff and rivers, and evaporation rate largely depends on the available energy and especially the radiation received. In water-limited regions, there is an excess of energy (e.g. solar radiation), and the actual evaporation rate can be close to the rainfall (Farquhar and Roderick 2007). Grapegrowers from different parts of the world have a very different view of their environment. The distinction between water-limited versus energy-limited worlds is not completely consistent because winters, for example, in water-limited areas will, in many cases, be part of the energy-limited 'world' (Figure 1 based on Budyko (1974) and a conceptual analysis of Farquhar and Roderick (2007)). Following this analysis, the actual evaporation rate, Ea, must be less than or equal to evaporative demand, ETp, and also less than or equal to precipitation, P (Figure 1). The water-limited regions or the water-limited part of the season (which could be part of both general areas) are on the left, and the energy-limited regions (or parts of the season) are on the right of the figure.

Material and methods

In order to evaluate different grapegrowing regions with respect to observed changes on precipitation patterns and ETp and in order to validate or disprove general observations on changes across the planet (Farquhar and Roderick 2007), the data of seven winegrowing areas in five countries in the northern and southern hemispheres across a large climatic transect were analysed.

Climatic data for this analysis were provided by the German Weather Service (Deutscher Wetterdienst) for the location Geisenheim in Germany (50.0°N, 8.1°E) in a temperate climate; the French INRA CLIMATIK, Agroclim project for the locations Dijon, Burgundy (47.2°N, 5.2°E), temperate, and Avignon (43.9°N, 4.9°E) in a Mediterranean climate; the US California data provision system on integrated pest management for Oakville, Napa Valley, CA (38.3°N, 122.3°W), a Mediterranean climate situation; the Marlborough Research Station for Blenheim, New Zealand (41.5°S, 173.9°E), a cool climate maritime region; the Australian Bureau of Meteorology for Williamstown, Adelaide Hills (34.7°S, 138.9°E) and Hobart, Tasmania (42.83°S, 147.5°E). Data were seasonally separated into precipitation and ETp 'summer' for the growing season (May–October for the northern hemisphere, October–May for the southern hemisphere), which in agro-meteorological terms is defined as the 'hydrological summer' (Bormann 2011), and the 'off-season' (November–April for the northern hemisphere, April–November for the southern hemisphere), the 'hydrological winter'. In the case of the German data predictions for precipitation rates and ETp were used based on model-outputs of a regionalised version of the STARII model of the Potsdam Institute of Climate Impact (Orlowsky et al. 2008). STARII constructs time series from 2007–2060 by resampling of observed weather data according to trend informations of the global climate model ECHAM5/OM (A1B) (Jacob 2005). This approach provides physical consistency of the combination of the weather variables and is in close agreement compared to the statistics of observed climatology (Orlowsky et al. 2008).

Results and discussion

The general expectation (also very prevalent in the popular press) is that as the world warms because of increased greenhouse forcing there will be a widespread increase in evaporative demand. This has been challenged by data proving the contrary and by a lack of scientific basis put forward by several scientists (i.e Farquhar and Roderick 2007). Peterson et al. (1995) were the first to publish the results from 190 sites in the former Soviet Union, where they found decreasing pan evaporation rates in the European sector, a decline in Siberia, and no trend in the Asian part. Since then many other reports from different parts of the world have been published but none has explicitly looked at grapegrowing regions.

Observed and predicted summer trends for areas in Europe and California

Figure 2A shows observed (calculated according to Penman-Monteith) and predicted changes in ETp during the growing season (May-October) for the temperate winegrowing region of the Rheingau (Geisenheim, Germany, 50.0° N, 8.0° E) from 1958 until 2060 (Schultz and Hofmann 2016). To smooth out temporal variability, 10-year running mean values were used. There is a clear increase in the difference between ETp and precipitation rate during the growing season already observed during the past 55 years and this development will continue in the future as predicted using a regionalised version of the STARII model (Orlowsky et al. 2008) (Figure 2A). A similar increase in ETp was also observed for the Mediterranean region near Avignon, France, since the mid-1970s, but with no observed change for about the last 20 years (Figure 2B). Available data for the Napa Valley in California show that ETp has not changed for approximately 30 years despite concomitant observations on rising temperatures.

Obvious from Figure 2A are the cyclic patterns of both ETp and precipitation rates, both for the period of observation and the projec-

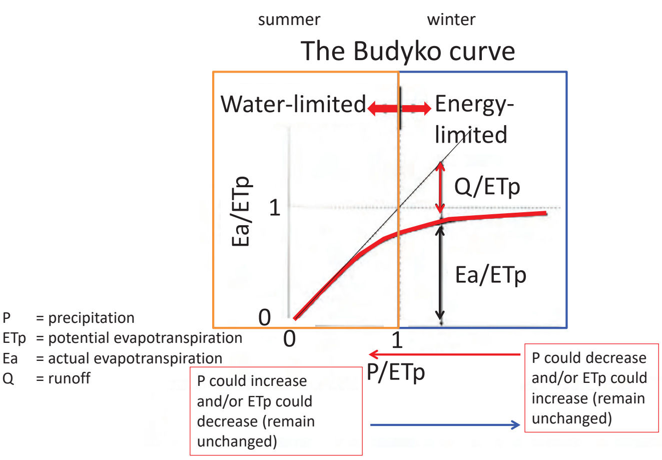

Figure 1. Inter-relationship between average precipitation (P), actual (Ea) and potential (ETp) evapo-transpiration and runoff (Q) and how season and climate change could affect this inter-relationship depending on the region. Grapegrowing areas are represented in both water- and energy-limited areas and the effect of climate change might be substantially different for different parts of the world. The original curve is known as the Budyko curve (Budyko 1974) and the presented figure is an adaptation from Farquhar and Roderick (2007).

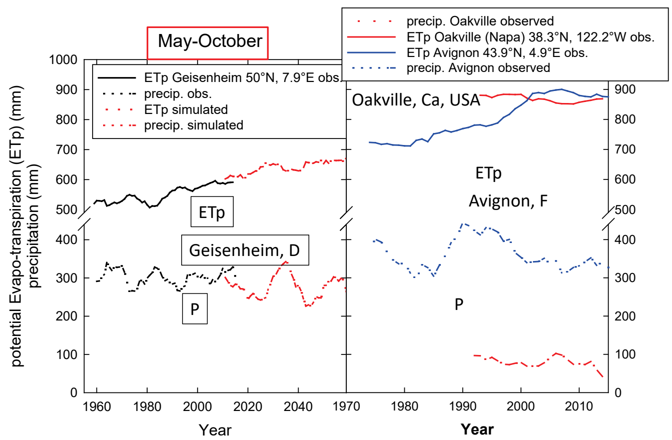

Figure 2. Observed and simulated precipitation and potential evapotranspiration for the hydrological summer (May-October) for Geisenheim in the Rheingau region (Germany, 50°N, 8°E) (A, left panel). Potential evapotranspiration rates for the observed time period (1958–2013) were calculated according to Penman-Monteith. Simulations were conducted with the STARII model of the Potsdam Institute of Climate Impact using the medium realisation run (Orlowsky et al. 2008). In the right panel B, observed ETp and precipitation data are shown for two Mediterranean type climate locations, one in Avignon, France, the other at Oakville in the Napa Valley, California. Data show 10-year running mean values. Observed data were from the Deutsche Wetterdienst, Germany, the French INRA CLIMATIK, Agroclim database and the US California data provision system on integrated pest management at the University of California, Davis.

tions until 2060. These cycles may be related to solar cycles which have been partly responsible for the warming during the first half of the last century but not during the second half (Stott et al. 2003). However, there is some uncertainty as to whether these cycles do continue to have an impact on the temporal development of warming on Earth and consequently on evaporation (Stott et al. 2003) but the data do show that variability and the development of extremes will become more likely despite cyclic variations (Figure 2A) (IPCC 2014). These cycles have an important effect on how climate change is perceived by humans since they can somewhat mask long-term trends (when precipitation is increasing or ETp is decreasing for several years) or on the contrary suggest a speed-up in these trends (Figure 2A).

Precipitation trends in Avignon have undergone some fluctuations but there was no distinct decrease observed, similar to summer precipitation in the Napa Valley, albeit on a much lower level (Figure 2B). If ETp predictions for the cool climate area of Germany (50°N) will be correct, then summer ETp values by the middle of the current century will be similar to Avignon (43.9°N) in the 1970s at lower precipitation rates.

Observed trends for Australian and California regions (summer and winter)

Analysing data from two Australian regions, Williamstown in the Adelaide Hills, and Hobart with a long data record for Tasmania, it is obvious that neither ETp nor precipitation have changed substantially over the time period of available data confirming other data from Australian sites (Roderick and Farquhar 2004) (Figure 3). The long-term data set for Adelaide Hills shows that ETp decreased between the 1970s and the 1990s during both winter and summer before increasing again to the early ETp values. This might have been related to the phenomenon of global dimming, a reduction in solar radiation observed in many areas during that particular period caused by increased cloudiness and aerosols (Wild et al. 2005; Hofmann and Schultz 2010). Precipitation rates also show no clear trend with a slight decrease during winter for the Adelaide Hills (left panel, Figure 3). Similarly, ETp during winter and summer of the Napa Valley location did not change appreciably (Figure 3), yet winter precipitation has almost been halved over the past 25 years, moving the area from an energy-limited towards a water-limited part on the Budyko curve (Figure 1). Despite a 'natural' focus on the developments within the growing season, changes in the water budget during the 'off-season' seem to become more important (Figure 3 left panel). Regardless of the fact that during winter and spring precipitation rates are exceeding ETp, the 'gap' between these two factors determining the soil water balance is decreasing in some areas (IPCC 2014). This suggests that for this particular region winter precipitation will eventually be matched by winter ETp with important consequences for the amount of water stored in the soils at the beginning of the growing season. It may also have consequences for the use of cover crops during the winter.

The phenomenon that ETp remains stable or decreases in many regions even in the post-global dimming period has been related to different combinations of effects, yet the most pronounced effect seems that the wind speed in many areas has decreased (Farquhar and Roderick 2007). A recent paper on the situation in China showed that wind speed has declined by 25–30% since the 1990s (Liu et al. 2014) and a decrease of similar magnitude has been observed for the Cape region in South Africa (Hoffmann et al. 2011). This has been implicated in the worldwide decrease in evaporative demand (McVicar et al. 2012). Data on wind speed are not easily available, but over the same time period, wind speed has not changed in several German regions (data not shown) and in some even an increase has been observed (Bormann 2011), which could be part of the explanation of different trends for different areas.

Observed trends for cool climate regions in Germany, France and New Zealand (winter and summer)

Aside of Mediterranean-type, low summer rainfall climates (water-limited) with a more or less continuous decline in water availability over most of the growing season, temporary water deficits also commonly occur in temperate, summer rainfall regions, particularly on vineyard sites with shallow soils and low water holding capacity (i.e. van Leeuwen et al. 2010). As compared to an irrigated vineyard situation in moderate or even hot climates, the natural cycles of stress and relief can be much more pronounced albeit completely unpredictable in frequency, duration and severity in these areas and are naturally part of the 'terroir' and the year to year variation in wine quality. Most classic European grapegrowing regions are unirrigated and examples are given for three classical cool climate regions and the observed trends in ETp and precipitation during winter and summer (Figure 4). Despite being classified as cool climate regions, both precipitation and ETp differ vastly. Blenheim in New Zealand has the highest ETp both in winter and in summer. Blenheim and Geisenheim in Germany show a continuing increase in ETp whereas Dijon in Burgundy showed a strong increase starting in the 1990s

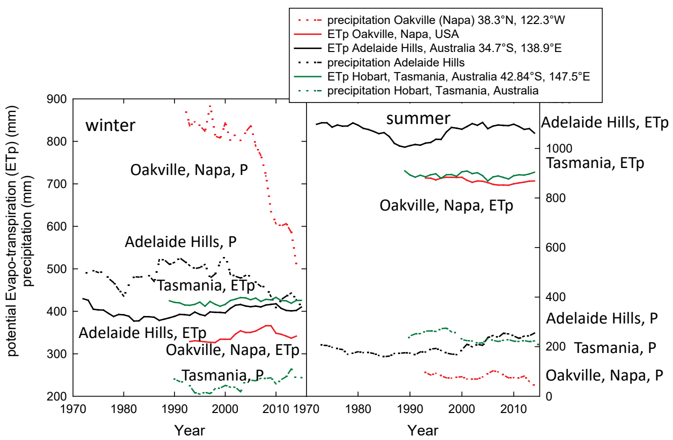

Figure 3. Observed precipitation and potential evapotranspiration for the winter (left panel) and summer periods (right panel) for Oakville, Napa Valley, California (USA, 38.3°N, 122.3°W), Williamstown, Adelaide Hills (Australia, 34.7°S, 138.9°E) and Hobart, Tasmania (Australia, 42.8°S, 147.5°E). Data show 10-year running mean values. Observed data were from the US California data provision system on integrated pest management at the University of California, Davis and the Australian Bureau of Meteorology.

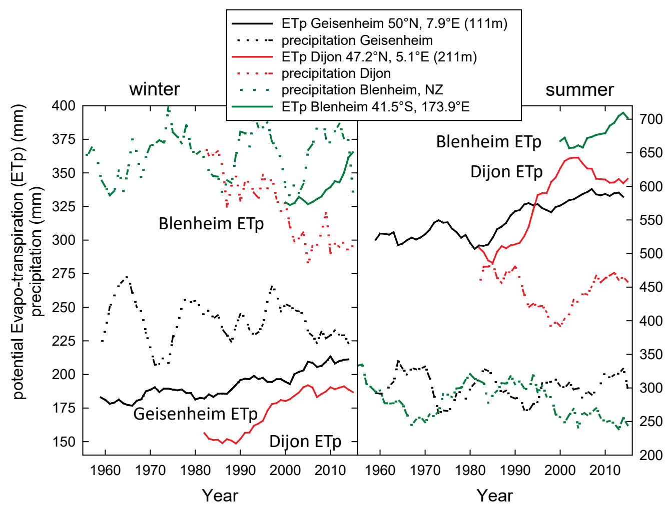

Figure 4. Observed precipitation and potential evapotranspiration for the winter (left panel) and summer periods (right panel) for Blenheim, New Zealand (41.5°S, 173.9°E), Geisenheim, Germany (50.0°N, 7.9°E) and Dijon, Burgundy, France (47.2°N, 5.1°E). Data show 10-year running mean values. Observed data were from the Marlborough Research Centre, New Zealand, Deutscher Wetterdienst, Germany, and the French INRA CLIMATIK, Agroclim database.

with no change or even a decline over the past 10–15 years (Figure 4). Roderick and Farquhar (2005) have reported decreasing pan evaporation for New Zealand and site specification might be decisive. Precipitation follows a cyclic trend in all regions and in all seasons with a strong decrease in winter precipitation in Dijon over the last 35 years (Figure 4 left panel). For the two other regions trends in precipitation are less obvious. In general precipitation and ETp are inversely correlated which would be according to theory (Farquhar and Roderick 2007) with the exception of Blenheim in the winter (Figure 4 left panel).

Conclusions

The data show that generalisations with respect to global developments are not possible and that each individual region needs to be analysed with respect to observed trends and also with respect to expected developments (Hofmann et al. 2014). The reasons for different developments in ETp seem to be complex and little understood. Trends might also be influenced by the drawing of moisture from water bodies which could balance the increases in temperature. According to the Budyko hypothesis, change in actual evaporation in dry regions is dominated by change in precipitation rather than potential evaporation. In humid regions, such as the three examples given here, the change in actual evaporation is controlled by change in potential evaporation rather than precipitation, which would mean that the development of water deficit will become more likely in the future. Of all regions analysed, none has shown a continued decrease in ETp or an increase in precipitation as observed for other parts of the world (Farquhar and Roderick 2007). Rising CO_2 concentration with its effect on stomatal closure and thus potential reduction in water use may also play a role in changes in the balance between precipitation and ETp (Gedney et al. 2006).

Acknowledgements

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Regional adaptation: alternative varieties in Australia

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Abstract

Careful consideration of what we grow, how we grow it and in which environmental conditions will be paramount for the future quality of Australian wine in the face of a changing climate. With almost all of Australia's 65 wine regions planted predominantly to a handful of varieties, there is much room for diversification. The industry can benefit by embracing broader plant material options which offer unique and symbiotic site/variety matching that can help growers buffer against adverse conditions, increase grape quality and reduce inputs required in both vineyard and winery. These varieties offer an alternative to the traditional international suite of French grapes that flourished in the New World in the 19th and 20th centuries. Some have been in Australia for decades but have not been widely cultivated, others have been introduced more recently. How and where we utilise these 'alternative' grape varieties to produce distinctive, quality wines will help define the style, reputation and sustainability of Australian wine in the 21st century.

Webcast of this presentation available at http://bit.ly/16thChalmers.

Introduction

The story of Chalmers and alternative grape varieties is a valuable one to consider because it can act as a precursor to wider adaption to climate change across the country. From our base in the warm, dry Murray Darling region, the Chalmers family have evolved from nurserymen to vignerons and eventually wine producers over the last three decades. Equipped with years of local agricultural experience and natural 'green thumbs', Bruce and Jenni Chalmers thoroughly understood the area and how to get the best out of the soils and environment in terms of primary production. Working predominantly with traditional international varieties in their earliest viticultural pursuits, Bruce and Jenni adapted their cultural techniques to achieve maximum sustainability and optimum quality. The combination of these techniques allowed Chalmers Vineyard in Euston, NSW to grow commercial crops averaging 25 tonnes per hectare with less than four megalitres of irrigation per hectare per annum. When combined with annual rainfall in a very dry year such as 2006-2007, the total water received by the vineyard for the year (July to June) was just 478 mm, about the same as a dry-grown vineyard in Santorini, Greece (Climate: Thira).

Technical viticultural management strategies and smart vineyard architecture are two ways to improve quality and efficiency but consideration of which varieties and clones to grow is another. Due to the nursery arm of the business, the Chalmers vineyards at Euston included many different clones and varieties, both of more commonly cultivated French varieties and a large collection of Italian varieties that Chalmers imported directly. The 35 different varieties and 88 unique clones imported are indigenous to various parts of Italy from north to south, many had evolved over hundreds of years to perform in specific conditions. The majority of these varieties were established in the early 2000s and began bearing in 2004, right in the middle of the millennium drought. Full maturity analysis was collected and flavour tasting in the field was undertaken on every selection; some of the results were very impressive. This is the process by which varieties were selected to be vinified. Crafting and evaluating wines was the logical next step in testing the success of these grapes in their new surroundings.

What are we looking for in a grape variety?

We are seeking attributes that can help with adaption to generally drier and warmer conditions as well as extreme weather events such as prolonged heatwaves or intense summer rainstorms.

There are thousands of known *Vitis vinifera* varieties across Europe, only a fraction of which are utilised in wine production across the globe today. Those varieties have adapted and evolved over many years to a multitude of different conditions in a wide variety of regions, soil types and climates. This has created great diversity in vine stocks. This vast botanical resource provides many options for commercial use in Australia: we only need to work out which ones will best suit our own individual backyards.

At Chalmers we were well placed for experimentation because of the nursery source block. Unlike most adventurous winegrowers having to start from scratch, choose a variety then source and plant it, we had a whole host of varieties and clones already established and were able to simply choose the highest quality grapes with the best analysis and flavours to make wine. Over the years of trials, through drought and downpour, we have refined a list of grape characteristics that we think are important for quality wine production in a future affected by global warming.

Drought tolerance

Of course drought tolerance is important to consider for both irrigated and dry-grown vineyards. Dry-grown vineyards with no access to water will be forced to adapt very quickly with no means of supplementing decreasing natural rainfall. All of our research has taken place in irrigated vineyards. There are two main reasons to decrease the need for irrigation water in a warm to hot climate: to reduce pressure on natural water resources; and to reduce costs.

Understanding a vine's ability to grow using minimal water, without resulting in collapse of the plant or fruit in hot conditions, is key to making smart vineyard planting decisions as future water availability and natural rainfall will vary due to climate change.

Heat tolerance

Heat tolerance is as important as drought tolerance. Because they are linked they are often considered together as one attribute but it is important to understand the difference.

Drought tolerance is the vine's capacity to withstand water stress. Heat tolerance is the physical resilience of the grapevine to extreme temperatures, that is the ability of the vine to withstand a heat spike or heatwave without the foliage or fruit collapsing. Because hydration of the plant is linked to heat tolerance, of course drought tolerance is related, but there are other physical factors which affect the ability of a vine and its fruit to withstand extreme heat:

- Large berried varieties fare well because they don't desiccate as readily
- Thick-skinned and loose-clustered varieties can generally better withstand heat

- Some varieties don't sunburn as readily as others
- Transpiration rate and canopy characteristics also vary.

Disease tolerance

The ability of a vine and its fruit to tolerate disease can partly be manipulated by vineyard management practices but it is also governed by genetic vine attributes. Disease tolerance in *V. vinifera* varieties is generally due to the following factors:

- Thick skins to protect the berry
- Loose bunches to allow airflow
- Open canopy to allow airflow
- Bunch positioning to avoid crowding.

Late ripening

Varieties that have long vegetative cycles and ripen late, well into autumn, are an advantage in warmer conditions for several reasons. They can help reduce the 'compressed vintage' effect which is creating massive pressure on winery infrastructure and logistics across the country. Late ripening varieties can also avoid damage from the extreme heat spikes and heatwaves of summer as fruit is still quite immature at this early stage and not so delicate. Ripening in the cooler autumn months can also result in a better balance between sugar and phenolic ripeness and more complex, less alcoholic wines.

Early ripening

One way to avoid heat damage is to harvest before the advent of extreme weather. Very early ripening varieties can avoid much of the summer heat or unseasonal rain storms, retaining freshness and remaining clean at the time of picking. Early ripening varieties can help alleviate compressed vintages and also improve cash flow by offering the opportunity for production of early release wines.

High natural acidity

In terms of wine quality this is very high on the list of favourable attributes. The main negatives of producing wine in hot climates are overripe, jammy fruit and high alcohol. Choosing a variety which naturally retains acidity through to harvest, even in the hottest conditions, will hold fruit in better condition as it approaches phenolic maturity and yield much more vibrant and balanced wines. In many cases, pH adjustment in the winery by acid addition is not required.

Rootstock

It is also important to consider the characteristics of the rootstock to which the *vinifera* scion is grafted because rootstock can affect ripening time, drought tolerance and yield/canopy management for disease resistance.

Which varieties are stand-out performers?

Since 2003 Chalmers have vinified most of the varieties imported, either through our commercial brands or experimental winery. All varieties have been grown in the Murray Darling region, and a selection of 25 of them in Heathcote. Our 13 vintages have included the second driest year ever recorded (2006: 123 mm, Mildura) and the two wettest years ever recorded (2010: 591 mm and 2011: 657 mm, Mildura) (Australian Bureau of Meterology). The following are a selection of the varieties which have consistently performed the best in the field and produced the best quality wines.

Fiano

Fiano (Figure 1) is the top performing grape in both our Murray Darling and Heathcote, Vic. vineyards in almost every vintage. Originating from Avellino in Campania, this variety is generally grown at 300–600 m altitude in its home region where summers are warm (22.6°C mean July temperature) and relatively dry with cool nights. Most of the 775 mm annual rainfall (Climate: Avellino) falls from November to April. It is also planted in Puglia and Sicily at low altitudes with some success.

In both Murray Darling and Heathcote, Fiano is naturally quite balanced in terms of canopy to crop ratio. It is planted at 2,222 vines/ha in the Murray Darling with two bilateral spur-cordons on two wire vertical trellis and 4,545 vines/ha in Heathcote in a single unilateral arched-cane VSP set-up. It adapts very well to both scenarios and soil types which seems to indicate that it is very adaptable to most situations. The fruit can be sensitive to sunburn if it is overexposed in extreme heat conditions but the canopy is upward and outward growing so it usually shades itself very well. Fiano is a very hardy variety with thick skins and good disease tolerance. In the extremely wet vintage of 2011 we had a parcel of Fiano fruit allocated to a customer who didn't want to harvest it until very late. It was left on the vine for a further four weeks past the harvest date of our own grapes and suffered no ill-effect due to disease despite the last fungicide application being several months prior. Furthermore, there was little change in either sugar concentration or titratable acidity during that time.

Table 1 shows compositional data for the 2015 vintage.

Fiano's naturally high acidity means that it often requires no acidification in the winery, even from the Murray Darling site. We believe this is a contributing factor to the quality of Fiano wines from warmer sites. It also has relatively small berries for a warm climate grape so the intensity of flavour in the juice is very good. It's a real winemaker's grape with the ability to be made into many styles from lean, racy, tank fermented young release styles to barrel fermented and aged wines. It also responds well to skin contact. Chalmers have made Fiano since 2005 and at a recent vertical of all vintages from both Murray Darling and Heathcote the wines showed extremely well, proving the propensity for ageing of Fiano is also very good. At Chalmers we also make a sparkling wine from the variety which requires no acidification or dosage to achieve perfect balance.

Figure 1. Fiano

Table 1. Compositional data for Fiano from the 2015 vintage, Chalmers vineyards

Heathcote	30/1/15	6/2/15	9/2/15	12/2/15	16/2/15
Baumé	11.3	11.8	12.2	12.6	13.1
pH	2.90	2.99	2.95	3.29	3.34
TA		13.3	13.2	9.0	8.2
Murray Darling	**19/1/15**	**27/1/15**	**5/2/15**	**9/2/15**	**12/2/15**
Baumé	10.8	12.0	12.7	13.2	13.3
pH	3.33	3.36	3.47	3.52	3.55
TA	7.8	7.0	6.0	5.8	5.9

Vermentino

Vermentino (Figure 2) is an Italian white variety whose most famous production area is Sardinia. It is also cultivated in Liguria and parts of Tuscany in Italy, and Provence and Corsica in France where it is known as Rolle. In its Italian homeland it has only one area of Denominazione di Origine Controllata e Garantita (DOCG) status in Gallura on the northern tip of the island of Sardinia, a mountainous region stretching south and inland from the coast with an elevation up to 1,362 m. The other regions in Sardinia that grow Vermentino are lower in elevation and sometimes very close to the sea. Although the summers in Sardinia can be quite arid with little or no rainfall from June to August, vineyards there have been traditionally un-irrigated although most now have 'emergency' irrigation for extreme events.

This variety made quite a splash when Chalmers produced the first Australian wine from the variety in 2004 under the Murray Darling Collection banner. Since then it has been widely taken up with 92 producers in Australia making Vermentino wines in 2016 (ANZ WID 2016). At Chalmers vineyards Vermentino grows as double bilateral spur-cordon at 2,222 vines/ha in Merbein and 4,545 vines/ha on single unilateral spur-cordon VSP in Heathcote. A hot climate variety with medium consistency of skins, it can be sensitive to disease in humid conditions so good airflow and open canopy are important. With large bunches and big berries it can withstand heat well. Some bunch exposure to achieve the trademark golden blush is desirable and a good indication of maturity. If night-time temperatures are elevated it can rapidly drop acidity as it approaches maturity.

Table 2 shows compositional data from the 2015 vintage.

What is impressive about Vermentino is its ability to produce fresh, chalky and structured wines in warm conditions and at commercially viable crop levels. In fact, a decent crop load on the vine usually gives better balanced fruit. Vermentino can reach full flavour development at relatively low sugar levels and usually holds its acid reasonably well. Harvest timing is very important with Vermentino as early harvest (10-11.5°Baumé) usually gives lean, 'lemony' wines while later harvest (12+°Baumé) gives more 'ripe stone fruit' notes. The classic characters of Vermentino wine are 'sea-spray' and 'dried herbs'. Vermentino wine is very suited to the Australian summer lifestyle. It is a good alternative to some of the traditional French varieties grown under hot conditions and produces a far more consumer-friendly and environmentally responsible product: a great choice for inland grape-growing for premium wines.

Figure 2. Vermentino

Table 2. Compositional data for Vermentino from the 2015 vintage, Chalmers vineyards

Heathcote	6/2/15	9/2/15	12/2/15	16/2/15	27/2/15
Baumé	11.0	11.4	11.6	12.4	13.6
pH	3.11	3.15	3.2	3.22	3.36
TA	11.0	9.8	7.7	7.3	7.1
Murray Darling	**19/1/15**	**27/1/15**	**5/2/15**	**9/2/15**	**16/2/15**
Baumé	9.1	10.4	11.5	12.5	13.0
pH	3.378	3.42	3.52	3.67	3.72
TA	9.1	6.7	5.7	5.2	5.2

Aglianico

Aglianico (Figure 3) grows well and makes fantastic wines at both the Murray Darling and Heathcote sites. A native of Campania, from a similar area to Fiano, it also grows in Basilicata further south. At home on volcanic hillsides, the highly tannic and acidic grape makes structured wines suited to ageing and is considered the most noble of all southern Italian red wine varieties.

Aglianico has a very long vegetative cycle: this can be a problem when it is grown on elevated sites that can have snow in early November. The variety has adapted well to drier conditions in Australia, although it can show some signs of suffering in prolonged extreme heat or drought. The variety is very tolerant of powdery mildew but susceptible to downy; and also sour rot in wet years so it is best grown in a system with good ventilation. Chalmers are growing Aglianico on single cordon VSP in both locations, and permanent cordon/spur pruned. Heathcote is 4,545 vines/ha and 225 m elevation in complex rocky Cambrian-era soil, Merbein is 2,222 vines/ha and 50 m elevation with red sand over limestone.

Table 3 shows compositional data for Aglianico for the 2015 vintage.

Currently we are still selling a 2005 Chalmers Aglianico wine which was produced from fruit grown in the Murray Darling region, in a hot dry year; even after a decade in bottle it looks amazing. It is light bodied but with good structure and acid to hold it in condition. More recently we have made more fruit-forward, young drinking examples from the Merbein vineyard as well. What the wines have in common is a lightness, elegance and freshness not usually associated with hot climate reds. Even the Heathcote wines are medium bodied at best but complex and full of interest and multi-layered. The potential for

Figure 3. Aglianico - Heathcote

Table 3. Compositional data for Aglianico from the 2015 vintage, Chalmers vineyards

Heathcote	25/2/15	4/3/15	11/3/15	24/3/15	6/4/15
Baumé	11.0	11.4	11.6		13.5
pH	3.08	3.34	3.34		3.54
TA	11.7	8.4	7.7		7.4
Murray Darling	**3/2/15**	**12/2/15**	**16/2/15/**	**19/2/15**	**1/3/15**
Baumé	10.10	11.5	11.6	11.7	13.5
pH	3.26	3.42	3.51	3.59	3.92
TA	9.63	7.1	6.6	5.7	5.2

this variety to make the kind of reds we typically associate with cool climates, but in hot areas, makes it a good choice for future viticulture in a warmer Australia.

Nero d'Avola

Nero d'Avola (Figure 4) was imported into Australia by Chalmers in 2000. Cultivated in inland hot regions since 2005, and becoming more widely planted in the last few years, this variety is already gathering momentum. A Sicilian native from the south-east of the island, it is used for producing all kinds of wines from rosé to heavy oaked reds and everything in between. The best wines come from around Vittoria where the Cerasuolo di Vittoria DOCG region lies. Vittoria is characterised by red soils and limestone so the adaption of this grape to inland viticulture seems logical, although it is grown in other parts of Sicily too. More elevated rocky sites are said to give structure to the wine while the sandier sites are said to produce fruitier and more transparent wines.

Nero d'Avola is drought tolerant and is mainly grown unirrigated in Sicily despite the low summer rainfall. However, the foliage and fruit can suffer from prolonged extreme heatwaves. Downward growth habit of the canopy, in combination with the potential for large bunches and high yields resulting in a congested fruit zone, can create a disease risk in wet seasons so irrigation needs to be handled carefully. It prefers short pruning in a reasonably expanded training system. Chalmers grows Nero d'Avola at both vineyards; in Heathcote it is planted at 4,545 vines/ha and is VSP trained to a unilateral cordon which is spur pruned to one bud. In Merbein it is grown in a more commercial style at 2,222 vines/ha on a two wire vertical spur/cordon system. Keeping the yield and vigour in check is the most important management tool with Nero d'Avola. Being a moderately late ripener, too much crop will hold back maturity.

Table 4 shows compositional data for Nero d'Avola for the 2015 vintage.

Figure 4. Nero d'Avola

Table 4. Compositional data for Nero d'Avola from the 2015 vintage, Chalmers vineyards

Heathcote	16/2/15	25/2/15	4/3/15	11/3/15	18/3/15
Baumé	11.5	12.4	12.8	13.4	13.7
pH	3.04	3.22	3.51	3.60	3.55
TA	9.3	8.6	7.2	6.9	6.5
Murray Darling	**9/2/15**	**9/2/15**	**12/2/15**	**16/2/15**	**19/2/15**
Baumé	10.0	11.3	11.6	11.7	12.0
pH	3.41	3.51	3.7	3.70	3.79
TA	8.1	7.1	7.6	6.9	5.7

Nero d'Avola can reach phenolic ripeness at reasonably low Baumé giving the opportunity to produce fresh and vibrant wine styles. The flavour profile of the grape is interesting with notes of 'cherry', 'cola', 'tar' and 'dried herbs'. The most attractive wines are made in a clean, fresh approachable style but well made examples can age in the mid-term. This variety is well adapted to hot climate viticulture with excellent drought tolerance and low Baumé at maturity. The potential for more heavy downpours in summer as a consequence of the changing climate could be detrimental to Nero d'Avola and would need to be considered in the management strategy. As a wine it makes wonderful rosé and excellent medium bodied, savoury reds so it is also on-trend for what the modern consumer is drinking.

What next?

In terms of homoclime data, all of the four varieties described above make perfect sense for Australian warm to hot climate grapegrowing. Of course homoclime research is a logical basis for planting decisions about alternative varieties. Growing Sicilian varieties in the Murray Darling, or growing Friuli varieties in Mornington Peninsula, seems to make perfect sense. But one of the most interesting and exciting things we have learned from our trials is that this is not always true.

Take Schioppettino, for instance, a rare red grape from the north-eastern Italian region of Friuli Venezia-Giulia. Schioppettino (Figure 5) is cultivated mainly in one valley in the region of Prepotto where the annual rainfall is 1,248 mm, average summer temperature is 22.1°C and average winter temperature is 3.4°C (Climate: Prepotto)[1]. In Italy the high acid, late ripening grape can give herbal and hard edged wines in cool years. The important factors in this variety's ripening are exposure and warmth during summer to achieve ripeness before the cold wet autumn sets in – transpose that to inland Australia and it produces delicious wines where summer warmth offers great ripening conditions, a drop in temperature at night provides relief, hotter conditions bring forward grape maturity which means the grape is less susceptible to prolonged heatwaves and drought. While in Italy they favour an oaked aged wine for this grape, we have found the soft tannin and bright acid to be fantastic for making a spicy, vibrant, young drinking red. The low alcohol of Schioppettino wines is seen as a negative in Italy while the health debate and consumer trends demand lighter wines here. On paper it wouldn't seem appropriate but Schioppettino is making some fantastic wines at both our Merbein and Heathcote sites.

Another exciting variety is Malvasia Istriana (Figure 6), a semi-aromatic white variety from Friuli. It is from a climate of warm summers and high annual rainfall (1,186 mm) (Climate: Gorizia) with rain events spread right throughout the year including frequent thunderstorms in summer. Proximity to the Adriatic also stabilises the weather with a maritime effect in many vineyards, so dry inland

Figure 5. Schioppettino

Figure 6. Malvasia Istriana

Australia hardly seems the place for Malvasia Istriana to thrive. The texts say it prefers wide spacing and long cane pruning but at Chalmers in both Merbein (1,222 vines/ha) and Heathcote (4,545 vines/ha) we have had success on spur/cordon, especially because of the ability to reduce the yield by leaving fewer buds. But the wine is the most exciting part of the story. Malvasia Istriana makes a fresh, light and aromatic wine with 'apple' and 'citrus' notes plus aromatic spices such as 'cardamom', 'clove' and 'bay'. The drier warm conditions here result in early harvest. Also, the wine can be released young.

Both these varieties have huge potential for small- to medium-sized wine producers, particularly given current on-premise wine drinking trends which align perfectly with the styles. But perhaps the most exciting lesson to come from all this is that thinking outside the square opens up the door to so many new options. There are many opportunities to find varieties which can fit perfectly with all the unique combinations of soil, climate, exposure and rainfall that we have in our winegrowing areas to create a new generation of regional Australian wines that broaden our offering and express each region more uniquely.

At Chalmers we have made a commitment to continue to explore the varieties of Italy and to import promising varieties to Australia. In 2015 we received the mother plants out of quarantine for 10 new varieties, mostly white, which we selected for import in 2011: Ansonica, Pecorino, Falanghina, Verdicchio, Grechetto, Ribolla Gialla and a new clone of Vermentino from Liguria (whites); Nero di Troia, Teroldego and Piedirosso (reds). In the next few years we will be putting these varieties through their paces in our vineyards and winery as we learn all we can about how they might perform in our backyard. The aim is not only to make the best wines we can with the lightest environmental footprint possible, but to inspire others to do the same and offer growers and winemakers across the country the opportunity to also find the grapes that best fit their terroir, ideals and palates.

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Practical options to manage vintage compression

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Abstract

Recent vintages have been characterised by rapidly maturing fruit and the compression of the ripening window, with varieties that used to ripen over 4–6 weeks in the 1990s, now maturing over a much shorter time period. This places significant pressure on harvest logistics, with growers struggling to find sufficient harvesters and wineries being forced to delay harvest due to a lack of fermenter capacity. Delays in harvest may result in yield loss due to berry dehydration, elevated sugar concentration/potential alcohol, negative impacts on fruit composition and undesirable changes in wine style.

A range of management practices are available that offer the potential to delay and spread fruit maturity. These include:

- *double pruning – where conventionally winter-pruned vines are pruned for a second time between 20 and 35 days after flowering and develop a new canopy*
- *delayed pruning – where the vines are pruned once at or shortly after budburst, effectively delaying budburst by up to three weeks and maturity by up two weeks*
- *applying plant growth regulators (especially auxins) prior to veraison to delay the start of the ripening process*
- *reducing the leaf to fruit ratio by trimming (or leaf plucking) to delay veraison and slow sugar accumulation in the fruit*
- *inhibiting photosynthesis through film-forming agents to block the stomata, resulting in a delay in veraison.*

The opportunity presented by each of these management techniques is reviewed in terms of their ability to modify maturity and their subsequent effects on grapevine productivity and fruit/wine quality and style.

Webcast of this presentation available at http://bit.ly/16thPetrie.

Introduction

Shifting phenological development is the most conspicuous biological effect of recent warming, with advanced maturity of grapevines being reported for Europe, North America and Australia (Duchêne et al. 2012; Petrie and Sadras 2008; Wolfe et al. 2005). Between 1993 and 2006, maturity of grapevines in Australia advanced between 0.5 and 3.0 dy^{-1} or 9.3 ± 2.67 $d°C^{-1}$ across a range of regions (Petrie and Sadras 2008).

Warmer temperatures and an advancement in maturity potentially impact on fruit quality and wine style, often causing 'unbalanced fruit' where high sugar levels are reached before optimum colour (and potentially flavour) development has been achieved (Sadras and Moran 2012). Associated with the advancement in maturity there have also been anecdotal reports of compression of the harvest period, with different varieties grown in the same region now reaching optimal maturity at similar dates and a narrower peak period over which a single variety matures (Coulter et al. 2015). Given the capital-intensive nature of the wine industry, climatic drivers that compress harvests have the potential to affect financial viability.

Vintage compression

To date the anecdotal reports of more compressed vintages have been difficult to validate and quantify. However, the analysis of commercial maturity data, dating from 1995 to 2014, from a major Australian wine company offered the opportunity to investigate these reports further. The sugar accumulation of individual blocks (based either on grower-reported values or samples delivered to the winery maturity analysis laboratory) across a region was tracked and the date when each block reached a total soluble solids (TSS) of 12°Baumé was interpolated from the maturity samples collected immediately above and below this level. Basing maturity assessment on TSS as opposed to date of harvest gives a more accurate assessment of the impact of climate on fruit maturity as it is independent of human decisions. These decisions can be influenced by other factors such as target wine style or winery capacity.

Results of this analysis showed a continuation of a trend first reported by Petrie and Sadras (2008) and Sadras et al. (2014a). At McLaren Vale, for example, the average date that Chardonnay reached 12°Baumé has advanced at 1.3 days per year and Cabernet Sauvignon has advanced at 1.9 days per year (Figure 1). Analysis of the longer data sequence highlighted not only the advancement in maturity, but also that in many regions the later varieties (i.e. Shiraz and Cabernet Sauvignon) were advancing in maturity at a faster rate than the earlier ripening Chardonnay. This means that times of maturity for Chardonnay and Cabernet are converging. In the early 1990s the range in dates between peak maturity of these two varieties in McLaren Vale was just over 20 days; it is now averaging closer to five days and the Shiraz also needs to be processed during this period (Figure 1). Note that while McLaren Vale was used in this example, due to there being sufficient data covering the three major varieties, a similar trend was observed across many Australian regions.

Individual varieties are also reaching maturity over a shorter period within one region. Using the same approach described above

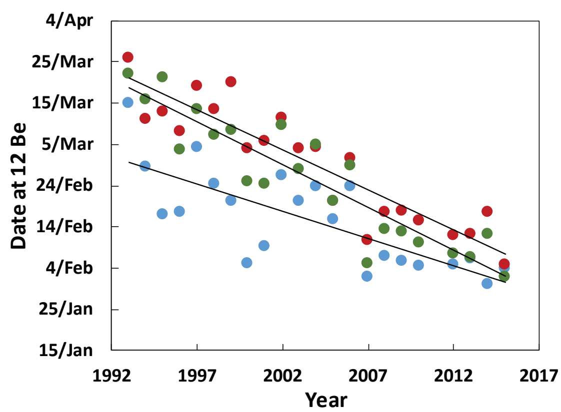

Figure 1. The advancement in the date at which 12°Baumé was reached for vineyards in McLaren Vale. Chardonnay – Blue; Cabernet Sauvignon – Red; Shiraz – Green

we tracked the time it took for the Shiraz blocks across the Barossa region (both Barossa and Eden Valley) to reach maturity (12°Be). During the late 1990s the bulk of the Shiraz harvest matured over a 30-day period; but this reduced to a 15-day window by the mid-2010s (Figure 2). Once again this increases pressure on vineyard and winery infrastructure. While the results are not presented, in the Barossa the shortening of the vintage period has also occurred gradually over time.

This analysis does not allow separation of the effects of warming and changes in management practices. However, there have not been step-changes in management during the study period; reduced yield is often suggested as a driver of earlier fruit maturity (e.g. Pearce and Coombe 2004) but there were no consistent yield trends observed across the regions included in this study. Regardless of the causes, the advancement in maturity and reduction in the duration of the window of peak maturity illustrate the challenges faced by wineries to process fruit over a shorter and more intense period.

Options to delay and spread fruit maturity

A range of viticultural practices is needed to counteract warming effects on vine development and berry attributes; these techniques aim to delay fruit maturity to a more familiar (later) part of the season. Obviously, the primary concern of growers and wineries would be the impact of these management practices on fruit quality, which has been assessed for several of the techniques. If the management practices are applied to a portion of vineyard, additional benefits may include an improvement in harvest logistics as the peak of fruit maturity can be spread over a longer period. Extending the maturity period for red varieties, even by a week, may allow an extra red fermentation cycle to be completed thereby improving the utilisation of red fermenters. The risk of yield or quality loss to inclement weather conditions can also be reduced due to spreading of sensitive stages of development. This insulates against the whole vineyard being affected by a heatwave at flowering or rainfall immediately prior to harvest.

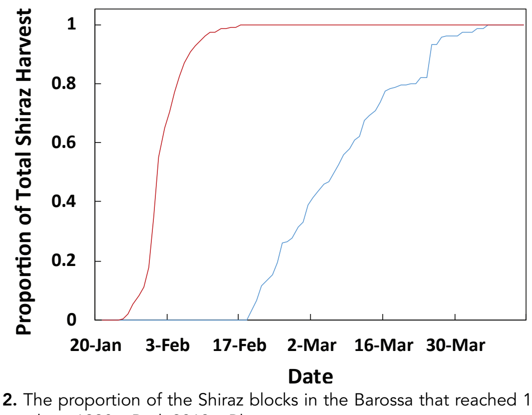

Figure 2. The proportion of the Shiraz blocks in the Barossa that reached 12°Baumé on a given date. 1998 – Red; 2013 – Blue

Figure 3. Shiraz vines soon after double pruning in the Barossa Valley. Photo courtesy of Martin Moran, SARDI

Double pruning

Double pruning involves pruning the vines in winter as per normal practice, and for a second time once the canopy has developed in late November or early December (Figure 3) when the new season's shoots are large enough to grow and support a new canopy and the buds well enough developed that the inflorescence primordia have initiated (i.e. when this bud breaks the shoot will carry a bunch). This is normally when the shoots are 20–25 leaves long (Dry 1987) or 20–35 days post-flowering (Gu et al. 2012). Lateral shoots and developing bunches need to be removed in the second pruning and care taken to ensure that no new laterals develop as these will inhibit the second budburst. Double pruning is currently being evaluated by Casella Family Brands in Riverina region (Andrew McLean, pers. comm. 2017) and was previously used commercially by Primo Estate in South Australia (Dry 1987). It has also been trialled successfully in the Central Valley of California (Gu et al. 2012). At Roseworthy, the harvest of Shiraz was delayed from mid-February for grapes pruned at the normal time through to mid-April and as late as mid-May depending on the date of the second pruning. Similarly, in the Central Valley of California, Cabernet Sauvignon maturity was delayed from late-August (equivalent to late-February in the southern hemisphere) through until mid- to late-October (equivalent to April) (Figure 4). Double pruning can have significant direct and indirect costs which include the repeated costs of pruning as well as a loss in yield of up to 60% (Dry 1987; Gu et al. 2012). Of all the practices that could potentially delay fruit maturity, double pruning has the potential to delay harvest by the most (over two months), thus limiting this practice to the hottest regions. At many sites this technique has the potential to delay maturity to the point where the fruit will not be ripe enough for harvest prior to the onset of winter.

Delayed pruning

Unlike double pruning, which requires vines to be pruned twice, delayed pruning delays maturity by pruning vines at or shortly after budburst. Delayed pruning was originally developed as a technique to help avoid frost damage (Friend et al. 2011) or to move flowering of vines grown in a cool climate into warmer conditions and thus improve yield (Friend and Trought 2007). It has also been successfully adapted to delay fruit maturity and spread harvest (Frioni et al. 2016; Petrie et al. 2017; Sadras et al. 2016; Sadras et al. 2014b). The later the vine is pruned the further that maturity is delayed. For Shiraz grown in the Barossa, pruning when the shoots growing from the tips of the canes are approximately 7 cm long (Figure 5) can delay maturity by approximately 2 weeks, while pruning at budburst can

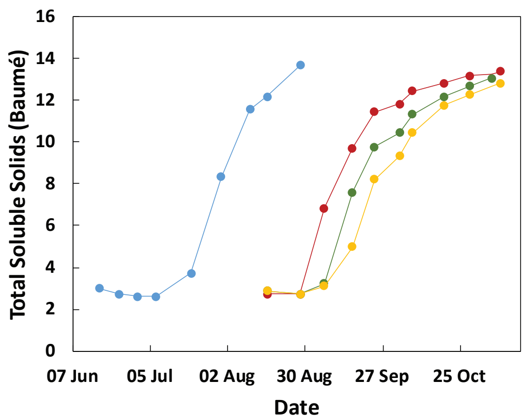

Figure 4. Sugar accumulation of Cabernet Sauvignon grapevines grown at Fresno, California, subjected to double pruning at a range of dates. Control (Blue), double pruned 21 days post-flowering (red), double pruned 28 days post-flowering (green), and double pruned 35 days post-flowering (black). Double pruning treatments had a significant effect on fruit sugar concentration ($p<0.05$). Figure adapted from Gu et al. (2012).

delay maturity by approximately one week (Figures 6 and 7). Delaying pruning significantly later than when the shoots at the tips of the cane are 7 cm long can reduce canopy development and yield. Tests with Barossa Shiraz showed late pruning had no effect on yield in four out of seven cases, increased yield in two and reduced yield in one (Sadras et al. 2016). Small-lot wines made from vines pruned during winter and when the shoots were 7 cm long were tasted as part of an industry workshop. At one site the wine made from the vines pruned during winter showed more intense colour, 'bitterness', 'earthy' and 'savoury' flavours than spring-pruned wines (Moran et al. 2015). At the second site wines made from the winter-pruned treatments showed less intense sweetness and ripe flavours but more acidity when compared to wines made from the vines pruned after budburst (Moran et al. 2015).

Figure 5. Shiraz vines shortly after delayed pruning when the shoots were 7 cm long (right) and the winter-pruned control (left)

Figure 6. Shiraz vines at veraison; winter-pruned control (left) and delayed pruning when the shoots were 7 cm long (right)

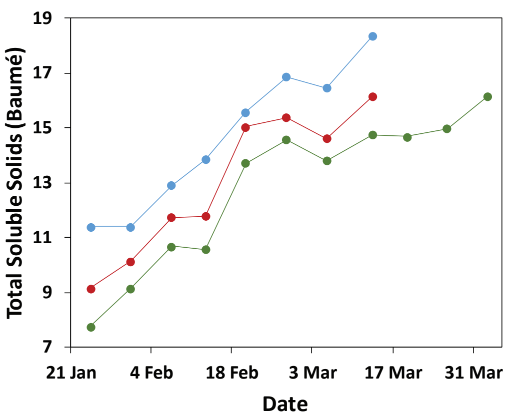

Figure 7. Sugar accumulation by Shiraz vines grown in the Barossa Valley, Australia, pruned during dormancy, at and shortly after budburst. Control (blue), pruned at budburst (red) and pruned when the shoots were 7 cm long (green). Delayed pruning had a significant effect on fruit sugar accumulation (p<0.05).

Plant growth regulators

Depending on the stage of development and the concentration, plant growth regulators can be used to advance or delay ripening of a range of fruit crops including grapes (Davies and Böttcher 2009). To delay grape maturity, the most success has been achieved by applying the synthetic auxin 1-naphthaleneacetic acid (NAA) prior to veraison (Figure 8) (Böttcher et al. 2011); however, other auxins (indoleacetic acid, IAA) and synthetic auxin analogs (e.g. benzothiazole-2-oxyacetic acid) are also effective. In trials fruit maturity has been delayed by more than 20 days with multiple applications of NAA (Figure 9). In some of the trials NAA applications increased average berry weight at the later stages of ripening and this would likely result in an increase in yield (Böttcher et al. 2011, 2012); similar trends have been seen with other fruit crops where NAA has been applied prior to

Figure 8. Shiraz vines showing the contrast in maturity between the control (top) and pre-veraison NAA treatment (bottom). Photo courtesy of Chris Davies and Christine Böttcher, CSIRO and Treasury Wine Estates

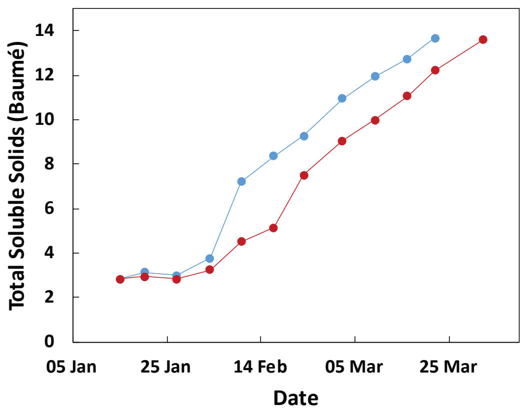

Figure 9. Delaying of ripening in Shiraz berries grown in the Adelaide Hills, Australia, resulting from pre-veraison treatment with NAA. Control (blue) treated with 0.05% Tween 20 solution and NAA (red) treated with 50 mg/L NAA in 0.05% Tween 20 solution. Delayed pruning had a significant effect on fruit sugar accumulation (p<0.05). Figure adapted from Böttcher et al. (2011).

the start of the ripening process. When trial Shiraz wines have been assessed from grapes with delayed maturity due to NAA treatment, either no impact on wine sensory properties was recorded (Böttcher et al. 2011) or a range of sensory attributes were affected including an increase in 'peppery' character in the wines (Davies et al. 2015). One of the advantages of using a plant growth regulator to delay maturity is that they can be applied relatively late in the season. This means the decision to delay maturity can be made once the seasonal conditions have been assessed. For example, in a cool season the plant growth regulator may not be used in some regions as there is a risk that fruit will not fully ripen. NAA is currently registered for use on apples and pears in Australia, for fruit thinning early in the season and to prevent fruit drop close to harvest, but not for use on grapes. Considerable trial work and residue studies would be required for NAA to be granted registration in vineyards.

Defoliation

The impact of canopy size (leaf area) has long been studied in the context of vineyard productivity and fruit quality; for example, the 7 to 12 cm^2 of leaf area that is reported to be required to adequately ripen a gram of fruit (Jackson and Lombard 1993; Smart and Robinson 1991). This has made manipulating the source (leaf area) to sink (crop size) ratio a strong candidate to delay fruit maturity across a range of environments (Parker et al. 2016; Whiting 2011; Bobeica et al. 2015). The expectation is that vines with a low source to sink ratio will show a delay in ripening (i.e. small canopies, large crops or both). A range of techniques has been used to defoliate the canopy, with trimming and mechanical leaf removal likely to be the most cost-effective. The defoliation is normally completed after fruit set, as earlier leaf removal is likely to reduce set and yield, meaning the desired change in the source to sink ratio will not occur. In an Australian context, Whiting (2011) looked at the effectiveness of leaf removal and trimming on Shiraz grown in Bendigo (Figure 10). Harvest was delayed by approximately 21 days (Figure 11) and there was a small reduction in yield. Small-lot wine was assessed informally: the winemakers involved had a strong preference for the control treatments (John Whiting, pers. comm. 2016). Two physiological mechanisms can compensate for small source to sink ratios: mobilisation of trunk and root reserve carbohydrates (Candolfi-Vasconcelos et al. 1994) and enhanced photosynthesis by the remaining foliage (Petrie et al. 2003). This means that the level of defoliation needs to be quite extreme (Figure 10) to be effective at delaying maturity; Whiting (2011) removed two thirds of the canopy. Removing a large portion of the canopy is also likely to affect the yield in the following year as the reserves that are used to ripen the fruit would normally be used to develop the fruit and canopy in spring (Holzapfel et al. 2006).

Figure 10. Shiraz vines with two-thirds leaf removal. Photo courtesy of John Whiting, John Whiting Viticulture

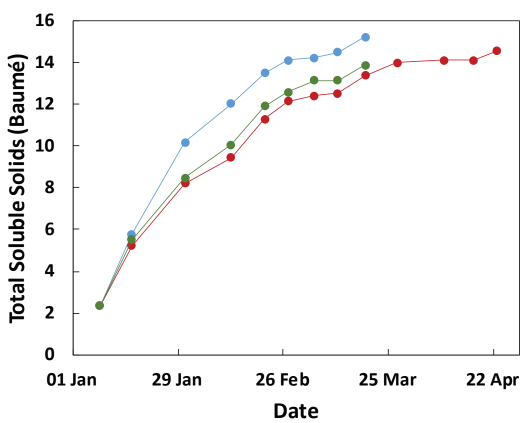

Figure 11. Sugar accumulation between fruit set and veraison in Shiraz vines grown in Bendigo, Australia in response to defoliation. Control (blue), defoliated by leaf removal (red) and defoliated by trimming (green). Defoliation had a significant effect on fruit sugar accumulation ($p<0.05$). Figure adapted from Whiting (2011).

Film-forming agents

While leaf removal reduces current vine photosynthesis irreversibly and may have some carry-over effect in the following season, a range of film-forming agents can be used to temporarily reduce photosynthesis. These techniques have the potential to delay maturity, while leaving the canopy in place to prevent fruit sunburn. The film-forming agents work by blocking the stomata (pores on the leaves) that regulate the flow of both carbon dioxide into the leaf (photosynthesis) and flow of water out of the leaf (transpiration) (Palliotti et al. 2013). The impact of the film-forming agents is temporary as they break down or are washed from the leaves. This means that multiple applications are likely to be needed to have the desired effect on fruit maturity. Di-1-p-menthene (Vapor Guard®) has been used effectively to delay the maturity of Sangiovese in Umbria, Italy (Palliotti et al. 2013) and Barbera, in Piacenza, Italy (Gatti et al. 2016) by at least 15 days (Figure 12). The impact of these treatments on wine style was not reported. High rates of horticultural oils, which are normally applied as part of a powdery mildew management program, can also reduce photosynthesis and delay fruit maturity (Finger et al. 2002), but when used at lower rates these are less effective. By reducing transpiration, this technique may prevent evaporative cooling and exacerbate leaf damage during heatwaves.

Conclusions

Warming conditions continue to promote the earlier maturity of wine-grapes both in Australia and internationally. These conditions

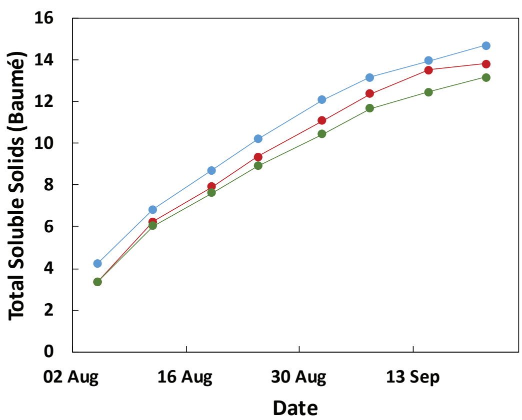

Figure 12. The impact of application of a film-forming antitranspirant on sugar accumulation by Barbera vines grown in Piacenza, Italy. Control (blue) anti-transparent applied pre-veraison (red) and both pre-flowering and pre-veraison (green). Application had a significant effect on fruit sugar accumulation ($p<0.05$). Figure adapted from Gatti et al. (2011).

also appear to be causing a compression of the harvest period which places considerable pressure on harvest and processing infrastructure. A range of techniques have been developed which show potential to delay fruit maturity and spread the harvest if they are only applied to a portion of a winery's intake. These techniques might involve trade-offs with yield, fruit composition and wine style and quality. Producers are recommended to try one or a range of these methods to see which is the best fit for their production system and are reminded that any impacts of delayed maturity on yield and quality will interact strongly with seasonal conditions.

Acknowledgements

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The changing landscape for sparkling wine production

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Abstract

Sparkling wine has a long history in Australia, dating back to the 1840s in South Australia and Victoria. Many brands that are still well known appeared from the 1870s onwards. These wines were generally made from grape varieties not noted for sparkling wines and grown in warm viticulture regions.

The real revolution in the quality of Australian sparkling wines commenced a century later in the 1980s, based on the planting of Chardonnay, Pinot Noir and Pinot Meunier in the emerging cool climate regions. The move to cooler sites was driven by the desire to improve wine quality rather than to compensate for a warming climate and remained so until the last decade. In the relatively short period of 40 years, Australia has proved very capable of producing very high quality sparkling wine and this wine sector has matured with the key producers having established distinctive styles for their respective brands.

The increasing commercial demand for cool climate sparkling wine has resulted in continued significant planting in many regions with progressively higher altitude and/or more southerly latitude. The establishment of vineyards in these cool climates does impart a level of business comfort based on the assumption that they are somewhat protected from climate change. However, climate change should not only be considered in terms of 'global warming', as the situation becomes far more complex when predicted increases in extremes of weather are factored in. Significant changes to vineyard resource requirements are also likely, with increased irrigation demand to be a major factor.

Both new and established sparkling wine regions across the world will be obliged to manage the effects of climate change by modifying their viticulture and winemaking practices in order to maintain their existing market identity. Major changes such as varieties and source region will be resisted to maintain current business models and subsequent evolution will progress over numerous decades.

Webcast of this presentation available at http://bit.ly/16thCarr.

Introduction

Firstly, I believe there is a need to clarify that this paper should not be viewed as a scientific research document but rather as the observations and interpretations of events past, present and potentially future of a sparkling winemaker. As a starting point it is appropriate to consider wine style and quality in terms of their intrinsic link to both terroir and winemaking. My preferred definition of terroir is from John Gladstones' second publication *Wine, Terroir and Climate Change* (2011), which is itself from the French writer Lalville (1990):

> That is the vine's whole natural environment, the combination of climate, topography, geology and soil that bear on its growth and the characteristics of its grape and wines. Local yeast and microflora may also play a part. (Gladstones 2011)

Therefore, it is reasonable to suggest that horticultural interventions—such as terracing, drainage, fertility and irrigation management—must be included as influencing factors, and hence overall terroir should be considered a combination of the natural and modified environment.

The winemaking processes are fundamental to the wine style but not intrinsically part of the terroir, so it is common to see different styles from the same varieties from the same region.

Terroir does not have any stipulation of area in its definition and hence may be measured in magnitudes of kilometers or meters depending on the approach of the producer or established regulatory governance.

In addition to the wine's character, terroir is often of great significance in the marketplace, that is, wines defined by origin are important to consumers as it suggests a style of wine (however broad that might be) but it does not always relate to quality in a totally predictable way.

The formal appellations of Europe are prime examples of the above, and now premium wines from around the globe commonly use 'sense of place' as a tool to define their wine's style and infer quality with the aim of improving their marketability in an ever more complex global market.

The production of Australian wine has followed the path of pioneers of viticulture and winemaking into new regions and grape varieties over a relatively short time frame; this can be divided into two distinct phases as follows.

Early history

Sparkling wine production commenced very early within Australia's history and can be traced back to the 1840s in New South Wales, South Australia and Victoria. Many of these ventures failed due to lack of funds, expertise and the use of unsuitable grape varieties and winemaking equipment. There was, however, a strong desire to make quality wine within the new colonies as can be seen in some current references, for example, from Smith (2007), *The Culture of the Grape Vine and the Orange in Australia and New Zealand* (Suttor and Jullien 1843):

> Some good wines are now made, they have a champagne, peculiar claret, sauterne, sherry and other light wines.

Another reference in the *Calcutta Englishman,* 25th May 1851:

> We hear that they have successfully imitated champagne and they must be sure to have a market for it, if tolerably good, in every quarter of the globe.

So the scene is set to pursue the production of sparkling wines and many ventures were to commence in the 19th century. Some examples of the more notable producers and their respective history are listed below. As previously mentioned, a lot of these had a short business life; however, many involved famous names and brands of which some are still relevant today (Smith 2007).

1840s	The Prospect Farm Vineyard produces sparkling wine (1847), in Tasmania.
1860s	Dr Louis Lawrence Smith establishes the 'Victorian Champagne Company' in Melbourne producing sparkling wines with labels such as 'Crème de Bouzy' and 'Perle d'Australie'.

1870s	Joseph and Henry Best commence winemaking in Great Western, Victoria.
	In South Australia, J.E. Seppelt, Thomas Hardy and Samuel Smith commence sparkling winemaking.
	Patrick Auld establishes the Auldana vineyard on the eastern outskirts of Adelaide and in 1842 shipped two pipes of Auldana base wine to Moet and Chandon, which was subsequently returned as finished sparkling wine and this was awarded first prize in its class at the 1881 Adelaide Wine Show.
1880s	Hans Irvine purchases Best's company in Great Western (1888) and later produces wine labelled as 'Special Reserve Champagne', 'Sparkling Burgundy' and 'Sparkling Hock'.
	Edmond Mazure becomes winemaker at the Auldana winery in Adelaide, South Australia.
1890s	'Minchinbury' is established at Rooty Hill in New South Wales, Leo Buring makes his first sparkling wine at this site in 1903.
	Thomas Hardy investigates the Penola district with the aim of producing lighter wines and concludes the district is highly suitable for producing excellent 'champagne'.
1910s	Hans Irvine sells to Seppelt and 'Great Western Champagne' becomes a significant commercial brand.
1920s	Edmond Mazure's 'La Perouse Cellars' is sold and changes its trading name to 'Romalo'.
	Hurtle Walker is appointed manager of Romalo in 1926.
1960s	Hurtle Walker retires and his son Norman takes over as sparkling winemaker and manager.
1970s	Romalo owned by Wynn Winegrowers is changed to the Seaview Champagne Cellars.

The grape varieties quoted for use for sparkling wine throughout the 19th century could be classed in modern terms as 'not fit for purpose' and in references to this time included Chasselas, Marsanne, Pinaeu, Peno Noire, Greenarch and Rheesling.

This approach continued for over a century through to the 1970s when a large range of commercially successful sparkling wines were made from varieties such as Palomino, Pedro, Grenache, Riesling, Semillon, Sultana, Trebbiano and Shiraz which were mainly grown in the warm viticulture areas.

The quality revolution – varietal and cool climate

Chardonnay and Pinot Noir did not appear in significant quantities in Australia until the late 1970s when there was a massive increase in planting across many regions to support both still and sparkling styles at most price points.

The national records show that from very humble beginnings in 1972/3 for Chardonnay (12 ha) and Pinot Noir (16 ha) that bearing area had grown to be in the order of 26,000 and 4,000 ha respectively by 2004/5 (Figure 1).

Although Australia had always revered Champagne as the global benchmark for premium sparkling wine, the local winemaking community had not taken the appropriate steps to move towards producing wines of comparable quality. The next significant change commenced from the 1980s with the development of vineyards planted to the proven varieties of Chardonnay, Pinot Noir and (to a much lesser degree) Pinot Meunier in the recognised cold climate regions.

In the earlier times of this development, the plantings were driven by the shorter-term business desire to make high quality sparkling wines and that consideration of climate change was significantly later.

Having stated that Champagne was considered the world quality benchmark, and assuming that climate is the primary factor of influence of terroir, then it would follow that the basis for the evolution of premium Australian sparkling wine would be vineyards of a similar temperature regime to Champagne.

There are two climate indices often used to assess the suitability of sites for viticulture. Both are calculated from temperature data over the seven-month growing period of their respective hemisphere (southern, Oct to Apr; northern, Apr to Oct).

- Heat Summation (Gladstones 1992, 2011) – a measure of the heat input to the vineyard over the growing season. There are numerous mathematical conditions and corrections used within the formula with the scale of results specified in 'Heat Degree Days' (HDD).
- Growing Season Temperature (Gladstones 1992, 2011; Jones 2006) – the average of the maximum and minimum temperature experienced by the vine throughout the growing season.

It is evident that both of these indices have varying degrees of support and criticism; however, they are essential tools for comparison between regions/sites.

One would expect regions with similar indices to Champagne (HDD, 1000/1200 or GST, 13.4/15.2) to be able to produce fruit suitable for the production of premium sparkling wine; a few examples are shown in Table 1.

The table is purposely not extensive as these are generalised data and hence I believe should only be used as a guide. As 'terroir' has no area definition, the examples indicate the potential of a region and assessment of individual sites will require multiple correction factors or weather recording stations on the actual site to ensure the most accurate data.

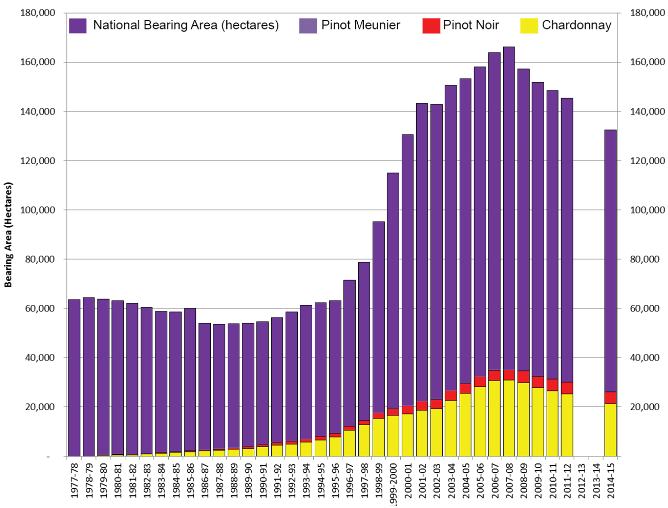

Figure 1. Bearing area (ha) of Chardonnay, Pinot Noir, Pinot Meunier and all varieties Australia, 1997/8 to 2014/15. Source: Anderson (2015)

Table 1. Heat Summation and Growing Season Average Temperature Indices for premium sparkling wine regions of France, England, New Zealand and Australia

Location	Temperature Summation (HDD)	Growing Season Mean Temperature (°C)
Reims (Champagne) – France	1191	14.7
Oxford – England	1061	13.5
Bushy Park – Tasmania	1097	14.4
Hobart – Tasmania	1158	14.5
St Helens – Tasmania	1264	14.8
Heywood (Drumborg) – Victoria	1159	15.6
Stirling (Adelaide Hills) – South Australia	1213	15.6
Blenheim – New Zealand	1265	15.8
Healesville (Yarra Valley) – Victoria	1352	15.9

Source: Gladstones (1992)

Reverting to the chosen description of terroir, being the combination of natural and modified environment, then there are many other factors that influence the terroir of a specific site which will affect the phenology of the vine and maturation of rate of the fruit:

Latitude

- at higher latitudes extended sunlight exposure with lower intensity due to oblique sun angle

Altitude

- with increasing altitude 0.6°C average temperature decrease for each 100 m
- and greater light intensity due to reduced air turbidity

Topography of vineyard and surrounds

- proximity to large bodies or water (continental or maritime) and the resultant impact on diurnal range
- slope, air drainage/cold air pooling
- aspect, incidence of sunlight/prevailing winds

Soil type

- energy reflective or absorbing types and respective impacts on day/night temperatures
- fertility effects for canopy density and hence fruit exposure.
- drainage and water holding capacity
- subsoils

Atmospheric conditions

- rainfall, frequency and volume
- cloud cover (light intensity and duration)
- humidity (water stress levels)

Vineyard management

- landscaping
- canopy structure/light infiltration
- water management, irrigation/drainage
- fertility
- crop yield

These factors do not act as separate entities and each specific site is a result of a single permutation of the vast number possible from the above. Therefore, it is not surprising that wines produced from all of the recognised premium regions show significant differences down to the level of individual vineyard patches.

There are many cool climate regions that have experienced extensive planting since the 1980s from which the following have been the most significant in driving successful premium sparkling wine brands:

- South Australia: Adelaide Hills
- New South Wales: Tumbarumba, Orange
- Victoria: Yarra Valley, King Valley, Alpine Valleys, Macedon, Strathbogie Ranges
- Tasmania.

Historical planting records are neither always available nor accurate (for example the Yarra Valley had significant areas planted from own cuttings in the 1990s rather than the more traceable figures for vine nurseries). However, data for Tasmania are more readily captured as it is classed as a single region (Australian GI) and may be used as an example of the industry focus on cool climate.

Over the last three decades the Tasmanian bearing vineyard area has increased from 47 ha in 1986 to over 1400 ha by 2015 with an estimate of varietal proportion being: Pinot Noir (41%), Chardonnay (18%), Sauvignon Blanc (17%), Pinot Gris (10%) and Riesling (8%) (Figure 2). Sparkling wine constitutes 35% of the total Tasmanian wine production volume (Wine Tasmania 2016).

Australian winemakers in general have not favoured Pinot Meunier in their sparkling blends and hence planting area for this variety is relatively small and often not reported. This is very much in contrast to Champagne where Pinot Meunier constitutes approximately 30% of production and is frequently a major component of the multi-vintage blends. It is difficult to explain the local reservation for the use of this variety but the opportunity still remains for its later inclusion.

Since the cool climate resource has become established the sparkling wine industry has matured as producers have developed their winemaking to build their distinctive styles across a range of price points and this now provides considerable choice to the consumer for premium Australian sparkling wine.

The consumption of sparkling wine in Australia has two distinct trends (Table 2).

A declining commercial (<$15 retail) sector: this is apparently due to the consumer moving to alternative wine types (Sauvignon Blanc, Pinot Gris/Grigio and Moscato) and other beverages of current popularity (cider and craft beer).

Significant growth of premium sales ($15 retail): this is driven by consumers seeking 'affordable indulgence' in the middle price points and those choosing the quality and sophistication at significantly higher prices (>$30). The continued strong growth of Champagne sales to 8.1 million bottles in 2015 (Lam 2016; Young 2016) highlights Australia's market demand for premium sparkling wine even in times that are considered financially difficult. The rate of growth of both domestic and imported premium sparkling wine sends a positive signal for future business opportunity.

The future and climate change

The current experience with changing weather conditions and the future prognosis of increasing global temperature will no doubt influence viticulture, and hence wine style, from the recognised regions and drive the establishment of new areas with cooler climates that are less influenced by these changes for the medium-term.

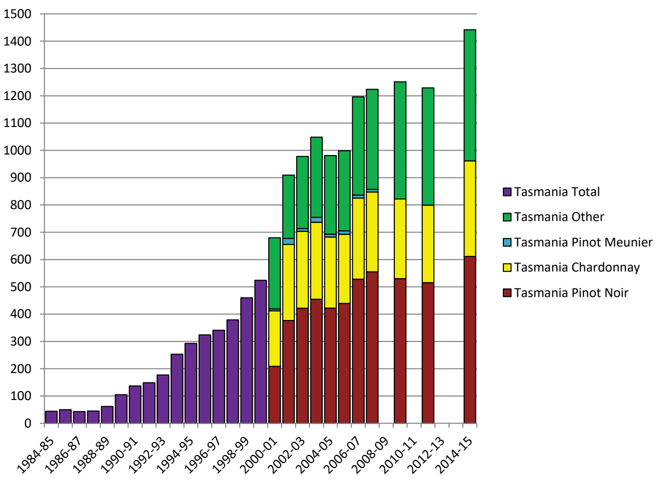

Figure 2. Bearing area (ha) of Chardonnay, Pinot Noir, Pinot Meunier and all varieties, Tasmania, 1997/8 to 2014/15. Source: Anderson (2015)

Table 2. A snapshot of the sparkling wine market

SPARKLING WINE PRODUCTION			
Global	211		
Champagne	24.5		
Australian	4.9		
AUSTRALIAN DOMESTIC MARKET			
(CAGR = Compound Annual Growth Rate)			
Australian Sparkling	3.7 (-2.45 CAGR 14/16)		
		Commercial <$15	3.1 (-4.4% CAGR 14/16)
		Premium >$15	0.6 (+10.1% CAGR 14/16)
Total Imports	1.5		
		Champagne	0.68 (+ 24% in 2015)

Sources: IWSR (2015); Bureau du Champagne Australia (2016); Wine facts – Wine Australia

Grape varieties can be grouped into preferred temperature ranges that will achieve perceived optimum maturity. One such analysis is shown in Figure 3.

The forecast temperature increase for the next decades is very significant when compared to the current varietal classifications which have a 2° to 3°C bandwidth, i.e. 0.2° to 1.1°C by 2030 and 0.4° to 2.6°C by 2050.

Presuming this will be an accumulative change over the course of time, i.e. 1.1°C by 2030 is in the order of 0.08°C per year and this gives the opportunity for the grapegrower to evaluate and activate options to offset this change, such as:

1. Modify vineyard management techniques
   - include or increase irrigation
   - increase canopy density to achieve additional shading of the fruit zone
   - review row orientation
   - apply solar reflecting products
2. Change grape varieties to suit the new temperature regime
3. Establish new vineyards in regions currently below the optimum temperatures for specific varieties, e.g. higher altitudes, southerly aspects (for the southern hemisphere).

It is really quite confronting to consider that the projected 1.1°C increase in the average temperature by 2030 requires approximately a 200 m increase in altitude (assuming 0.6°C/100 m) to compensate. This would hardly seem practical in many areas that either have no higher ground suitable for viticulture due to their low topography or the vineyards are currently planted near the top of the high country.

As previously mentioned, terroir gives wine a sense of place and perceived quality/style so I would fully expect that existing wines and brands will look to prolong this often hard-gained market presence.

The wine consumer is conservative and hence often resistant to change and, although new varieties and styles may be heavily promoted by the media as being exciting, the volumes and marketplace following is typically small.

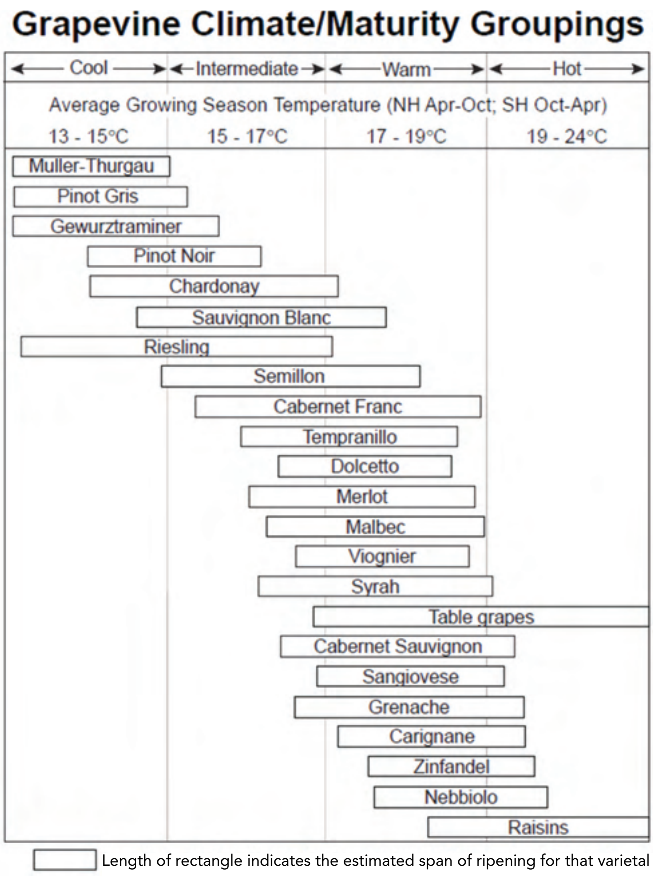

Figure 3. Grape variety maturity groups. Source: Jones et al. (2005)

Over history many wine types have built kudos and business models based on the current perception of their respective varieties and regions. There will be a great reluctance to change and hence modification of the current practices in both the vineyard and winery will be the initial response. This would include practices such as irrigation (if possible), canopy management and harvest. Changes of varieties and a regional shift (particularly where prescribed by regulation) are currently very difficult to contemplate in terms of the financial impact and market perception.

When you consider that a vineyard has a 5- to 10-year timeline to return development costs and generally a 50-year working life, I would expect changes to be measured in order to maximise the financial return of the existing development.

So, we are currently obliged to continue to work with the ever-increasing commercial difficulties of:

- Earlier and compressed vintages – it appears that one day earlier per year is generally considered the likely trend
- Generally higher water demand – current experience shows that some vineyards planted within the last 20 years have insufficient irrigation infrastructure and/or water supply to maintain a fully active canopy
- Increased extreme and unpredictable weather events giving greater variability between vintages and increased risk of failure.

Figure 4 shows the influence of increasing temperature on phenology in a vineyard in the upper Yarra Valley: over the last 20 years harvest has become approximately three weeks earlier.

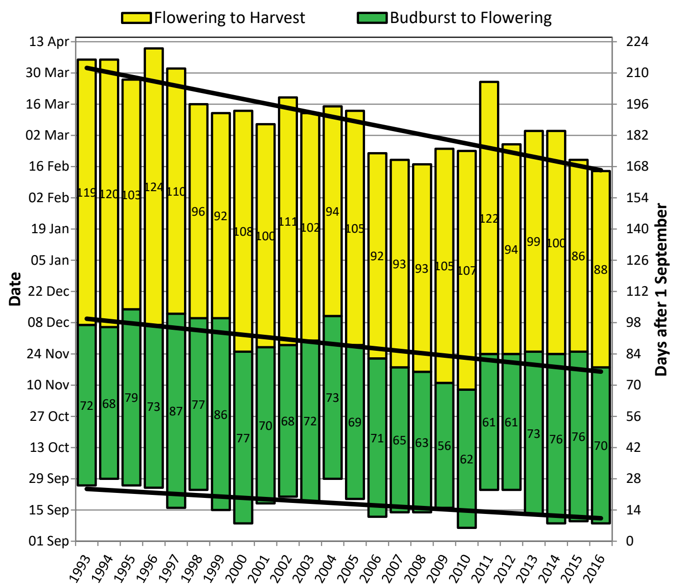

Figure 4. Phenological dates and duration for budburst to flowering and flowering to harvest; 1993-2016. Beenak vineyard, Yarra Valley (source: Accolade Wines)

Table 3. Heat summation and growing season average temperature of selected Australian regions

Growing Region	Heat Summation (HDD)	Growing Season Average Temperature (GST) (°C)
Tasmania	1158	14.5
Henty – Victoria	1159	15.6
Adelaide Hills – South Australia	1213	15.6
Yarra Valley – Victoria	1352	15.9
Padthaway – South Australia	1479	17.4
Barossa Valley – South Australia	1486	17.6
Clare Valley – South Australia	1613	18.4
Riverland – South Australia	1745	20.1

Source: Gladstones (1992)

Considering the current range of sparkling wine made from Pinot Noir and Chardonnay grown within the broad range of climate in south-eastern Australia, we have a clear picture of the influence of increasing temperature on this wine style. Although these varieties have optimal Growing Season Average Temperatures in the order of 14°C to 17°C for premium sparkling wine, they are obviously able to grow in all the regional examples in Table 3 and produce commercially successful wines. Therefore the changes in style that will result for each region due to increasing temperature is broadly indicated by regional products that are currently on the retail shelf. Applying the projected temperature increase directly to the GST is most likely a sound estimate of the style change, i.e. 0.6°C to 1.1°C by 2030 and 0.4°C to 2.6°C by 2050.

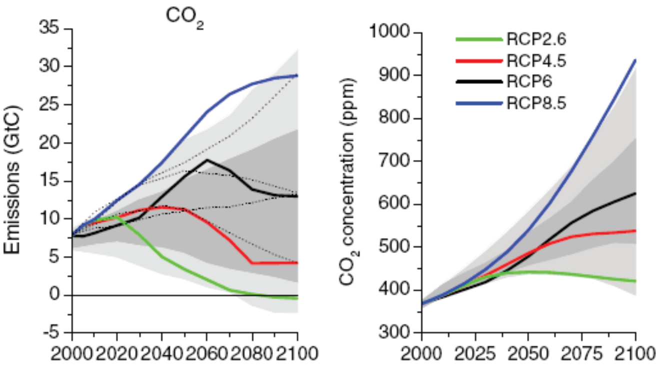

Figure 5. Emissions of CO_2 across the RCPs (left) and trends in concentrations of carbon dioxide (right). Grey area indicates the 98th and 90th percentiles (light/dark grey) of the values from the literature). The dotted lines indicate four of the SRES marker scenarios. Source: Climate Change in Australia (2016)

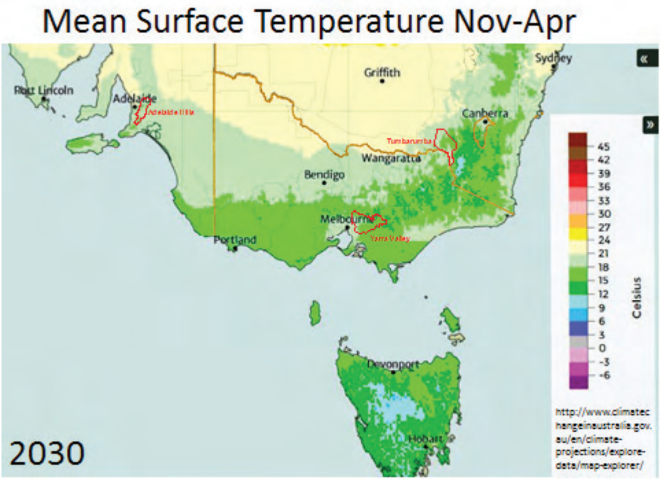

Figure 6. 2030 predicted mean Surface Temperature, November to April, RCP 4.5

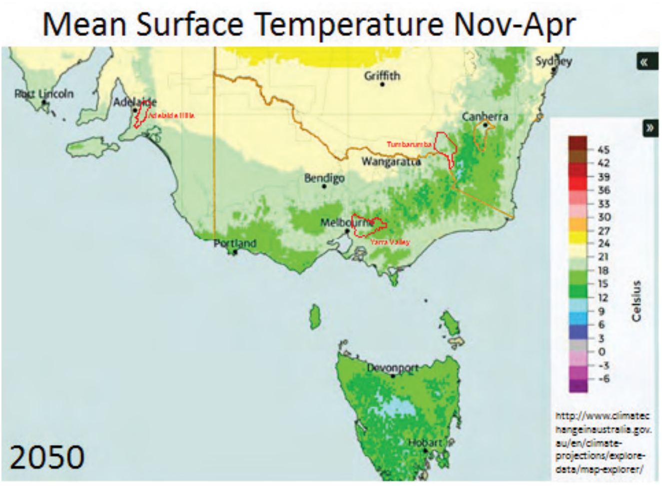

Figure 7. 2050 predicted mean Surface Temperature, November to April, RCP 4.5

I expect that the rate and final extent of these changes are as yet unknown (despite vigorous and high quality modelling) due to a multitude of possible influences ranging from the overall global policy all the way through to individual vineyard management.

One model of the projected temperature increase for the south-eastern Australian vineyard regions is depicted in Figures 5 to 10 with the 12°C to 15°C range being most relevant to premium sparkling production. It is truly disconcerting to visualise the predicted extent of the decline of area within this temperature band.

These charts are extracts from the CSIRO website, Representative Climate Future Framework (Clarke et al. 2011; Whetton et al. 2012). This emission rate is expressed by the Representative Concentration Pathway (RCP) and ranges between 8.5 (effectively zero curbing of emission rates) and 2.6 (a very ambitious mitigation program).

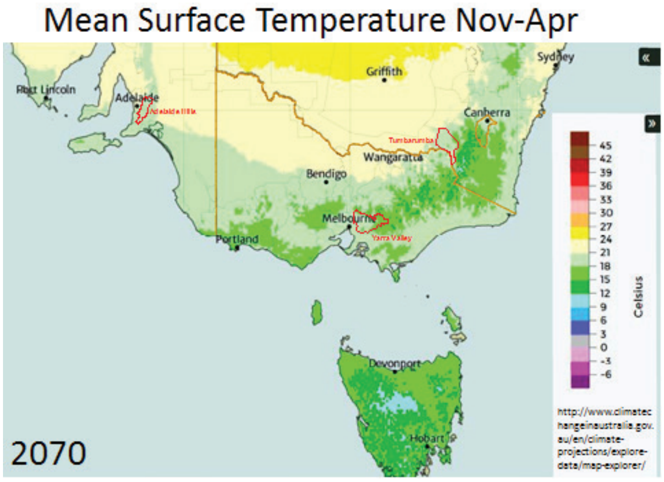

Figure 8. 2070 predicted mean Surface Temperature, November to April, RCP 4.5

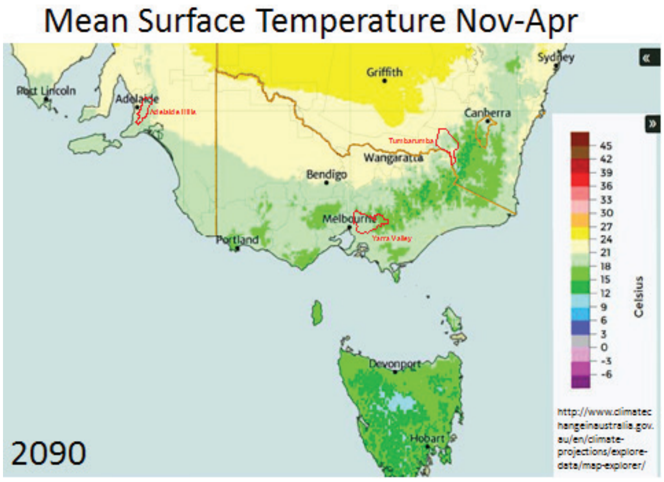

Figure 9. 2090 predicted mean Surface Temperature, November to April, RCP 4.5

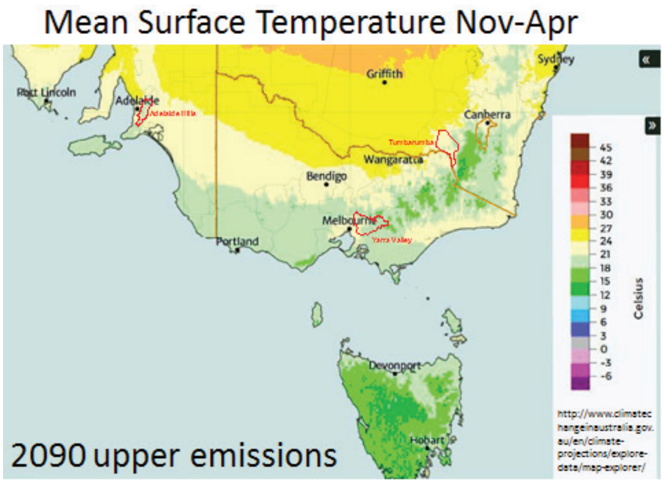

Figure 10. 2090 predicted mean Surface Temperature, November to April, RCP 8.5 and uninhibited greenhouse emissions

Figures 6 to 9 are scenarios (2030, 2050, 2070, 2090) based on a recommended intermediate emission rate RCP of 4.5 with Figure 10 being 2090 with uninhibited greenhouse emissions with an RCP of 8.5 (Dr Peter Hayman, SARDI, pers. comm.).

Conclusion

There has been a truly remarkable change in viticulture and oenology for sparkling production in Australia since the 1840s. I suggest the evolution has been even more significant since the 1980s with the focus on premium cool climate styles. In less than 40 years the production of premium sparkling wine has developed to a stage where there are many distinctive brands that are world quality at their respective price points.

The immediate future remains very positive as quality continues to improve from influences such as development of new cool climate sites, increasing maturity of vineyards and more knowledgeable winemaking.

The long-term future of Australian premium wine faces the same climate-driven uncertainties as does global agriculture. It would appear that the journey is far from over.

Acknowledgements

To prepare this paper I have drawn on the expertise of many industry experts in their respective fields of viticulture, winemaking, journalism and market analysis. There are many views represented and I have aimed to distill them to present a single interpretation of past, current and potentially future events.

To name a few on the topic of viticulture, thanks go to Dr Andrew Pirie and to past and present colleagues, Ray Guerin, Alex Sas, Craig Callec and, in particular, Warren Birchmore who has been of invaluable assistance in accessing much of the data for this presentation.

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Breeding for disease resistant varieties

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Abstract

Grapevine cultivars in production suffer from various biotic stress factors requiring intense plant protection treatments. In order to reduce these costly treatments, grapevine breeders set out to select resistant cultivars. Selection mainly addresses the two mildew fungi: powdery mildew (PM, Erysiphe necator*) and downy mildew (DM,* Plasmopara viticola*) combined with good wine quality. Examples from the breeding program at Geilweilerhof are given.*

To achieve more durable resistance, combinations of resistance loci are required. For the time being, stacking of resistances, i.e. the combination of two (or in future three) resistance loci against a single pathogen, should be envisaged. From their portfolio, breeders can make use of three resistance loci for PM (Run1, Ren 1, Ren3) and four for DM (Rpv1, Rpv3, Rpv10, Rpv12), which are found in elite genetic background. Other genetic loci remain to be introgressed into Vitis vinifera *genetic background showing good viticultural performance. The mildew loci should be combined with other traits, for example, conferring* Botrytis *resilience, late ripening or other resistances. A strategy will be discussed based on locus specific homozygous (LSH) lines for 2&2 resistances (e.g. Run1, Ren1 & Rpv1, Rpv3) passing on the resistance loci to the entire progeny.*

Webcast of this presentation available at http://bit.ly/16thToepfer.

Introduction

Since the end of the 19th century plant protection against powdery mildew (*Erysiphe necator*) and downy mildew (*Plasmopara viticola*) has been obligatory in a wide range of vineyards in Europe and around the world. Preventively, winegrowers apply fungicides in order to avoid disease formation and mildew epidemics. Breeding for mildew resistance is considered to be one possible contribution that keeps the mildews in check and reduces the risk of crop failure, as well as the demand of plant protection efforts. Recent success in breeding fungal resistant cultivars shows that, generally speaking, more than 50 per cent of the plant fungicidal treatments can be reduced. First reports of downy mildew strains overcoming resistance (Peressotti et al. 2010, Delmas et al. 2016), as well as experiences gained for other crops (e.g. Mundt et al. 2014), show that further efforts are required to reduce plant protection requirements.

During the last decade breeders have received considerable support from the scientific community by developing markers for marker assisted selection (for summary see www.vivc.de > database search > data on breeding and genetics). For downy mildew more than a dozen loci have been reported. The loci Rpv1, Rpv3, Rpv10, and Rpv12 for downy mildew have been introduced into elite genetic background, i.e. introgressed into *V. vinifera*, and genotypes show good wine quality as well as good viticultural performance. Such elite genotypes or newly-selected cultivars can directly be used as crossing parents for the selection of new cultivars. Similarly, almost a dozen loci have been identified for powdery mildew. The loci Run1, Ren1, and Ren3 are in elite genetic background. The loci Ren4 (Mahanil et al. 2012) and Ren6 (Pap et al. 2016), which seem to be interesting loci for powdery mildew resistance, need some further adaptation to elite genetic background. However, breeders today are in a rather comfortable situation as they can make use of several resistant loci. It is their challenge to combine (stack) several loci to achieve more durability of resistances and combine this trait with other required characteristics.

First experiences with new cultivars in Germany

It took about 120 years for Alexis Millardet's postulate to become reality. He proposed that it should be possible to combine the quality of the European wine-grape with the resistance of American wild grapes. Generations of breeders around the world followed his suggestion and created resistant interspecific hybrids from wild species of American as well as Asian origin and *V. vinifera* cultivars. Changing the *V. vinifera* parent to avoid inbreeding depression by consecutive pseudo-backcrosses (pBC) is necessary to remove the undesired wild characteristics. In Germany the first convincing cultivars were introduced into the market in 1995. Since then German breeders have developed and released more than 30 new cultivars. Table 1 shows these cultivars and the resistance loci found. This list indicates the very narrow genetic basis of resistance that resulted from using continuously advanced breeding lines and new cultivars to improve wine quality. To keep these breeding programs focused on quality, introgressions of new resistance loci were not developed. Thus, only single loci are found in the current cultivars (1&1, Figure 1). As far as marker analyses can tell, for downy mildew either Rpv3 or Rpv10, and for powdery mildew only Ren3, were used. Comparing the ratings of the official German variety list (2015) (Table 1) there is a tendency for plants carrying Rpv10 to be slightly more resistant compared with plants that carry Rpv3. This observation is supported by a comparison of leaf disk assays of plants carrying the individual resistance loci that are currently available (R. Eibach, unpublished).

For a perennial crop like grapevines, disease resistance becomes increasingly important. New cultivars on the German market are a

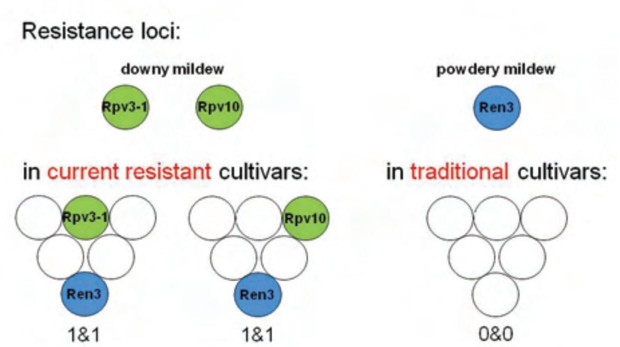

Figure 1. Categories of cultivars and the resistance loci identified therein which are on the German market. Coloured circles indicate the presents of resistance. Only single loci (1&1), one for downy mildew (green) and one for powdery mildew (blue), are found in the cultivars. Traditional cultivars instead do not show resistant loci (classified as 0&0)

great achievement from breeding and environmental perspectives as they permit a reduction in plant protection treatments of more than 50%. Reflecting on 30 years of breeding experience and using new cultivars in Germany, one can summarise (i) winegrowers have accepted the new cultivars but (ii) they are still hesitant to use them due to a lack of consumer demand. A major reason is that consumers lean towards buying known varietal wines instead of unknown products, e.g. Riesling versus Felicia. As a result, new grapevine cultivars need some marketing efforts. Many boutique wineries are successful in selling wines from new cultivars as they can provide more explanation and have more time to create consumer attention. To get the information widespread is, however, a long process. From this lesson, breeders have identified both the scientific and the market challenges.

The most recent cultivar from JKI Geilweilerhof is the breeding strain Gf.1993-22-6, denominated as Calardis Blanc (Figure 2) according to the historical description Calardiswilre for Geilweilerhof. It is a white wine variety with a good aroma between Sauvignon Blanc and Traminer, which is attractive to consumers. The winegrower finds improved downy mildew resistance due to two resistance loci, one powdery mildew locus (2&1), black rot resistance, as well as high *Botrytis* resilience favoured by small and tight berries and loose-cluster architecture. A later ripening grape, similar to Riesling, makes Calardis Blanc the appropriate answer to climate change demands compared with the previously selected new cultivars. Calardis Blanc is the starting point of the second generation of resistant cultivars in Germany which will show combinations of resistances (e.g. 2&2) and provide answers to problems arising due to climate change.

New approaches in breeding methology

Marker assisted selection opens up new vistas for grapevine breeding. It provides the opportunity to combine and monitor resistance loci throughout the breeding steps. Figure 3 shows the mildew resist-

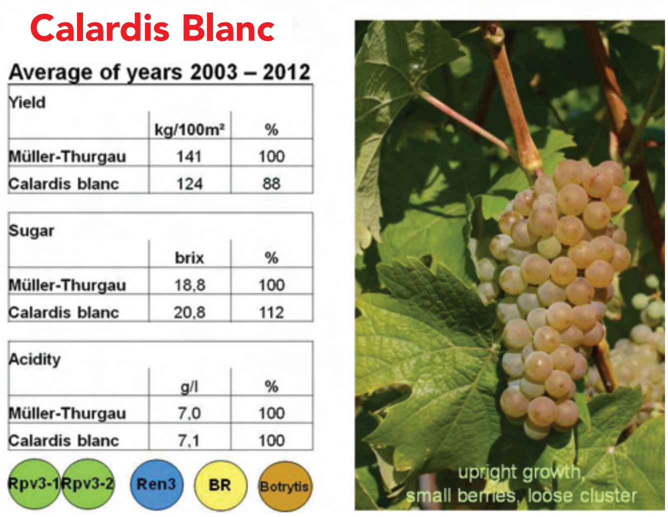

Figure 2. Calardis Blanc (from the historical description Calardiswilre for Geilweilerhof) is a new cultivar showing combined resistances and is a prototype for some trait combinations.

Table 1. Cultivars registered in the German variety list (2015). Plants were rated for resistance (1 = resistant, 9 = susceptible) in the vineyard upon cultivation with reduced fungicide application. Listing follows the resistance loci to downy and powdery mildew which were found according to marker analyses. Rating for *Botrytis* resilience is also given. B = white; N = black

Cultivar	Year of protection/ admission		Parents	Berry colour	DM	PM	Botrytis	Rpv3	Rpv10	Ren3
Phoenix	1992	1992	Bacchus x Villard Blanc	B	2	4	6	X		X
Orion	1994	1994	Optima x Villard Blanc	B	2	5	5	X		X
Regent	1994	1995	Diana x Chambourcin	N	3	3	4	X		X
Staufer	1994	1994	Bacchus x Villard Blanc	B	2	5	5	X		X
Sirius	1995	1995	Bacchus x Villard Blanc	B	2	5	4	X		X
Johanniter	1997	2001	Weisser Riesling x (Seyve Villard 12- 481 x (Pinot gris x Weißer Gutedel)	B	2	3	4	X		X
Helios	2004	2005	Merzling x (Seyve Villard 12-481 x Müller Thurgau)	B	3	3	4	X		X
Prior	2004	2008	(Joannes Seyve 234-16 x Pinot Noir) x Bronner	N	2	3	3	X		X
Villaris	2004	2011	Sirius x Vidal Blanc	B	2	4	5	X		X
Pinotin	2007	2014	Cabernet Sauvignon x Regent	N	2	3	3	X		X
Calandro	2009	2011	Domina x Regent	N	3	4	7	X		X
Muscaris	2012	2013	Solaris x Gelber Muskateller	B	2	3	4	X		X
Bronner	1997	1999	Merzling x (Zarya Severa x St. Laurent)	B	2	4	3		X	X
Solaris	2001	2004	Merzling x (Zarya Severa x Muscat Ottonel)	B	3	3	5		X	X
Cabernet Carol	2004	2008	Cabernet Sauvignon x Solaris	N	2	3	6		X	X
Cabernet Cortis	2004	2008	Cabernet Sauvignon x Solaris	N	2	3	4		X	X
Monarch	2004	2008	Solaris x Dornfelder	N	2	4	3		X	X
Souvignier Gris	2012	2013	Cabernet Sauvignon x Bronner	B	2	3	2		X	X
Rondo	1997	1999	Zarya Severa x St. Laurent	N	3	5	4		X	
Cabernet Carbon	2004	2008	Cabernet Sauvignon x Bronner	N	2	5	3		X	
Baron	2005	2012	Cabernet Sauvignon x Bronner	N	2	-	3		X	
Merzling	1995	1995	Seyve Villard 5-276 x (Riesling x Pinot gris)	B	4	4	4			X
Hibernal	1997	1999	(Chancellor x Weisser Riesling)F2	B	6	5	3			X
Prinzipal	1997	1999	Hibernal x Ehrenfelser	B	7	5	3			X
Saphira	1999	2004	Arnsburger x Seyve Villard 1-72	B	7	6	4			X
Reberger	2004	2011	Regent x Limberger	N	5	4	5			X
Allegro	2006	2009	Chancellor x Rondo	N	3	3	3			X
Bolero	2006	2008	(Rotberger x Reichensteiner) x Chancellor	N	3	3	4			X
Accent	2007	2010	Kolor x Chancellor	N	3	4	3			X
Piroso	2005	2010	(Portugieser x Heroldrebe) x (Deckrot x Freiburg 589-54)	N	2	4	4	n.t.	n.t.	n.t.

ance loci that are currently available in elite genetic background. Interestingly, the ratings for powdery mildew are better than the ratings for downy mildew loci. A further strong locus for powdery mildew resistance was recently described as Ren6 from *V. piasezkii*, an Asian species thus far not used in grapevine breeding (Pap et al. 2016). Breeders expect that it will be possible in the long term to build up genotypes with high resistance against powdery mildew. In contrast, the rating indicated in Figure 3 clearly shows that for downy mildew, further good sources of resistance are missing.

Having in hand the loci indicated in Figure 3, breeders have started to combine different loci (Eibach et al. 2007). Not knowing the mechanisms they combined two resistances from different origins for each of the two mildews (2&2). Lines showing 3&3 loci are envisaged. In one step further from these activities plant lines with homozygous resistance loci (LSH-lines) have been selected at Geilweilerhof (2-2&2-2) and offer the possibility to create large offspring generations which are uniform in terms of resistance (Figure 4). In combination with markers for the flower sex locus (Fechter et al. 2012), breeding lines can be selected that simplify crossing schemes based on female genotypes which need no emasculation prior to crossing. As a consequence, hand work is reduced although the number of seeds can be increased. This finally raises the chances of selecting superior genotypes. Grapevine breeding at the beginning of the 21st century

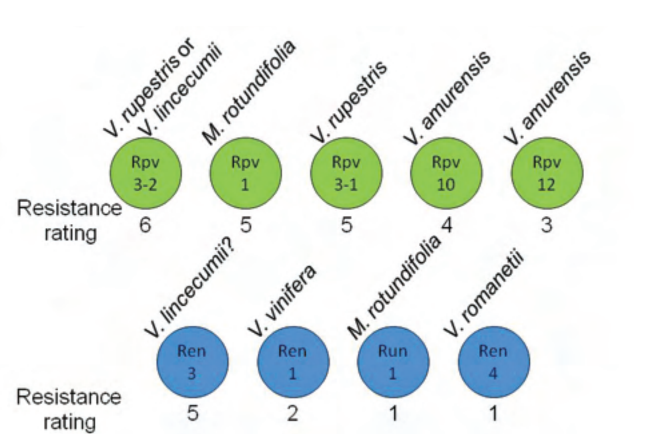

Figure 3. Loci for downy (green) and powdery mildew (blue) and the rating of resistance (1 = resistant, 9 = susceptible) analysed on leaf discs or detached leaf assays. The species origin of resistance is indicated

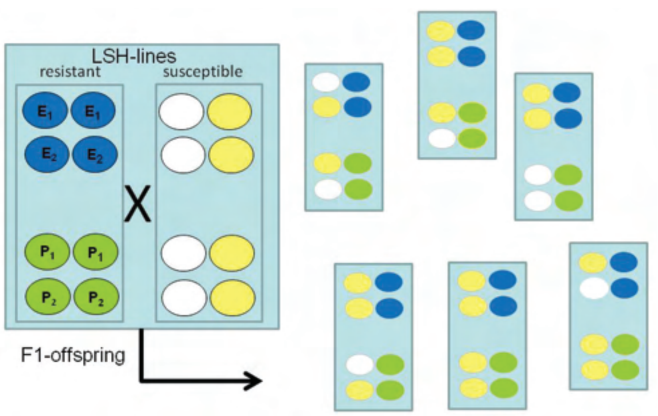

Figure 4. Increased breeding efficiency is expected from locus specific homozygous (LSH) lines. These lines show resistant loci in a homozygous state (2-2&2-2). The entire offspring can be screened and selected for genotypes showing good yield, viticultural performance and quality traits. The resistances are found in the entire offspring and consequently need not be selected for mildew resistance.

offers unprecedented opportunities. Marker development for mildew resistance is a milestone, but markers for other traits such as *Botrytis* resilience, phenology, yield, and quality parameters are still to be identified.

Techniques of genome sequencing and genome analysis (genotyping tools) have proven to be crucial for progress in grapevine breeding. Marker assisted selection permits the combination of resistance loci on demand. Currently techniques for phenotyping plants are rapidly evolving and need to be elaborated and adapted to the specificities of grapevines (e.g. Kicherer et al. 2015). In combination with genome analysis they will result in new markers and thus selection schemes for speeding up grapevine breeding.

Conclusion

Grapevine breeding has resulted in cultivars that have shown good field performance, even with a reduced plant protection regime (reduction potential >50%). The new tools of marker assisted selection have paved the way to combine resistance loci. They will soon result in cultivars that show 2&2 mildew resistance loci that are a good step towards greater durability of resistance – a crucial trait for a perennial plant. A combination of more loci 3&3 (e.g. Rpv1, Rpv10, Rpv12 & Run1, Ren1, Ren3) is possible and will be a function of time. Furthermore, the stacking of different resistant loci within the category 3&3 (e.g. Rpv3-2, Rpv10, Rpv12 & Ren1, Ren4, Ren6) in another cultivar is envisaged. Such diversity is expected to additionally contribute to durability of resistance.

Acknowledgements

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Regional evaluation of Chardonnay and Shiraz clones

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Abstract

Variability in wine characteristics between clones is accepted and offers an opportunity for winemakers to select clones to match desired wine styles. However, for Shiraz and Chardonnay, the two major varieties planted in Australia, clonal diversity has not been widely exploited and there are now large areas planted to a single clone of each. Climate change projections indicate existing vineyards will experience changed conditions in the future; will clones assist in mitigating future impacts and how might clonal wines change as regions become warmer and drier?

The 'Clones for climate change' project is using existing differences in the present climate between widely geographically separated sites as a surrogate for climate change to gain some insights about how clones may perform in the future. The Shiraz and Chardonnay clones in this study are planted across the Riverland, Barossa Valley (SA), Henty, Grampians (Vic.), Margaret River and Great Southern (WA). In addition to the collection of many viticultural measurements and climate data, duplicate lots of 50 kg of grapes were made into wine under a standard process for the 2014, 2015 and 2016 vintages and evaluated by trained tasting panels. First season data revealed differences in sensory properties of the same clone from the coolest to the warmest sites with a clone x site interaction for some attributes. Principal component analysis revealed the wines from clones at some sites were clustered but at other sites the predominant clonal attributes varied. For some attributes there were significant differences between clones that were consistent across all sites. These data and the 2015 results will be presented and the implications for climate change will be discussed.

Webcast of this presentation available at http://bit.ly/16thMcCarthy.

Introduction

Extensive clonal evaluation has been an important part of the European wine industry and intra-varietal diversity is commonly assessed to identify superior planting material for wine quality, disease, drought and heat tolerance, flavour parameters for market development and adaptability to climate change. The Australian wine industry relies on a relatively small number of international grapevine varieties for 95% of its production, and, within those, a small number of imported clones for some varieties, e.g. Chardonnay. For some major wine-grape varieties Australia holds a unique repository of vine material derived from very old vineyards, some pre-dating the spread of phylloxera in Europe and Australia, e.g. Shiraz. Other countries have expressed interest in obtaining some of Australia's 'heritage' material. In an international context germplasm evaluation is seen as a key aspect of product development and there is a great opportunity for the Australian industry to gain more information about the clonal material being used across regions.

Previous clonal evaluations in Australia primarily examined quantitative, rather than qualitative, performance during a period when improving productivity and eliminating virus-like diseases was seen as the main objective. Most of this work was undertaken in the 1980s and 1990s and little clonal trial work has been conducted in the past ten years. Some more recent replicated clonal trials established in 2005 by the Riverland Vine Improvement Committee are still under evaluation, and older established trials are still available for assessment, particularly for a closer examination of wine quality parameters.

Chardonnay and Shiraz represent the two major wine-grape varieties in Australia. Both varieties are grown in a wide range of climatic sites for different wine styles and market price points. Some vineyards have been planting a range of clones to broaden the availability of varying wine characteristics offered by different clones. Information that is available on aroma and flavour profiles of clones is largely anecdotal and very few systematic and standardised evaluations have been conducted. Of more recent interest is the impact of climate change on the performance of varieties and clones particularly on wine aroma and flavour profile. There has been a general trend for seasonal warming as evidenced by substantially earlier harvests over the past 15 seasons or so. Given the difficulty of monitoring changes in aroma and flavour profiles of wines over a long period of gradual temperature increases, a project was established to utilise temperature differences between regions as a surrogate for climate change.

The clones

Mature plantings (minimum ten years old) of a range of clones of each of Chardonnay and Shiraz were selected across regions in southern Australia. The clones were in replicated trial sites and/or were in blocks planted in close proximity to each other. In Australia, very old plantings of Chardonnay are rare and most of the expansion of Chardonnay plantings in the past 40 years has been based on imported clones from the USA and France. Clones from the USA were imported in the 1960s and 1970s and were primarily selected for high yields. Most clones from France were imported in the 1980s onwards and were primarily selected for consistency of yield and wine quality. The Shiraz clones are all based on Australian selections from South Australia (SA), New South Wales (NSW), Victoria (Vic.) and Western Australia (WA). Not all clones in this study are represented in each region as some popular local examples were included to broaden the intra-site comparisons. The clones in the trials are described in Table 1.

The regions

Five regions for Chardonnay and four regions for Shiraz, covering a mix of hot, warm, maritime and cool climates were selected. The Chardonnay plantings were in the Riverland (SA), Margaret River (WA), Great Southern (WA), Grampians (Vic.) and Henty (Vic.) regions, and the Shiraz plantings were in the Riverland (SA), Barossa Valley (SA), Margaret River (WA) and Grampians (Vic.) regions. The clones present at each site are listed in Table 2. Long-term Day Degree means are mostly based on 25 to 30 years of data prior to 1992 (Table 3). Henty is the coolest region and Riverland the warmest, with the other regions falling in between the two extremes. Day Degrees (DD base 10°C) across the regions for the two seasons reported here follow a similar trend to the long-term means but are substantially

Table 1. Chardonnay and Shiraz clones included in the study

Clonal designation	Comments
Chardonnay[1]	
Bernard 76	Selected in Burgundy region. Consistent good yield, above average quality.
Bernard 78	Selected in Burgundy region. High production of below average quality.
Bernard 95	Selected in Burgundy region. Vigorous, slightly below average production of good quality. Susceptible to coulure and millerandage.
Bernard 96	Selected in Burgundy region. Very vigorous, high production of good quality.
Bernard 277	Selected in Burgundy. Good production of very high quality.
FV I10V1	Selected in California as high yielding from vines originally from France. Also known as Foundation Plant Service (FPS) 06 and clone 6 in New Zealand.
FV I10V5	Selected in California as high yielding from vines originally from France. Also known as FPS 08.
Gingin	Mass selected from Valencia Wines at Gingin, WA. Original cuttings from Swan Research Station of undetermined origin.
Shiraz	
SA 1654	Original vines planted 1940 on Nuriootpa Research Centre. Selected in 1960s. Widely planted.
BVRC 12	Selection from Barossa Valley (SA) vineyard in 1970s.
BVRC 30	Selection from Barossa Valley (SA) vineyard in 1970s.
SARDI 7	Selection from old northern Barossa Valley (SA) vineyard in 1986.
SARDI 4	Selection from old central Barossa Valley (SA) vineyard in 1986.
R6W	Selection from '1860s' Shiraz block at Tahbilk Wines (Vic.) in the 1970s. Known as R6WV28 or R6V28 in SA.
PT 15	Selection from pruning trial (PT) at Griffith Research Station (NSW) in 1960s.
PT 23	Selection from pruning trial (PT) at Griffith Research Station (NSW) in 1960s.
WA selection	Local mass selection, possibly from the Swan Valley (WA).
Bests selection	Mass selection from Bests Vineyard (Vic.) established 1866. Possibly from the Busby collection.

[1]Information on Bernard clones from Bernard (1987)

Table 2. Chardonnay and Shiraz clones represented in each region

Chardonnay Clone	Region				
	Riverland	Grampians	Margaret River	Henty	Great Southern
Bernard 277	✓	✓	✓	✓	✓
Bernard 76	✓	✓	✓	✓	✓
Bernard 78		✓		✓	
Bernard 95	✓	✓	✓	✓	✓
Bernard 96	✓	✓	✓	✓	✓
FV I10V1			✓	✓	✓
FV I10V5		✓		✓	
Gin Gin			✓		

Shiraz Clone	Region			
	Riverland	Barossa Valley	Grampians	Margaret River
BVRC 12	✓	✓	✓	✓
BVRC 30	✓	✓	✓	
SA 1654	✓	✓	✓	✓
PT 15		✓	✓	✓
PT 23	✓		✓	
R6W	✓	✓	✓	
WA selection				✓
Bests selection			✓	

higher reflecting the increase in temperatures associated with climate change. The 2014–15 season was slightly cooler (within 40 DD) in the Henty, Grampians, Barossa Valley and Riverland regions, much cooler (by 120 DD) in the Margaret River region, and much warmer (by 132 DD) in the Great Southern region.

Vine performance

The vines in the trial were monitored regularly through the season. Dates of phenological stages were determined for budburst, flowering and veraison. Several canopy measurements were obtained just after veraison, such as canopy dimensions, leaf area index and canopy porosity (the latter two using the VitiCanopy app developed by the University of Adelaide). At harvest berry samples were collected for juice analysis, bunch samples for determining bunch compactness, and crop weights and components of yield obtained. Target sugar concentrations of 22.5°Brix (12.5°Baumé) for the Chardonnay and 24.3°Brix (13.5°Baumé) for Shiraz were set. Since the trials were located on commercial vineyards, some flexibility with the target sugar concentrations was required to fit with the vineyard harvest schedule. The main focus of this report is to describe the wine quality attributes for the clones and regions and the detailed seasonal data will be provided in subsequent reports.

Winemaking and sensory description

Lots of 100 kg of fruit from each clone were harvested for subsequent winemaking. Grapes were either transported to the crushing facility immediately after harvest or stored in a cool room overnight (for the trials in Victoria) before transport to the winery. The grapes from SA and Victoria were processed at the Wine Innovation Cluster (WIC) Winemaking Facility, Waite Campus, Adelaide, and the grapes in WA were crushed, fermented and stabilised in a DAFWA winemaking laboratory at Bunbury before transport to the WIC winery for finishing off and bottling. Where possible duplicate ferments of 50 kg each followed the same prescribed winemaking process in vessels of matching material and volumes. Winemaking protocols were designed to minimise human intervention in the finished wines. All wines were finished off and bottled at the WIC winery. The wines went through malo-lactic fermentation and were not exposed to any wood prior to bottling.

After informal screening for suitability for sensory analysis, a panel of eight or ten assessors, all part of the Australian Wine Research Institute (AWRI) trained descriptive analysis panel, determined appropriate descriptors and assessed suitable standards for aroma attributes before the formal sensory sessions (Tables 4 and 5). Around 30 descriptors were used and the intensity of the descriptors for appearance, aroma and palate were rated from 0 to 10 (1 = low, 9 = high). Each wine was presented two or three times (depending on the variety) over five or six sessions. All judges were found to be performing to an acceptable standard in regard to agreement with the panel mean and discrimination across samples. Relatively few attributes showed significant differences between fermentation repli-

Table 3. Day Degrees (°C) for six regions involved in the trials

Region	Nearest Bureau of Meteorology site	Day Degrees (°C)		
		Long-term[1]	2013–14	2014–15
Henty	Dartmoor	1180	1380	1339
Grampians	Ararat	1349	1450	1427
Great Southern	Mount Barker	1441	1747	1879
Margaret River	Witchcliffe	1599	1833	1713
Barossa Valley	Nuriootpa	1577	1849	1818
Riverland	Loxton	2148	2216	2213

[1]Gladstones (1992)

cates or presentation replicates. An Analysis of Variance (ANOVA) was carried out of the sensory data and Tukey's Honest Significant Difference (HSD) values (2013–14) or Fisher's Least Significant Difference (LSD) values (2014–15) were calculated for comparisons of means. Principal Component Analysis (PCA) was conducted on mean values averaged over panellists and replicates, using a correlation matrix.

Results and discussion

Sensory assessment 2013–14 wines

Chardonnay

No grapes were harvested from the Henty region due to insufficient crop mainly due to very cool weather during flowering resulting in poor fruit set. Of the 28 sensory attributes analysed across the remaining four regions there were significant differences ($p<0.05$) between clones for 18 attributes, and a further two attributes were significant to $p<0.1$. The PCA used all sensory attributes with significance $p<0.1$ and the loadings for PC1 and PC2 accounted for 41.1% of the variation in the data set (Figure 1). The wines plotted to the right of the figure were rated higher in 'floral' and 'confection' aromas (primarily Margaret River and Great Southern, and one clone from Riverland), while those to the left were rated lower in those attributes and higher in 'box hedge', 'passionfruit', 'vegetal' and 'sweaty'/'cheesy' aromas (primarily the Grampians region, and one clone from Riverland). Wines in the upper half of the figure were higher in 'citrus' flavour, 'acidity' and 'astringency' (some clones from Margaret River and Great Southern), whilst those in the lower half were higher in 'stone fruit' flavour, and 'hotness' and 'sweetness' on the palate (some clones from Great Southern, one from Grampians and two from the Riverland).

Table 4. Descriptors developed for assessment of Chardonnay clonal wines

Attribute	Definition/synonyms
Appearance	
Yellow colour intensity	Yellow colour
Aroma	
Overall fruit intensity aroma	Intensity of the fruit aromas in the sample
Tropical	Intensity of the aroma of tropical fruits: passionfruit pineapple, mango, melons, banana, mango, lychee, guava
Stone fruit	Intensity of the aroma of stone fruits: peach, apricot both fresh and dried, nectarine, pear
Citrus	Intensity of the aroma of citrus fruits: lemons, limes, grapefruit, oranges, mandarins
Confection	Intensity of the aroma of confection: musk, banana lolly, red lolly
Floral	Intensity of the aroma of flowers: violets, rose, geranium, jasmine and blossoms
Green	Intensity of the aroma of green grass, green stalks, green leaves, snow peas, fresh green vegetables
Herbal	Intensity of the aroma of eucalypt and mint
Vegetal	Intensity of the aroma of cooked vegetables, dirty tea towel, drain
Box hedge	Intensity of the aroma of box hedge
Flint	Intensity of the aroma of flint, wet stones, metals, toast, smoke
Sweaty/cheesy	Intensity of the aroma of sweat and cheese, barnyard
Savoury/meaty	Intensity of the aroma of savoury, meat, sausage roll, peanut oil, cooked rice, vegemite
Pungent	Intensity of the sensation of pungency and alcohol
Palate	
Overall fruit intensity palate	Intensity of the fruit flavours in the sample
Tropical	Intensity of the flavour of tropical fruits: pineapple, passionfruit, melon, mango, kiwifruit, guava, pawpaw, lychee
Stone fruit	Intensity of the flavour of stone fruits: peach, apricot both fresh and dried, nectarine
Citrus	Intensity of the flavour of citrus fruits: lemon, lime, mandarin, orange, including aftertaste
Confection	Intensity of the flavour of confectionery: red lolly, banana lolly, musk, including aftertaste
Green	Intensity of the flavour of green grass, green stalks, cucumber, green leaves, herbal and vegetal
Flint	Intensity of the flavour of flint, smoke
Viscosity	The perception of the body, weight or thickness of the wine in the mouth (low=watery, thin mouth-feel; high=thick mouth-feel)
Oily	The perception of oiliness and buttery mouth-feel in the mouth
Acid	Intensity of acid taste in the mouth, including aftertaste
Hotness	The intensity of alcohol hotness perceived in the mouth, after expectoration and the associated burning sensation (low=warm; high=hot, burning)
Astringency	The drying and mouth-puckering sensation in the mouth (low=coating teeth; medium=mouth-coating and drying; high=puckering, lasting astringency)
Bitter	The intensity of bitter taste perceived in the mouth, or after expectoration
Fruit AT	The lingering fruit flavour perceived in the mouth after expectorating, excluding citrus

Table 5. Descriptors developed for assessment of Shiraz clonal wines

Attribute	Definition/synonyms
Appearance	
Opacity	The degree to which light is not allowed to pass through a sample, colour intensity
Purple	Intensity of the purple colour in the sample
Brown	Intensity of the brown colour in the sample
Aroma	
Overall fruit intensity	Intensity of the fruit aromas in the sample
Red fruits	Intensity of the aroma of red fruits and berries: raspberries, strawberries, cranberries, redcurrants
Dark fruits	Intensity of the aroma of dark fruits and berries: blackberries, plums, black currants, cherries
Confection	Intensity of the aroma of confection: raspberry lollies, musk lollies
Floral	Intensity of the aroma of flowers: violets, roses, talc
Vanilla	Intensity of the aroma of vanilla
Sweet spice	Intensity of the aromas of various sweet spices: cinnamon, cloves, mixed spice, aniseed
Pepper	Intensity of the aroma of black and white pepper
Mint	Intensity of the aroma of mint, other fresh herbs
Green	Intensity of the aroma of green stalks, leaves, green capsicum, geranium
Earthy	Intensity of the aroma of earth, organic matter, dust, beetroot
Chemical/plastic	Intensity of the aroma of plastic, chemicals
Boiled egg	Intensity of the aroma of boiled egg, cooked veg, drains
Pungent	Intensity of the aroma and effect of alcohol
Palate	
Overall fruit intensity	Intensity of fruit flavours in the sample
Red fruits	Intensity of the flavour of red fruits and berries: raspberries, strawberries, cherries, cranberries
Dark fruits	Intensity of the flavour of various dark fruits: blackberries, currants, plums
Green	Intensity of the flavour of green stalks, green capsicum, green bean, herbs, rhubarb
Sweet	Intensity of the taste of sucrose
Salt	Intensity of the taste of salt
Viscosity	The perception of the body, weight or thickness of the wine in the mouth (low=watery, thin mouth-feel; high=oily, thick mouth-feel)
Acid	Intensity of acid taste in the mouth, including aftertaste
Hotness	The intensity of alcohol hotness perceived in the mouth, after expectoration and the associated burning sensation (low=warm; high=hot, burning)
Astringency	The drying and mouth-puckering sensation in the mouth. (low=coating teeth; medium=mouth-coating and drying; high=puckering, lasting astringency)
Bitter	The intensity of bitter taste perceived in the mouth, or after expectoration
Fruit AT	The lingering fruit flavour perceived in the mouth after expectorating

The grouping of two Great Southern clones (Gingin and I10V5) in a different quadrant of the PCA diagram may relate to greater sugar concentrations in the grapes at harvest and resultant higher alcohol levels in the wines. One clone from the Grampians region (KLV 76) also scored differently to the rest of the wines and it had the highest per cent alcohol. Differences in maturity or alcohol in the wines did not relate to the spread of wine scores in the Margaret River or Riverland regions.

The PCA results tend to group more by region than by clone. The clone x region effects were analysed across four clones common to the four regions. Eight attributes in the analysis showed a clone x region interaction (yellow colour, 'box hedge' aroma, 'sweaty'/'cheesy' aroma, 'spritz', 'sweetness', 'acidity', 'astringency' and 'bitterness') and four attributes showed a significant clone effect without a clone × region interaction. These attributes were 'pineapple' aroma, 'overall fruit' flavour, 'tropical fruit' flavour and 'viscosity' (Figure 2) and the clones were split into two groups. Clones 76 and 96 were relatively high for these four attributes across all sites and clones 95 and 277 were relatively low.

Shiraz

The ANOVA revealed significant differences ($p<0.05$) between clones for all 29 attributes analysed with most of them significant at $p<0.001$. The PCA analysis used all sensory attributes and the PC1 and PC2 loadings accounted for 60% of the variation in the data set (Figure 3). The wines plotted to the right of the figure were rated higher in opacity, the aromas of 'vanilla', 'dark fruit' (blackberries, plums, cherries, black currants, blueberries), 'overall fruit' and 'sweet spice', the flavours of 'dark fruit' and 'overall fruit', and 'viscosity', 'sweetness' and 'fruit' after-taste (primarily wines from Barossa Valley). The wines plotted to the left of the figure were rated higher in confection aroma and 'red fruit' (raspberries, strawberries, cranberries, red currants) aroma (primarily Grampians region wines). Wines in the upper section of the figure rated higher in brown colour, the aromas of 'chemical'/'plastic', 'boiled egg', 'earthy' and 'green', and 'hotness', 'bitter', 'salt' and 'pungency' (primarily Riverland region wines). Wines in the lower half of the figure were higher in purple colour, 'confection' aroma, 'floral' aroma, 'mint' aroma and 'red fruits' flavour (most of the Margaret River wines were in this section).

One clone from the Grampians region (MLG 23) appeared in a different quadrant to the rest of the clones and it had the highest sugar concentration at harvest, a high per cent alcohol and highest pH in the wine. One of the Margaret River clones (MR BVRC 12) was also in a different quadrant and it had the highest per cent alcohol and a high pH in the wine. One clone from the Barossa Valley (BV BVRC30) was also in a different half of the PCA diagram (predominantly 'green' flavour) and it had a low per cent alcohol and lowest pH in the wine indicating the grapes were less mature. The Barossa Valley clone

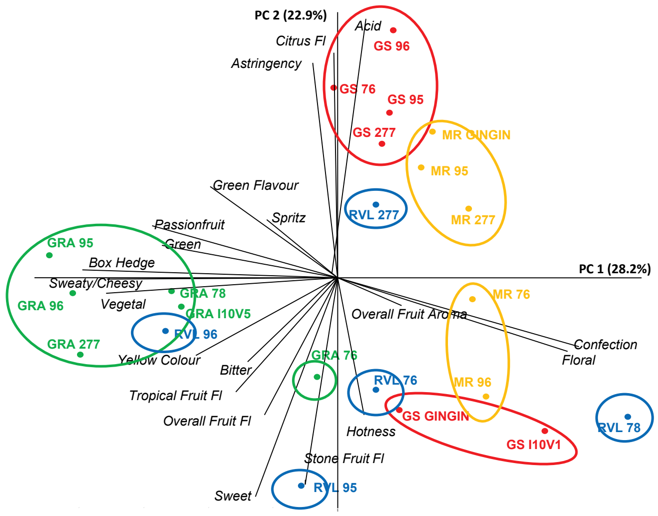

Figure 1. Scores and loadings bi-plot for PCA of attributes and treatments of Chardonnay clones in 2013–14, showing PC1 and PC2. GS = Great Southern, MR = Margaret River, RVL = Riverland, GRA = Grampians. Note that region and clone identifiers in Table 2 may differ from those in the figures due to different coding used during winemaking and sensory evaluation.

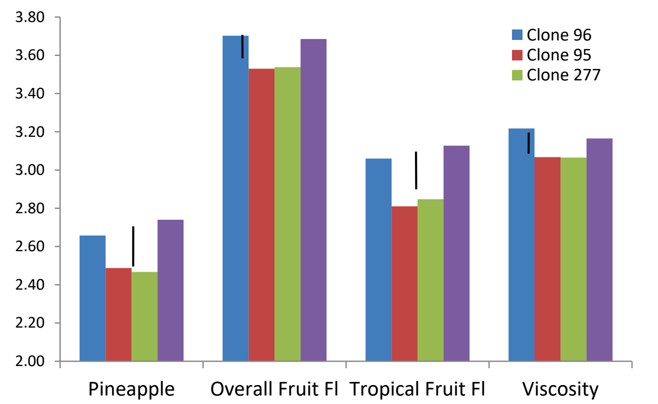

Figure 2. Mean values for four attributes ('pineapple' aroma, 'overall fruit' flavour, 'tropical fruit' flavour and 'viscosity') for four regions by clone in 2013–14 showing a significant clone effect without a significant clone x region interaction. Bars are LSD values (p = 0.05)

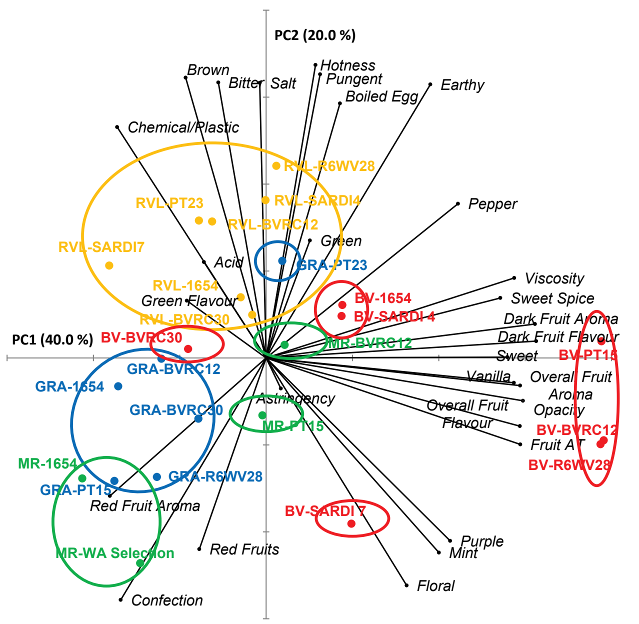

Figure 3. Scores and loadings of PCA bi-plot for nine clones of Shiraz in 2013–14 across four regions averaged across duplicate ferments. MR = Margaret River, RVL = Riverland, GRA = Grampians, BV = Barossa Valley

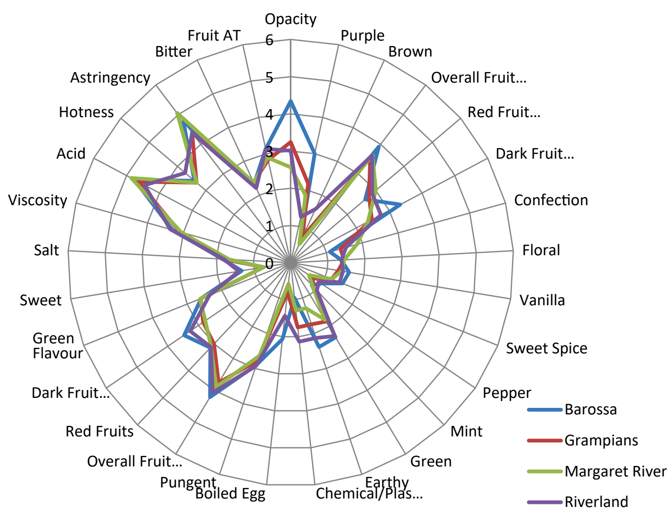

Figure 4. Radar plot of mean scores for attributes from Shiraz clone SA 1654 from all four regions in 2013–14

SARDI 7 also had low wine alcohol and pH and it was closely associated with 'floral' and 'mint' aromas and purple colour. The Riverland clones were grouped closely together and all wines had similar alcohol levels (the range from highest to lowest alcohol was 0.5%).

The PCA shows that region has more influence on the sensory attributes than clone. This was examined further for the SA 1654 clone over the four regions (Figure 4). For that clone some attributes showed relatively larger differences between regions, viz. opacity, purple and brown colour, the aromas of 'dark fruit', 'green', 'earthy', 'chemical'/'plastic' and 'boiled egg', 'dark fruit' flavour, and 'sweetness', 'hotness' and 'astringency' on the palate. There were little differences between regions for 'pungent', 'floral' and 'pepper' aromas, and 'salt', 'viscosity' and 'bitterness' on the palate.

Sensory assessment 2014–15 wines

Chardonnay

Some wines were excluded from the tastings as they were deemed unfit for sensory analysis. The ANOVA revealed significant differences ($p < 0.06$) between wines for all sensory attributes. The PCA analysis used all sensory attributes and the PC1 and PC2 loadings accounted for 51.8% of the variation in the data set (Figure 5). The wines plotted to the right of the figure rated higher in all the 'fruity' attributes (primarily wines from Great Southern and most from Margaret River). The attributes on the left of the figure were 'non-fruit' attributes: aromas of 'savoury'/'meaty', 'sweaty'/'cheesy' and 'vegetal', and 'flint' flavour (primarily wines from Riverland and some from Grampians). The upper half of the figure contained attributes high in 'oily' and 'viscosity' on the palate but none of the regions grouped in this section. The lower half of the figure reflected high scores for 'citrus' flavour, 'green' flavour, 'acidity' and 'astringency' (some of the Henty wines). Compared with the 2013–14 season, the clones were reasonably well grouped in the Riverland, Margaret River and Great Southern regions, but not as well grouped in the Grampians and Henty regions.

One of the Great Southern clones appears in a different quadrant to the others (GS GG = Gingin) and it had the highest sugar concentration at harvest and the highest per cent alcohol and highest pH in the wine. One of the Grampians clones also appears in a different quadrant a (ARM I10V5) and it had relatively lower sugar concentration at harvest, resulting in lowest alcohol and pH in the wine. One clone from Margaret River (MR 96) was in a different quadrant to the other clones and it had lower sugar concentration at harvest and the lowest alcohol in the wine. The clones from the Henty region were quite spread across two diagonally opposite quadrants. At the two extremes, I10V1 (DRM I10V1 – associated with yellow colour, 'fruit' after-taste, 'stone fruit' and 'tropical fruit' flavours, 'viscosity' and 'herbal' aroma) had one of the highest sugar concentrations and the highest per cent alcohol and pH in the wine, and I10V5 (DRM I10V5 – associated with 'acidity', 'green' flavour and 'astringency') had the lowest sugar concentration at harvest and the lowest alcohol and pH in the wine. There was no clear association between maturity and wine composition between the other five clones of Chardonnay in the Henty region. Clones from the Riverland region were closely grouped and there were minimal differences in maturity or wine alcohol (the range between highest and lowest alcohol was 0.5%).

One clone (76) was represented in all five regions and a radar plot of attributes with significant and nearly significant differences between clones is presented (Figure 6). It shows larger differences between regions for the attributes 'savoury'/'meaty' aroma, 'sweaty'/'cheesy' aroma, 'vegetal' aroma, 'floral' aroma, 'hotness', 'flint' flavour and 'oily' taste, and smaller differences between regions for 'acidity', 'tropical fruit' flavour, 'herbal' aroma, 'confection' aroma, 'overall fruit' aroma and yellow colour. The Riverland and Grampians regions have the highest means for 'vegetal' aroma, 'savoury'/'meaty' aroma and 'flint' flavour, while the Great Southern and Margaret River regions are highest in 'floral' aroma, 'confection' aroma and 'overall fruit' aroma, and lower in 'savoury'/'meaty' aroma, 'vegetal' aroma, 'flint' flavour and 'oily' palate. The Henty region was relatively low in 'savoury'/'meaty' aroma and 'sweaty'/'cheesy' aroma, and high in 'overall fruit' aroma.

Shiraz

The Riverland wines were assessed separately from the other three regions based on them having quite different chemical data and sensory properties, and some Margaret River wines were excluded from the main tasting as they were deemed unfit for sensory analysis. The ANOVA of the main tasting (excluding the Riverland wines) revealed significant differences ($p<0.05$) and nearly significant differences ($p< 0.1$) between wines for 27 of the 30 sensory attributes.

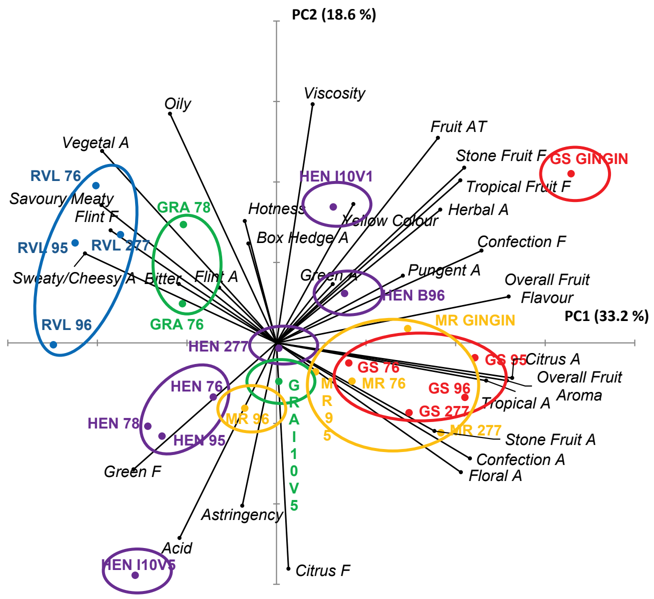

Figure 5. Scores and loadings bi-plot for PCA of attributes and treatments for Chardonnay clones in 2014–15. HEN = Henty region, MR = Margaret River, GS = Great Southern, RVL = Riverland, GRA = Grampians

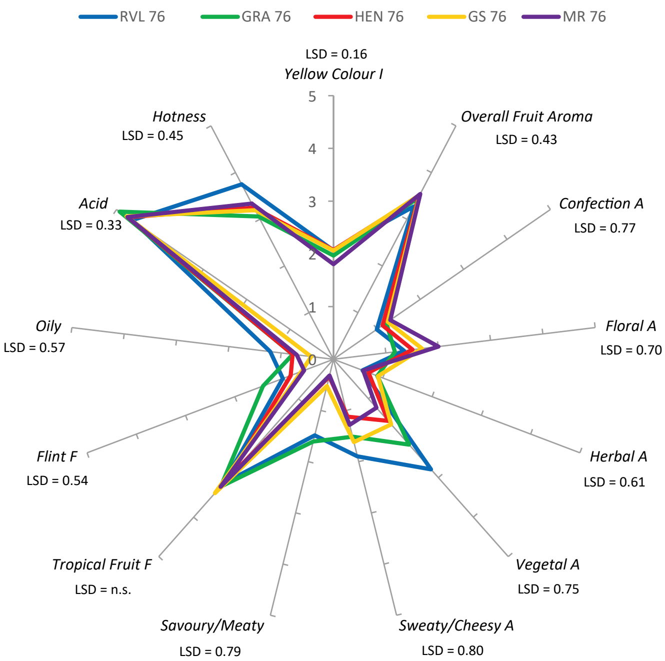

Figure 6. Radar plots for Chardonnay clone 76 in five regions for significant and nearly significant attributes. LSD values of the significant attributes are included. RVL = Riverland, GRA = Grampians, HEN = Henty, GS = Great Southern, MR = Margaret River

The PCA analysis used the significant and nearly significant (p<0.1) sensory attributes and the PC1 and PC2 loadings accounted for 71.1% of the variation in the data set (Figure 7). The wines plotted to the right of the figure rated higher in opacity, brown colour, the aromas of 'pepper', 'dark fruit', 'woody', 'pungent', 'sweet spice' and 'cooked fruit', 'dark fruit' and 'green' flavours, and 'viscosity', 'hotness', and 'acidity' (primarily wines from Barossa Valley). The attributes on the left of the figure were predominantly purple colour, aromas of 'red fruit' and 'confection'/'floral', and 'red fruit' flavour (primarily wines from the Grampians region). The upper half of the figure contained attributes high in 'overall fruit' aroma and 'fruit' aftertaste but none of the regions grouped in this section. The lower half of the figure reflected high scores for 'chocolate' flavour (three of the four Margaret River wines).

The WA selection grown in the Margaret River region (MR WA SELECT) is in a different quadrant compared with the remaining clones and it had the ripest fruit at harvest and produced a wine with the highest alcohol and pH. Whilst the Grampians region clones are grouped in the one quadrant, the SA 1654 clone (MLG 1654) is located away from the other clones (less purple colour, 'confection'/'floral' aroma, 'red fruit' aroma and 'red fruit' flavour) and it had the lowest sugar concentrations at harvest and lowest alcohol in the finished wine. The Barossa Valley clones are located across two quadrants. The two in the upper quadrant (BV BVRC12 and BV R6WV28) had the highest sugar concentrations at harvest and the highest per cent alcohols in the wines (BVRC12 also had the lowest wine pH and highest titratable acidity). The clone PT15 (BV PT15) shows a strong association with brown colour and this clone had the highest wine pH and lowest titratable acidity.

For the clone SA 1654 a radar plot of mean sensory scores for three regions in the main analysis demonstrated significant differences between the regions (Figure 8). The Barossa Valley wines had higher scores for opacity, brown colour, 'dark fruit' aroma and flavour, 'viscosity', 'acidity' and 'hotness', and lower scores for 'red fruit' aroma and 'chocolate' flavour. The Grampians region showed higher purple colour, aromas for 'red fruit', 'confection'/'floral', 'vanilla' and 'overall fruit', and 'red fruit' flavour, and low in brown colour, 'vegetal' aroma, 'dark fruit' flavour and 'bitterness'. The Margaret River wines were high in 'vegetal' aroma and 'chocolate' flavour, and low in opacity, 'cooked fruit' aroma, 'pungency', 'hotness' and 'fruit' after-taste.

The Riverland wines were assessed in a separate tasting and the ANOVA revealed significant differences (p<0.05) between the clones for eight of the 30 attributes, and a further five were nearly significant (p<0.1). The PC1 and PC2 loadings accounted for 57.2% of the variation of the data set in the PCA analysis (Figure 9). The clone R6WV28 was high in 'sweet spice' aroma, 'cooked fruit' aroma, 'dark fruit' aroma, 'earthy' aroma and 'overall fruit' flavour. SARDI 4 and SARDI 7 were high in 'green' aroma and 'chemical'/'plastic' aroma. BVRC 12 was high in opacity, and the clones SA 1654, BVRC 30 and PT 23 were not particularly high in any attribute. The spread of clones across the four quadrants was not associated with grape maturity or wine alcohol, pH or titratable acidity.

Seasonal effects

With only two seasons of data available only a preliminary analysis of the data between seasons was conducted for Chardonnay. Twenty-four sensory attributes across four clones common to three regions were analysed in a balanced factorial design using ANOVA. There were significant differences between the two seasons for 14 of the 24 attributes. The 2013–14 season produced significantly higher scores for yellow colour, the aromas of 'citrus', 'confection', 'box hedge' and 'herbal', 'pungency', 'viscosity' and 'fruit' after-taste, and significantly lower scores for the aromas of 'overall fruit', 'stone fruit' and 'vegetal', and the flavours of 'overall fruit', 'tropical' and 'stone fruit', and 'bitterness' compared with 2014–15. There were no significant differences between the two seasons for the aromas of 'confection', 'floral', 'green',

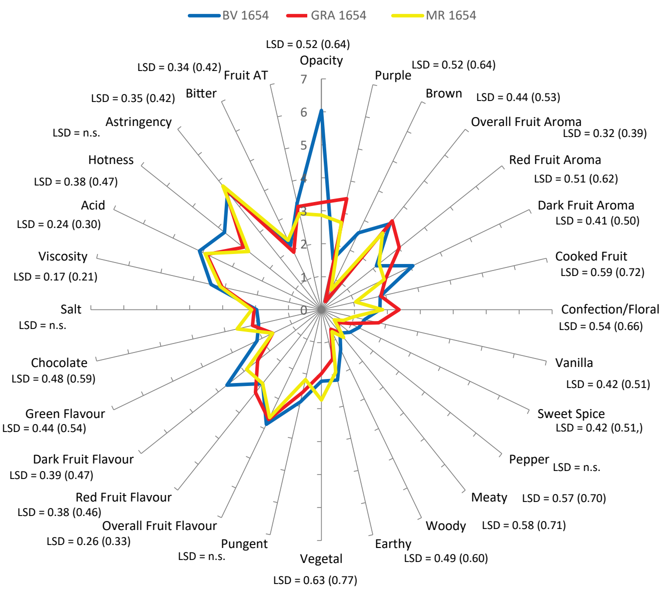

Figure 8. Radar plot of mean scores for attributes from Shiraz clone SA 1654 for each of three regions in 2014-15. MR = Margaret River, GRA = Grampians region, BV = Barossa Valley. LSD is for p=0.05. First LSD is for comparing regions where all wines had duplicate ferments (Barossa Valley and Grampians). LSD in brackets is for comparing regions with duplicate versus single ferments (Margaret River)

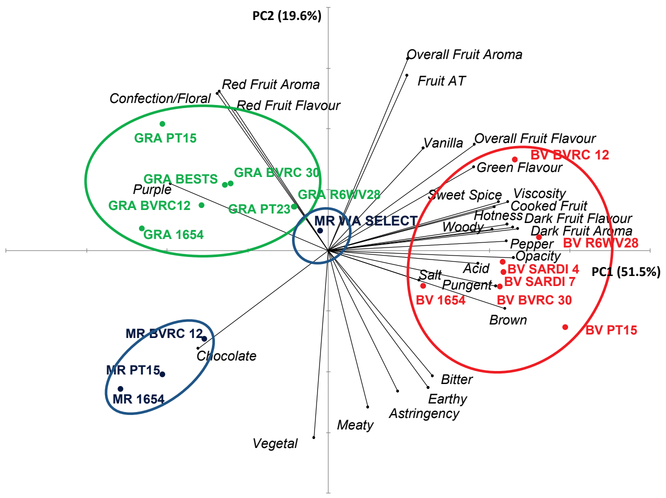

Figure 7. Scores and loadings for PCA bi-plot for Shiraz clones in 2014-15 in the main analysis. Scores are the average of duplicate fermentations. MR = Margaret River, GRA = Grampians region, BV = Barossa Valley

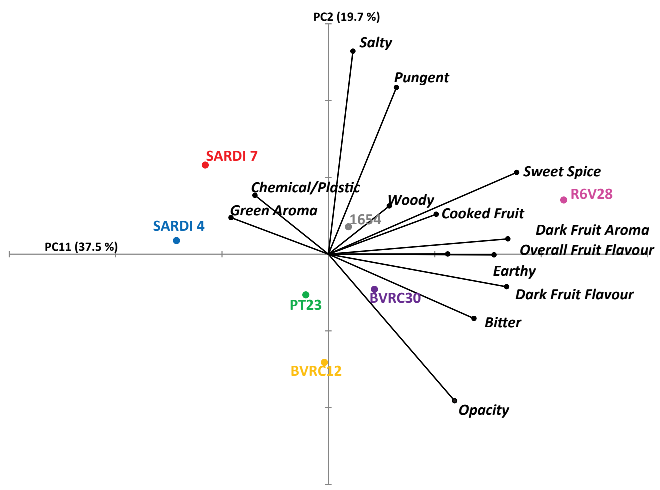

Figure 9. Scores and loadings for PCA bi-plot for significant attributes of the Riverland Shiraz clones 2014-15

'flint' and 'sweaty'/'cheesy', the flavours of 'citrus' and 'green', 'acidity', 'hotness' and 'astringency'. It is not clear what caused the significant differences since there were only minor differences between the two seasons (averaged across the three regions and four clones) for DD, harvest sugar concentrations, and wine alcohol, pH and titratable acidity.

There were significant differences between the regions for nine of the 24 sensory attributes. In most cases there were no significant differences between the Margaret River and Great Southern regions, and the Riverland region was significantly different to both those regions. The Riverland region had higher yellow colour, 'vegetal' aromas, 'sweaty'/'cheesy' aroma and 'hotness' on the palate, and lower 'citrus' flavour and the aromas of 'overall fruit', 'citrus', 'confection' and 'floral'. Where there were significant differences between Margaret River and Great Southern, Great Southern had higher yellow colour and 'citrus' flavour, and lower 'floral' aroma. There were some significant interactions between season and region for nine sensory attributes.

There were significant differences between clones for three sensory attributes. Clone 76 had the highest score for yellow colour, 'flint' aroma and 'tropical' flavour against some or all the other three clones. There was a significant interaction between season and clone for two sensory attributes.

Conclusions

Further wine sensory data for subsequent seasons is being collected and only a partial analysis of the results to date has been conducted so any conclusions here must be regarded as preliminary. For the two seasons so far there are significant differences between many wine sensory attributes related to clone, region and season. An analysis of a subset of the Chardonnay sensory attributes revealed that season produced the greatest number of significant differences (14), followed by region (9) and least of all clone (3) (when pooled over two seasons, three regions and four clones). Further analysis of the data is required to determine what climatic data and/or grape or wine characteristics may be associated with the seasonal differences. Significant differences between Chardonnay in the Riverland region and the two WA regions may well relate to temperature (mean DD over two seasons – Riverland 2,215, Margaret River 1,813 and Great Southern 1,773) since many of the changes in sensory attributes can be related to the hotter conditions in the Riverland, e.g. higher yellow colour and 'hotness' (perceived higher alcohol), lower 'citrus' flavour and lower aromas for 'citrus', 'confection', 'floral', and 'overall fruit' aroma.

Within a season and a region, differences in maturity and wine composition may be influencing some results and creating wines distinctive from the other clones. The target sugar concentration for harvesting has not always been achieved due to the difficulty in coordinating harvesting and delivery to the winemaking facility over long distances. In addition, the trials are in commercial vineyards and harvest is timed to fit with commercial winery requirements. Also, the period between veraison and harvest has become relatively short (around 20 days for some sites) and the rate of sugar accumulation quite high, over 5°Brix per week, making timing of harvesting difficult. Where grapes have been harvested at similar maturities within a region the regional wine scores tend to cluster around similar sensory attributes.

There are instances where clones show consistent sensory attributes across regions, e.g. with Chardonnay in 2013–14, clones 76 and 96 scored consistently higher for 'pineapple' aroma, 'overall fruit' flavour, 'tropical fruit' flavour and 'viscosity' than clones 95 and 277 across four regions. Clone 76 also showed higher 'tropical fruit' flavour over the two seasons suggesting this is a consistent trait with this clone. A more detailed examination of the data for the remaining seasons is required to elucidate further consistencies.

One of the ideas behind this project was to see if regional differences in climate could be used as a surrogate for climate change. The DD over the two seasons studied for the trial site in the Henty region (1,380 and 1,339) is now equivalent to the historical long-term mean (pre-1992) of the Grampians region (1,349), and the DD for the Grampians region for the two seasons in this study (1,450 and 1,427) are now equivalent to the long-term mean for the Great Southern region (1,441) (Gladstones 1992). The other three regions have also shown marked increases in seasonal DD compared with pre-1992. With Chardonnay, Margaret River and Great Southern, both similar DD, show comparable sensory profiles, generally higher in aromas of 'confection', 'floral', 'citrus', 'tropical' and 'stone fruit', 'citrus' flavour, and 'acidity'. The warmer Riverland region generally shows greater 'vegetal' and 'sweaty'/'cheesy' aromas and 'hotness' on the palate. Unexpectedly, the cooler Grampians region also shows 'vegetal' and 'sweaty'/'cheesy' aromas, but without the 'hotness' on the palate. The coolest region, Henty, in the one year of data available, showed predominant aromas of 'green', 'stone fruit', 'tropical' and 'herbal', 'green' flavours and 'acidity'. One might speculate that if the Henty region continues to warm up the Chardonnay will develop more 'confection', 'floral' and 'citrus' characters.

The DD for two of the sites with Shiraz are not that dissimilar (over two seasons: Margaret River 1,833 and 1,713 and Barossa Valley 1,849 and 1,818) yet the sensory profiles of the wines are quite different. Margaret River wines were high in the aromas of 'red fruit', 'chocolate' and 'vegetal', whilst the Barossa Valley wines predominated in aromas of 'dark fruit', 'sweet spice', 'cooked fruit' and 'overall fruit', 'dark fruit' and 'overall fruit' flavour, and 'hotness', 'viscosity', 'pungency' and 'fruit' after-taste. The sensory profiles of the Grampians region wines, the coolest site, were quite similar to those of the Margaret River wines (high in 'red fruit' aroma and low in opacity, 'dark fruit' and 'sweet spice' aromas, 'dark fruit' flavour, and 'viscosity' and 'hotness'). The sensory profile of the Riverland region wines, the warmest site, showed some similarities with the Barossa valley (high in aromas of 'dark fruit', 'vanilla' and 'sweet spice', 'dark fruit' flavour and 'viscosity' and 'hotness') but also higher brown colour, 'cooked fruit' and 'earthy' aromas. Given that the Margaret River site is relatively maritime compared with the other temperate sites, further analysis of the sensory differences between regions will need to consider other factors such as continentality of the climate.

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Genetic diversity in clones of Chardonnay

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Abstract

Since publication of two Pinot Noir genome sequences in 2007, genomics has increasingly contributed to the understanding of grapevine. This has been demonstrated through, for example, integration of genetic and physical maps for use in marker-assisted selection, ongoing annotation efforts and by more clearly defining the history of grape cultivar evolution. Despite its utility, the current Pinot Noir genome data is not representative of the genomic complexity typical of wine-grape cultivars deployed in the field and the available assembly is still highly fragmented. This is characteristic, not only of the grapevine reference genome, but of many genomes assembled using the dominant sequencing technology of the last decade. However, the genomics landscape has changed with the recent introduction of sequencing technologies that promise both improved representation of genomic complexity and increased assembly contiguity. The application of these new sequencing technologies to the assembly of a Chardonnay reference genome is presented. Chardonnay is the result of a centuries old cross between Pinot Noir and the now nearly completely displaced Gouais Blanc. There are many clones of Chardonnay exhibiting variation in a number of viticultural and oenological traits. The genetic diversity between Chardonnay clones, revealed through a comparison with a new Chardonnay reference, and the implications for vineyard diversity will be discussed.

Webcast of this presentation available at http://bit.ly/16thSchmidt.

Introduction

Chardonnay has an unusual genetic heritage, resulting from a cross, centuries ago in north-eastern France, between Pinot Noir and Gouais Blanc (Bowers et al. 1999). The first Chardonnay may have come to Australia as early as 1831 (Nicholas 2006) and this varietal now forms part of the history and popular mythology of winemaking in this country (Farmer 2008; Brebach 2013). The massive expansion of Chardonnay plantings in Australia in the early to mid-1980s coincided with the maturation of clonal selection programs in France, the USA and Australia and the subsequent regional trialling of those clones (Cirami 1993; Wolpert et al. 1994; Goldschmidt and Kenworthy 1995). Despite both the history of clonal selection programs and the relatively recent recognition that clonal selections can have a bearing on Chardonnay wine style, much of Australia's plantings of Chardonnay are a single clone, I10V1, also known as FPS06.

I10V1 was a consistent performer in many of these trials, showing promise as a high yielding clone with moderate cluster weight and vigorous canopy. The availability of virus-free clonal material at this time was a boon for the industry and the move from mass selected vineyards helped cement productivity gains in the sector. However, in retrospect it might now be considered that this phase of viticultural modernisation has limited Chardonnay diversity. The current availability of an expanded set of clones in Australia raises the possibility for a considered expansion of diversity in new or augmented Australian Chardonnay vineyards.

There are now many clones of Chardonnay available to Australian winemakers, exhibiting variation in a number of key viticultural and oenological traits (Cirami 1993; Bettiga 2003; Reynolds et al. 2004; Fidelibus et al. 2006; Anderson et al. 2008; Duchene et al. 2009). That clonal choice can be used to drive wine style is increasingly being recognised not only in the academic space but also in the popular press (Witt 2012). The genetic diversity within Chardonnay clones is being revealed through comparison with a new Chardonnay reference genome.

Since the concurrent publication of two Pinot Noir genomes in 2007 (Jaillon et al. 2007; Velasco et al. 2007) genomics has increasingly contributed to the understanding of grapevines. This has been demonstrated through, for example, integration of existing linkage maps with newer single nucleotide polymorphism (SNP) maps for use in marker assisted selection (Barba et al. 2014), with ongoing annotation efforts (Vitulo et al. 2014), and by more clearly defining the history of cultivar evolution (Vezzulli et al. 2012). Despite the utility of the current Pinot Noir reference genome, it does not represent the complexity typical of wine-grape cultivars deployed in the field and is still highly fragmented. A high degree of fragmentation is characteristic, not only of the grapevine reference genome, but of many genomes assembled using the dominant sequencing technology of the last decade (Snyder et al. 2015).

The team's initial approach to the construction of a reference genome for Chardonnay was, like almost all *de novo* assembled genomes, based on a mix of sequencing by synthesis short read information, mate pair sequence data and, a perhaps not so common approach, sequencing of pooled unbarcoded bacterial artificial chromosome (BAC) libraries. BAC libraries are typically constructed to contain very large inserts (>100,000 bp). The chances of overlapping BACs occurring in any pool were minimised by limiting the number of BACs in each pool. One hundred BAC pools were sequenced (10,000 BACs with an average insert size of 100 kbp) on an Illumina HiSeq 2000. It was anticipated that the large contigs that were generated following initial assembly of data derived using this approach coupled by scaffolding using large insert mate pair data would deliver a reference genome that was a substantial improvement to earlier reference genomes of grapevine. Similar approaches had been used to build reference genomes for oyster (Zhang et al. 2012) and Norway spruce (Nystedt et al. 2013). However, the highly heterozygous nature of grapevine coupled with the high fraction of repetitive sequence it contained confounded this attempt. It has since been shown that longer read lengths and indexing of individual BACs is required to reliably scaffold such long inserts without creating high percentages of chimeric fragments within the assembly (Taudien et al. 2011; Beier et al. 2016).

The genomic landscape has recently changed with two critical developments. The first is the maturation of single molecule long read sequencing technologies such as those developed by PacBio (Eid et al. 2009) and Oxford Nanopore (Lu et al. 2016). The second is the development of assembly strategies that are capable of accommodating this type of information thanks to haplotype-aware assemblers such as Falcon (Chin et al. 2016) and CANU (Koren et al. 2016).

The differences in output characteristics between PacBio and Illumina sequencing technologies are shown in Figure 1. The key feature that distinguishes the two data types aside from the obvious difference in read length is the variation in read length distribution. Short read sequencing is capable of read lengths up to 250 bp using current generation sequencers and all reads in the data set are of exactly the same length. Long read sequencing can have average read lengths 40 times greater than this but the longest reads can be more than 200 times as long. It is primarily these very long reads, greater than 10,000 bp in length, that substantially drive improvements in genome assembly.

Creating genome assemblies from these two very different data types is analogous to completing two jigsaw puzzles, one from a large number of identically sized very small pieces, the other from pieces that are much larger. Placing all those small pieces in a puzzle where there are many repetitive features (think of completing a puzzle that contains a lot of sky) is extremely difficult. Having larger pieces makes the job easier because single pieces can span regions of complexity and have a higher chance of containing sufficient information (colour clues for example) that help to identify the adjoining piece of the puzzle.

The primary effect of being able to assemble a genome from substantially larger pieces is a reduction in the degree of fragmentation of the assembly. Table 1 compares the genome assembly statistics for a range of sequencing techniques applied to grapevine. The minimum possible degree of fragmentation for a grapevine genome is nineteen, which corresponds to the total number of individual chromosomes in this species. The initial effort published by Jaillon et al (2007) resulted in a genome with a relatively low degree of fragmentation. However, this grapevine was an inbred haploid derivative of Pinot Noir bred specifically for genomics. Its low degree of heterozygosity permitted substantial contig consolidation despite the relatively short reads used in the assembly. At completion, this assembly was comprised of more than 14,000 contigs despite the ideal conditions of the source material.

Compare this to the contemporaneous effort of Velasco et al. (2007). Confronted with a cultivar of Pinot Noir used for grape production, with all the intra-organism variation that this entailed, a draft assembly at publication was comprised of more than 66,000 contigs. Seven years later another attempt, this time based on shorter read length, but high coverage Illumina data, was unable to improve on the assembly metrics of earlier efforts (Di Genova et al. 2014).

Jump to 2016 and genome assemblies of heterozygous wild type grapevines based on long read sequence data show greater than 60-fold reduction in fragmentation. These complete genomes are comprised of less than 1,000 contigs (Chin et al. 2016). Manual inspection of each genome element becomes possible. Such assemblies require vastly reduced resources directed toward manual curation and this increases the confidence in the structure of the assembly.

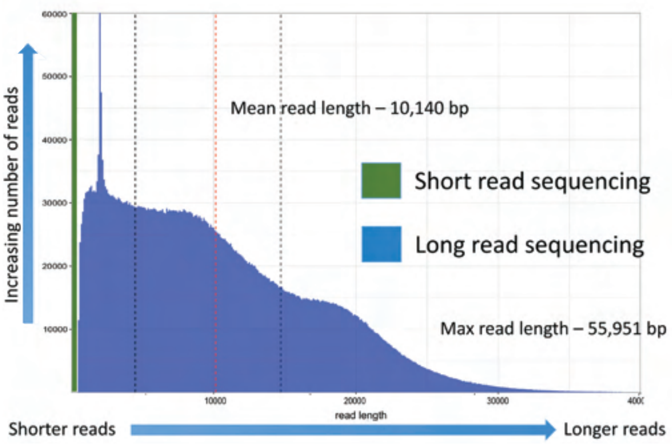

Figure 1. Comparison of read length distributions in Illumina short read and PacBio long read sequence data

Another advantage of long read based assemblies is that they can be haplotype resolved. This means that the two copies of the genome that make up a diploid organism can be disentangled to a large degree. Therefore, the reconstructed genome more accurately represents the true genomic diversity of the individual organism. From an operational point of view this has a key advantage. It allows a better understanding of the association between any two pairs of varying genomic information. The combined effect of multiple allelic variants on the phenotype of an organism is known as compound heterozygosity. When the association between genomic variants is known, it becomes possible to infer the degree of impact that a specific genetic alteration might have (Tewhey et al. 2011).

One of the primary uses for high quality genome reconstructions of the types that have been discussed here is to enable comparison between individuals. Using reference genomes constructed from long read data allows not only an improved contextual framework for the interpretation of SNPs but also the capacity to categorise structural rearrangements such as insertions and deletions, many of which result from the action of transposons. Therefore, the two primary genetic drivers of phenotypic diversity in clonally propagated plants such as grapevine can now be characterised in ways not possible using previous approaches.

How does all of this help the wine community make better use of the available grapevine resources? By understanding the variability between clones of a single variety, tools will be developed to distinguish varietal clones in the field through DNA testing. Insights are being gained into the role that vine propagation plays in generating genomic variation and the nature of grapevine genome stability. It will also be possible to probe the malleability of grapevine genomes in different climatic regions or in response to varying climate. And finally, this work will allow a fresh look at clonal germplasm and enable the benefits of clonal selection programs to be captured to help maximise vineyard diversity.

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Table 1. Grapevine genome assemblies derived from different sequencing technologies and associated assembly statistics

Sequencing project	Sequencing technology	N50 (million bases)	Number of contigs
Pinot Noir (Jaillon et al. 2007)	Sanger	0.102	14,577
Pinot Noir (Velasco et al. 2007)	Sanger and Roche 454	0.015	66,164
Sultanina (Di Genova et al. 2014)	Illumina (100 bp PE)	0.078	63,028
Chardonnay (AWRI 2014)	Illumina (100 bp PE)	0.113	11,282
Chardonnay (AWRI 2016)	PacBio	0.98	978
Cabernet Sauvignon (Chin et al. 2016)	PacBio	2.1	718

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Fungicide resistance in Australian viticulture

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Abstract

Powdery mildew, Botrytis bunch rot and downy mildew are the three most economically important diseases in Australian vineyards. To determine the incidence and severity of fungicide resistance in Australia, samples of all three diseases were collected from vineyards in the main viticultural regions of Australia and tested against a range of commonly used fungicides. Leaf disc bioassays were used to determine the EC_{50} values of Erysiphe necator *(powdery mildew) for sensitivity to pyraclostrobin, penconazole, myclobutanil and tetraconazole, and of* Plasmopara viticola *(downy mildew) to metalaxyl M, mandipropamid and pyraclostrobin. EC_{50} values, minimum inhibitory concentrations and discriminatory dose values were established for* Botrytis cinerea *isolates using a microtitre assay system and mycelial growth assay against fenhexamid, iprodione, pyrimethanil, boscalid, tebuconazole, azoxystrobin and fludioxonil. Representative isolates of each pathogen were genotyped for the presence of known mutations conferring resistance. The mutation linked with quinone outside inhibitor (QoI) fungicide resistance, G143A, was found in isolates of powdery mildew from most viticultural regions. While phenotypic resistance of* E. necator *to the demethylation inhibitor fungicides (DMIs) was not widespread, the mutation linked with resistance, Y136F, was present in approximately 60% of the powdery mildew isolates. Populations of* P. viticola *resistant to metalaxyl have been confirmed in all states except South Australia, and the allele conferring resistance to QoI has been detected.* B. cinerea *resistant populations to fenhexamid, iprodione, boscalid and pyrimethanil were detected. While 54% of sites had no resistance detected, two sites had populations resistant to all four fungicides. The results of the testing so far has confirmed the presence of resistant populations of these three pathogens to many fungicides throughout Australia. However, more work is needed to confirm how these results relate to the potential for field failure.*

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Introduction

Disease management for grapegrowers is critically dependant on fungicides to control the three most economically important diseases in Australian viticulture: powdery mildew caused by *Erysiphe necator*, downy mildew caused by *Plasmopara viticola*, and Botrytis bunch rot caused by *Botrytis cinerea*. Resistance or reduced sensitivity to some of the fungicides used in Australia has been reported for all three pathogens: *E. necator* to Demethylation Inhibitor (DMI) fungicides (Savocchia et al. 2004) and to Quinone outside Inhibitor (QoI) fungicides (Wicks et al. 2013); *P. viticola* to metalaxyl (Wicks et al. 2005); and *B. cinerea* to anilinopyrimidines (Sergeeva et al. 2002), dicarboximides and benzimidazoles (Hall et al. 2001). In filamentous fungi there are three principal known mechanisms of resistance: 1) target site modification, where point mutations in the fungicide target gene result in amino acid substitutions altering the structure of the target protein, and thus reducing the binding affinity of the fungicide to the enzyme. Point mutations have been observed to cause varying levels of cross resistance to different fungicides (Cools and Fraaije 2013); 2) overexpression of the target gene *cyp51* – increased production of the CYP51 enzyme results in a general reduction of sensitivity across all the DMIs (Ishii and Hollomon 2015); 3) increased efflux by overexpression of membrane-bound drug transporters, reducing the accumulation of fungicide at the target site – enhanced efflux tends to result in a phenotype of broad-spectrum resistance across the DMIs and unrelated fungicide classes (Ishii and Hollomon 2015).Testing was undertaken phenologically using leaf disc bioassays (Erickson and Wilcox 1997), a microtitre assay (Stammler and Speakman 2006) or mycelial growth agar assay (Leroux et al. 1999) to determine the EC_{50} (the concentration that inhibits growth by 50%) and the minimum inhibitory concentration values (MIC, the concentration that inhibits growth by 100%). Target gene molecular analysis was used to provide data to support phenotyping results and detect the mutant alleles which confer resistance (Walker et al. 2012).

This paper reports the results of a survey conducted throughout the main viticulture areas of Australia to determine the extent of resistance to selected fungicides in these three pathogens.

Powdery mildew

Leaves and bunches infected with powdery mildew were supplied by growers and consultants from the major grapegrowing regions around Australia. Plant material ranged in age from growth stage EL 19 to EL 36 with both recently sporulating and old colonies present. Powdery mildew was successfully subcultured from both leaves and bunches; however, much greater success was achieved with younger plant material. Fewer sporulating colonies were observed with older plant material and the viability of powdery mildew spores was lower in bunches with old infections resulting in difficulties obtaining a viable culture.

Isolates of powdery mildew were tested for sensitivity to the fungicides in the QoI and DMI groups using a leaf disc bioassay. Commercial grade fungicides, namely Cabrio® (pyraclostrobin 250g/L ai, BASF Australia Ltd), Topas® EC (penconazole 100g/L ai, Syngenta Crop Protection), Domark® (tetraconazole 40g/L ai, Sipcam) and Mycloss™ Xtra (myclobutanil 200g/L ai, Dow Agro Sciences), mixed in sterile double-distilled water (SDDW), were diluted to 5–6 different concentrations between 0.001 and 16 µg/mL, with SDDW used as the control.

Young, glossy leaves cv. Cabernet Sauvignon were collected from plants grown in a controlled environment room and surface sterilised in 0.5% bleach (White King®) for 3 mins, then washed 3–4 times in sterile distilled water. Ten mm diameter leaf discs were cut from the leaves and discs were placed immediately into a Petri dish containing SDDW until needed. Discs were removed from SDDW, randomised and placed abaxial surface upwards in a 140 mm diameter Petri dish lined with sterile filter paper containing 9 mL of fungicide at a given concentration. After soaking in fungicide solution for 30 mins for

Cabrio® and Topas® 100 EC and 60 mins for Mycloss™ Xtra (Wong and Wilcox 2002) discs were removed and blotted dry between two layers of sterile paper towel. Discs were placed adaxial surface upwards in 60 mm diameter Petri dishes containing 1.5% Water Agar (TWA) amended with 2.5 µL/mL of pimaricin (2.5% aqueous suspension, Sigma Aldrich). Three discs per dish and 3 dishes per fungicide and isolate combination were used.

The discs were left overnight and inoculated with *E. necator* the following morning. Each disc was inoculated in the centre with ~ 300 powdery mildew spores, by touching the end of a sterile cotton tip on to the surface of a 14-day-old sporulating colony of *E. necator* to collect spores on the cotton tip and depositing these spores on the centre of a disc by touching once with the cotton tip.

After 14 days incubation at 22°C, 12/12 hr day/night under fluorescent light, each leaf disc was assessed for the percentage of leaf area colonised by powdery mildew. The EC_{50} for each isolate was calculated by Probit analysis using Genstat 15th edition (VSN International, UK).

Fungal DNA was extracted from infected leaf material using a CTAB extraction method (Cubero et al. 1999). The subsequent DNA extractions were used as templates to amplify the complete *cyp51* gene (Délye et al. 1997) and the *cytb* region associated with the G143A mutation. Amplification was carried out using high-fidelity Phusion polymerase (New England Biolabs) according to the supplier's protocol. Un-purified amplified DNA was sent to Macrogen Inc. (Korea) for sequencing. *Cyp51* sequences were then aligned to a reference sequences (GenBank no. U72657.2) while *cytb* sequences from sensitive and resistant isolates were aligned to identify any mutations.

Our results indicate that resistance to QoI fungicides was widespread: 53% of the samples phenotypically tested using the bioassay showed reduced sensitivity, and the G143A mutation, which is associated with QoI resistance, was present in 86% of samples (of only 36 genotyped to date) (Table 1). Correlation of phenotypic test results with fungicide application in the last two years showed that a higher EC_{50} was evident in sites where pyraclostrobin has been applied in the previous 2 years (Figure 1).

Table 1. The percentage of sites sampled between 2013 and 2016 with isolates of *Erysiphe necator* showing reduced sensitivity in a leaf-disc assay and the detection of the gene associated with resistance to pyraclostrobin (Cabrio®), penconazole (Topas®), myclobutanil (Mycloss™ Xtra) and tetraconazole (Domark®)

Fungicide		No. sites tested	Sites with reduced sensitivity (%)	Sites with mutant present (%)
pyraclostrobin (Cabrio®)	2013/14[1]	36	36	86
	2014/16[2]	58	63	?
penconazole (Topas®)[1]		38	0	68
myclobutanil (Mycloss™ Xtra)[1]		66	14	84
tetraconazole (Domark®)[1]		21	0	82

[1]EC_{50} >1.0 µg/mL
[2]Discriminatory dose, >20% growth at 1.0 µg/mL

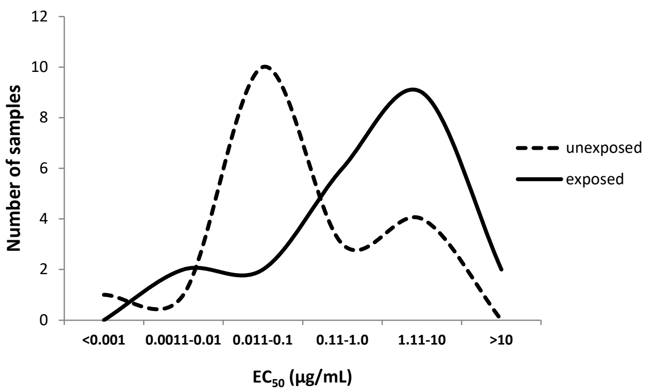

Figure 1. The frequency of EC_{50} values from samples of *Erysiphe necator* tested for sensitivity to Cabrio® (pyraclostrobin) in sites either exposed or not exposed to the fungicide in the previous two years.

The *cyp51* gene mutation, Y136F, which is linked with DMI resistance, was detected in 68%, 82% and 84% of samples tested against the DMI fungicides penconazole, tetraconazole and myclobutanil respectively. However in the leaf-disc assay, reduced sensitivity was detected in only 14% of the isolates tested with myclobutanil and none in those tested with either penconazole or tetraconazole.

Downy mildew

Leaves infected with downy mildew were supplied by growers and consultants from the major grapegrowing regions around Australia. There were only sporadic outbreaks in the first year of testing, and in the second year the infection was late in the season; of the 42 samples received only 18 were able to be tested.

A leaf-disc assay used to phenotype isolates for fungicide sensitivity was similar to that described for powdery mildew, using cvs. Sultana or Tempranillo. A 10 µL suspension (10^6 spores/mL) was placed onto each leaf disc and incubated for 24 h in the dark at room temperature (~22°C). The leaf discs were dried for 2–3 h then incubated at 23°C for 12 h light/dark. The per cent of leaf infection was assessed at 7 days and EC_{50} determined. The fungicides tested were mandipropamid (Revus®) at 0.001 to 10 µg/mL, metalaxyl M (Ridomil®) at 0.05 to 10 µg/mL, and pyraclostrobin (Cabrio®) at 0.001 to 0.1 µg/mL.

Fungal DNA was extracted from leaf tissue infected with downy mildew using a DNeasy® Plant Mini Kit (Qiagen) and the presence and frequency of the G143A mutation was determined using next generation sequencing of a 180 bp amplicon that surrounded the G143A mutation.

When tested on leaf material, resistance to metalaxyl was detected in 12 (67%) of the samples, confirming that resistant populations to metalaxyl now occur in Victoria, Western Australia and Tasmania as well as New South Wales. One sample showed reduced sensitivity to mandipropamid. Testing of pyraclostrobin on leaf material needs to be repeated; however, the G143A mutation was detected in three of the 23 sites tested.

Botrytis

A total of 742 single spore isolates of *B. cinerea* was established from grape material collected between 2013 and 2016 from 114 sites throughout Australia. A subset of 54 of these isolates was screened against six fungicides using the microtiter plate method to establish EC_{50}, MIC values and to define a discriminatory dose for each fungicide. Technical grade azoxystrobin, fenhexamid, boscalid, pyrimethanil, iprodione and tebuconazole was dissolved in absolute ethanol and seven dilutions between 0.01 and 10 µg/mL of each fungicide were evaluated. Re-testing of isolates that exhibited a significant reduction in sensitivity was carried out with at least 1 or more ranges of increased concentrations of fungicides, up to 150 µg/mL. In each well 0.5 µL of fungicide stock and 0.5 µL of 10% Tween20 was added to 94 µL of liquid media. Five µL of *B. cinerea* spore suspension (10^5/mL) was added to 95 µL of the media mixture resulting in a final concentration of 5000 spores/mL. Each plate was wrapped in parafilm to prevent evaporation and incubated. There were two biological replicates each with two technical replicates for each isolate. Immediately following the addition of the spore suspension the optical density (OD) was measured at 450 nm wavelength in a Synergy HT microplate reader (BioTek). After 72 h incubation at room temperature in darkness, or 96 h for pyrimethanil, the OD was again measured. Final OD values were adjusted by subtracting the readings taken immediately following the addition of the spore suspension. The EC_{50} was estimated by linear regression of percentage reduction in OD (compared to zero fungicide control) against the log concentration of the fungicide.

Results from the microtitre screen allowed discriminatory doses to be defined for each fungicide except for fludioxinil. Discriminatory dose values for fludioxinil were defined using a smaller scale microtitre screen using three isolates only. The discriminatory dose values were defined as 1 µg/ml fenhexamid, 3 µg/ml iprodione, 0.4 µg/ml pyrimethanil, 1 µg/ml boscalid, 5 µg/ml tebuconazole, 5 µg/ml azoxystrobin and 1 µg/ml fludioxinil. For some of the fungicides there was a clear delineation between the resistance and sensitive isolates, such as with axozystrobin (Figure 2). This is consistent with the qualitative resistance shown by this group of fungicides (Brent and Hollomon 2007). A wider range of sensitivities were found for other fungicides such as pyrimethanil (Figure 3).

Six hundred and ninety-two isolates were subsequently tested in a mycelial growth assay using discriminatory doses of fungicide active ingredient as previously described. After three days incubation in the dark, fungal growth was scored as either present (resistant isolate) or absent (sensitive isolate).

B. cinerea DNA was extracted from fungal cultures using a Qiagen biosprint method (Qiagen), sequenced and aligned to reference sequences to assess for known mutations. Alternatively a cleaved amplified polymorphic sequence (CAPS) method using restriction enzymes Taq I (Oshima et al. 2006) and Sma I was used to genotype a large number of isolates resistant to iprodione. A number of mutations were found in the target genes; G143A in the *cytb* gene (QoI target), H272R or H272Y in the sdhB gene (boscalid target), I365S (CAPS-Taq I) or Q369P (CAPs-Sma I)/N373S in the bos-1 gene (iprodione target), F412S in the erg27 gene (fenhexamid target), L8P or D416E in cgs gene (pyrimethanil target).

Botrytis cinerea resistant populations to fenhexamid, iprodione, pyrimethanil, boscalid, fludioxinil, tebuconazole and azoxystrobin

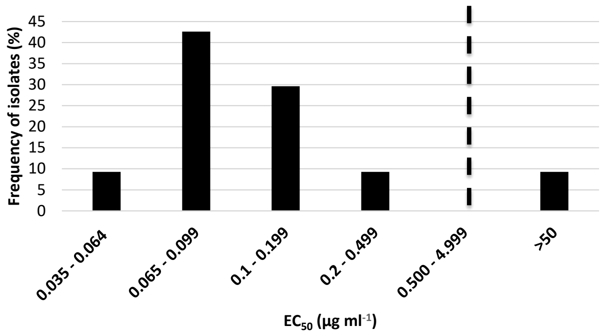

Figure 2. The frequency of EC_{50} values from samples of *Botrytis cinerea* tested for sensitivity to azoxystrobin by microplate test. The vertical dashed bar represents the discriminatory EC_{50} value between sensitive and resistant isolates. EC_{50} values higher than the discriminatory dose are associated with a mutation in the target gene that is linked with resistance.

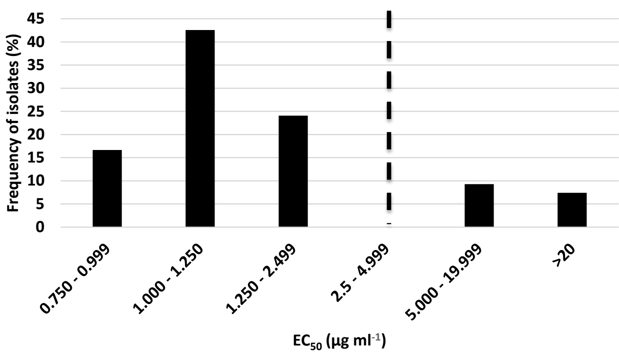

Figure 3. The frequency of EC_{50} values from samples of *Botrytis cinerea* tested for sensitivity to iprodione by microplate test. The vertical dashed bar represents the discriminatory EC_{50} value between sensitive and resistant isolates. EC_{50} values higher than the discriminatory dose are associated with a mutation in the target gene that is linked with resistance.

were detected in 2.0, 11.3, 7.1, 2.7, 0, 0.13, and 5.2% respectively of the 114 sites tested. While 43 (38%) sites had no resistance detected, 19 (17%) sites only had isolates that exhibited resistance to one fungicide group, 17 (15%) sites had at least one isolate resistant to two or three fungicide groups and 10 (9%) sites had at least one isolate resistant to four or five fungicide groups.

Conclusion

Field failure of fungicides has many potential causes, including incorrect timing of application and inadequate coverage. However, results of this research indicate resistant strains of these pathogens to currently used fungicides are present in Australian vineyards and careful management of fungicide programs is needed to ensure that these strains do not become a significant problem. Further research is needed to understand the relationship between these laboratory testing results and field efficacy. Significant gaps in knowledge have been identified during this project which need to be addressed for this relationship to be elucidated.

Current resistance management recommendations should be followed to delay or prevent resistance developing. Using alternative chemistry either as a mixing partner or in alternation is advisable; however, growers should consult with the agrochemical companies to ensure compatibility of mixing partners. While all DMI fungicides are within the same resistance management group, there are differences in the activity of each compound, and hence differences in the resistance levels in the populations. It is advisable not to rely on the same product for an extended period, but swap to an alternative DMI product within the applications allowed under the resistance management strategies.

To further reduce the risk of resistance developing, apply fungicides effectively at the correct rate at the correct time and with good coverage, and avoid applying single site action products when there is a heavy infestation of the disease.

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Innovations in the management of grapevine trunk diseases

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Abstract

The grapevine trunk diseases eutypa and botryosphaeria dieback contribute to grapevine decline worldwide, reducing vineyard productivity and longevity. The causal fungi infect vines through pruning wounds and colonise wood, causing dieback and eventual vine death and, in the case of eutypa dieback, stunting and yellowing of shoots and leaves. Due to the predominance of susceptible cultivars and ageing vineyards, trunk diseases are becoming more prevalent in Australia, threatening the wine industry which contributes $40 billion to the Australian economy. Innovations in the management of trunk diseases are providing positive outcomes for the long-term sustainability of the industry. Cultural practices, such as the timing of pruning to avoid rainfall, can reduce the likelihood of infection, and disease control can be achieved both preventatively (through pruning wound treatment) and curatively (by remedial surgery). Removal of infected wood allows vines to be rejuvenated, taking advantage of established root systems to return to full production within a few years. Early adoption of preventative strategies to protect pruning wounds from infection is critical to managing trunk diseases effectively into the future. Paints and pastes are registered for protection of large wounds made during reworking, along with fungicide and biocontrol products for spray application to annual pruning wounds. The timing of application of wound treatments is a major focus of current research which, together with knowledge being generated on inoculum dispersal, wound susceptibility, sources of disease tolerance in germplasm and the effects of water stress, will contribute to sustainable management of grapevine trunk diseases for vineyard longevity.

Webcast of this presentation available at http://bit.ly/16thSosnowski.

Symptoms

Eutypa dieback is evident in the appearance of stunted, yellowing shoots, cupped leaves with necrotic margins and cordon dieback (Figure 1). Foliar symptoms may vary from year to year (Sosnowski et al. 2007b). Bunches on stunted shoots ripen unevenly, are small and, in severe cases, shrivel and die. Cross-sections of cordons with stunted shoots reveal dark brown, wedge-shaped zones of dead wood (Figure 2). Several years after infection, cordons begin to die back and cankers (sunken dead areas) develop on the outside of cordons and trunks. Vines die when the infection girdles the trunk.

Symptoms of botryosphaeria dieback are similar to those of eutypa dieback with the exception of the characteristic foliar symptoms. Spurs often show reduced growth followed by dieback of cordons and trunks, with cross-sectional staining observed in infected cordons and trunks (Figure 3).

Figure 2. Wedge-shaped zone of brown, dead wood (left) and external canker (right) on trunk of grapevine with eutypa dieback

Figure 3. Botryosphaeria dieback symptoms comprising reduced foliage and dieback (top) and wedge-shaped zone of brown, dead wood (bottom)

Distribution

Eutypa and botryosphaeria dieback occur worldwide (Carter 1991; Úrbez Torres 2011). In Australia, surveys have confirmed the presence of eutypa dieback on grapevines in South Australia,

Figure 1. Eutypa dieback symptoms comprising stunted shoots, cupped leaves with dead margins and cordon dieback

Victoria, Tasmania and southern NSW. Botryosphaeria dieback has been reported in most major wine regions of Australia but is most common in the warm climatic regions of Western Australia and New South Wales (Taylor et al. 2005, Savocchia et al. 2007, Pitt et al. 2010).

Dieback has been recorded on vines as young as five years in south-eastern Australia (Figure 4) and eutypa dieback foliar symptoms on vines as young as seven years (data not shown). It can take 3–8 years for foliar symptoms to appear after infection has occurred (Carter 1978; Tey-Rulh et al. 1991), suggesting that infections may occur from the first year of pruning. Therefore, it is important to protect pruning wounds from infection from the first pruning season. Incidence of symptoms increase with age, with some vineyards recorded to have more than 80% of vines symptomatic by 15–20 years of age. As vines age, they are more likely to become infected via successive pruning or reworking events and symptoms become more severe as the fungus progressively colonises the wood of infected vines. However, some older vineyards have little dieback which may be due in part to varietal susceptibility but also the result of effective disease management.

Varietal susceptibility

All *Vitis vinifera* varieties can be infected but foliar and dieback symptoms can vary, and are most pronounced in the commonly planted Cabernet Sauvignon, Shiraz and Sauvignon Blanc (Figure 5; Sosnowski et al. 2013a; Sosnowski et al. 2016a). Furthermore, recent observations have revealed variation in expression of symptoms among clones of the same variety. Inoculation of wounds with eutypa and botryosphaeria dieback pathogens has confirmed that the extent of colonisation in canes varies among varieties and rootstocks, including scions of the same variety grafted onto different rootstocks. There is evidence that lignin content and xylem diameter may influence the susceptibility of varieties to infection by trunk disease (Rolshausen et al. 2008; Pouzoulet et al. 2013; Hamblin 2015; Hamblin et al. 2016). Further research in this area may provide decision support tools for new planting selections as part of integrated management of grapevine trunk diseases.

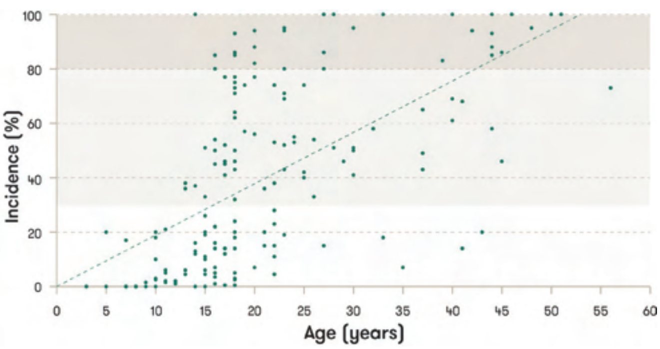

Figure 4. The effect of vineyard age on incidence of vines with cordon dieback from a survey in south-eastern Australia in 2012. Shading indicates potential level of yield loss based on Shiraz (Wicks and Davies 1999; dark grey-high >2.6 t/ha, grey-medium 0.8-2.6 t/ha, light grey-low <0.8 t/ha).

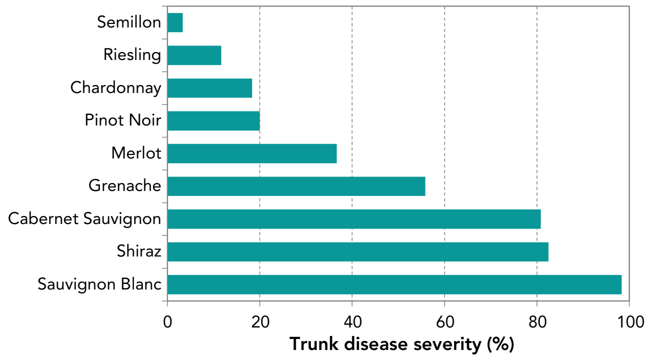

Figure 5. Mean severity of grapevine trunk disease observed over two seasons in 30 to 35-year old vines in the Nuriootpa Research Centre germplasm collection in Barossa Valley, South Australia

Disease cycle and predisposing factors

Spores are released from old, infected wood within two hours of at least 0.2 mm (botryosphaeria dieback) or 2 mm (eutypa dieback) of rain or irrigation, and continue to be released for up to 36 hours after rain has stopped (Carter 1991; Úrbez Torres 2011). Around 12 days later a new generation of spores can be produced and ready for release. Spores of *E. lata* can be carried in wind up to 50 km to infect open wounds (Ramos et al 1975; Petzoldt et al. 1983), whereas spores of botryosphaeria dieback pathogens are carried predominantly in rain splash for much shorter distances within a vineyard (Úrbez Torres 2011). Large wounds, typically on older vines, provide a greater surface area for spores to land on, take longer to heal and are considered more vulnerable to infection than small wounds on young vines. Mature spur-pruned vines have been reported to have greater incidence of eutypa dieback foliar symptoms but lower mortality than that of mature cane-pruned vines (Dumot et al. 2012). This may be due to a greater number of wounds on spur-pruned vines but, as wounds on cane-pruned vines are larger and nearer the crown, the fungus could rapidly spread into the trunk (Figure 6). The total wound surface area typical of various pruning styles is the subject of ongoing research.

Wounds are most susceptible to infection immediately after cuts are made and have been reported to remain susceptible to *E. lata* for up to six weeks (Chapius et al 1998*)*, and for up to four months to botryosphaeria dieback pathogens (Úrbez Torres 2011), when inoculated with large doses of spores. Recent preliminary data indicate that in Australia wounds on grapevines are most susceptible to infection by trunk disease pathogens during the first 2–3 weeks post-pruning, suggesting this to be the most important period for wound protection (Ayres et al. 2016b), and that susceptibility varies little throughout the pruning season. Future research will evaluate the duration of susceptibility of pruning wounds in different climatic zones and at much lower spore doses, more closely reflecting disease pressure in natural conditions.

Spore release is reported to be greatest later in the pruning season, but wounds are less susceptible in late winter and spring when they heal most quickly (Carter 1991). There is also more competition from

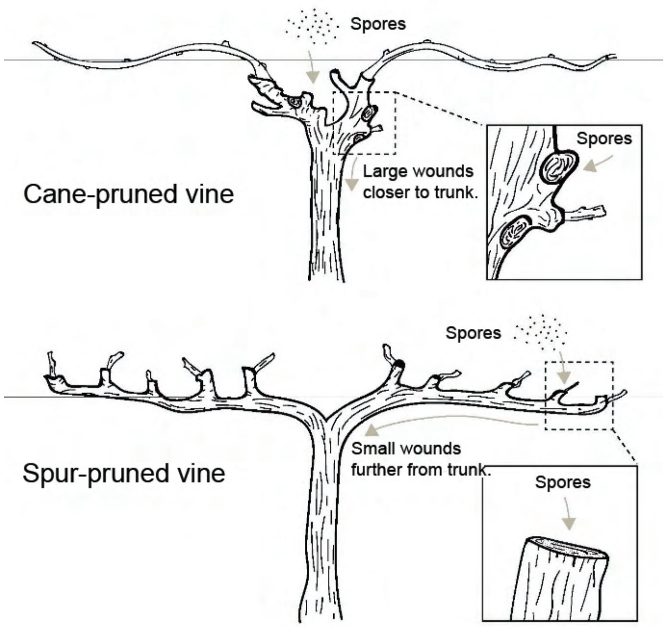

Figure 6. Spur- and cane-pruned vines, showing wound size and proximity to trunk, factors which have an influence on the progression of dieback (Wine Australia 2016)

naturally occurring beneficial microorganisms later in the season, and sap flow may 'flush out' spores from the vascular tissue. Recent evidence from wine regions around the world suggests that inoculum dispersal and wound susceptibility can vary greatly between different climatic environments (Úrbez Torres 2011). Research is underway to monitor spore dispersal in representative Australian climatic regions (Billones-Baaijens et al. 2016). Molecular techniques have been developed, Burkard spore traps deployed, and data are being collected to improve understanding of the timing and duration of spore release and its relationship with climatic conditions. This information will provide decision support for timing of pruning and wound protection treatments for management of trunk disease.

In mature vines, dieback due to *E. lata* has been reported to advance up to 5 cm/year (Sosnowski et al. 2007a), and the corresponding rate for botryosphaeria dieback pathogens is 4–8 cm/year (Pitt et al. 2013). Vines dieback towards the base of the trunk, reducing the transport of water and nutrients to foliage, and eventually die altogether. Foliar symptoms of eutypa dieback are caused by toxic fungal metabolites produced in the wood and transported to the foliage (Moller and Kasimatis 1981; Tey-Rulh et al. 1991; Molyneux et al. 2002; Mahoney et al. 2005).

Alternative hosts

E. lata is known to infect 88 perennial plant species, including stone fruit, pome fruit, citrus, black currant, fig, olive, pistachio, walnut, quince, persimmon, willow, poplar, oak, hawthorn, ivy, ceanothus, oleander, peppercorn and rose (Carter 1991). Dead, diseased branches of these plants may provide a source of spores for nearby vineyards. Apricot is a common host of *E. lata* and disease appears as gummosis. *E. lata* has not been recorded on native Australian plants. Species that cause botryosphaeria dieback are ubiquitous in the environment, and can infect a wide range of annual and perennial plant species, including Australian native species (Úrbez Torres 2011).

Cultural practice

Pruning in wet weather should be avoided and preferably delayed to late winter when wounds heal more rapidly and sap is flowing. Removal of dead wood from grapevines and alternative hosts in and around the vineyard will reduce the potential inoculum level. The amount of infection can be reduced by double pruning, a practice in which mechanical pre-pruning is used to leave long spurs in early winter followed by hand-pruning to short spurs in late winter (Weber et al. 2007). Contamination of pruning tools is not a major means of spreading trunk disease and the use of fungicide to protect wounds will eliminate possible infection. Removal of watershoots in spring may lead to sporadic infection (Lecomte and Bailey 2011), so it is recommended that shoot thinning in wet weather, when pathogen spores are present, be avoided.

Abiotic stress

Vines subjected to a combination of extreme heat or cold plus low or high soil moisture in a controlled environment growth chamber displayed more severe foliar symptoms than other treatments, but no difference in wood colonisation by *E. lata* was observed (Sosnowski et al. 2011a). Furthermore, field experiments suggested that water-stressed vines in a warm, dry environment may be more susceptible to infection of pruning wounds by *E. lata* than vines receiving standard watering. However, recent research contradicts this observation, showing no greater incidence of wound infection or colonisation of canes in water-stressed vines than in well-watered vines in a warm, dry environment (Sosnowski et al. 2016b). In fact, for *E. lata*, colonisation in well-watered vines was greater than in vines subjected to water deficit. Sosnowski et al. (2007b) also reported increased foliar symptoms following periods of high rainfall, and the physiological reasons for these observations are subject to ongoing research.

Wound protection

Table 1 lists the products available for use as pruning wound treatments and which have been registered to control eutypa dieback.

Paints and pastes, which need to be applied by hand, are the most effective treatments for preventing infection of pruning wounds and are recommended for use on large wounds. The application of acrylic paints and wound sealants containing fungicides, such as Greenseal paste (containing tebuconazole) and Garrison Rapid (cyproconazole + iodocarb), have been reported to control eutypa and botryosphaeria dieback pathogens effectively (Sosnowski et al. 2008; Pitt et al. 2012; Sosnowski et al. 2013b).

Fungicides can be applied efficiently to pruning wounds with commercial spray machines (Sosnowski et al 2013b; Ayres et al. 2014, 2016c; Figure 7), but it is important to direct nozzles to target the pruning wound zone and use high spray volumes (600 L/ha) to maximise coverage of wounds. Fluazinam, tebuconazole and pyraclostrobin have been reported to control infection of pruning wounds by eutypa and botryosphaeria pathogens (Pitt et al 2012; Sosnowski et al 2013b; Ayres et al. 2016c). The timing of application for these fungicides is currently under investigation, with preliminary data revealing both curative and preventative properties for each for the control of eutypa and botryosphaeria dieback pathogens (Ayres et al. 2016a).

Biological control agents, such as the fungi *Trichoderma* spp. and *Fusarium lateritium* and the bacterium *Bacillus subtilis,* have controlled *E. lata* in trials worldwide (Carter 1991), but the results have been variable and control is often less effective than fungicides, paints and pastes. Although biological control offers long-term protection, the time required for biological control agents to colonise the wound creates a window of susceptibility to infection by *E. lata*.

Table 1. Treatments available for application to wounds to control eutypa dieback. Follow instructions on label when using registered products.

Treatment	Trade name	Active ingredient	Application method
Paint/paste	Acrylic paint	n/a	Paint brush
	Greenseal™	Tebuconazole	Bottle top applicator
	Garrison Rapid®	Cyproconazole + Iodocarb	Bottle top applicator
Fungicide	Emblem®	Fluazinam	Sprayer
	Gelseal™	Tebuconazole	Sprayer
Biological	Vinevax™ Wound Dressing	*Trichoderma atroviride*	Paint brush/Sprayer

Figure 7. Application of pruning wound protectant with a recycle sprayer

Garlic extract and lactoferrin (an anti-microbial protein by-product from the dairy industry) were also effective as wound protectants in evaluation trials (Ayres et al. 2014, 2016c).

Remedial surgery

Vines showing foliar and/or dieback symptoms in spring should be tagged and all infected wood removed by remedial surgery at any time of the year. Discoloured cordon and trunk wood should be cut out and a further cut made at least 10–20 cm below to ensure all infected wood is removed. Making cuts low down on the trunk will improve the likelihood of eradicating the pathogen from the vine, which was demonstrated for eutypa dieback (Sosnowski et al. 2011b). Resulting wounds should be protected with paints and pastes, as described above. Cordons and trunks can be retrained from water-shoots, returning vines to full production within a few years (Figure 8). Current research is evaluating the effectiveness of remedial surgery for botryosphaeria dieback affected vines (Savocchia et al. 2016).

When infection has reached ground level in trunks of own-rooted vines, layering can be used to self-rejuvenate vines (Ahrens 2010). Healthy canes can be taken from a neighbouring vine to replace a diseased or dead vine.

Conclusions

Grapevine trunk diseases have become prevalent in Australia. Cultural practices are important as part of an integrated management strategy, and disease control is best achieved through early adoption of preventative strategies for wound protection. As a result of research, numerous products are now registered for wound protection and some can be applied efficiently using spray machinery. For vineyards with existing disease, removing infected wood by remedial surgery can provide curative control.

Current research is developing localised knowledge on inoculum dispersal, wound susceptibility and timing of fungicide application to provide optimal control of grapevine trunk diseases which, together with potential sources of disease resistance and better understanding of the effects of water stress, will contribute to vineyard longevity.

Future research efforts will be directed towards expanding our understanding of the dynamics of spore dispersal by grapevine trunk disease pathogens and integration of findings with developing technology for real-time spore threat alert systems. With recent advancement in the development of highly sensitive molecular diagnostic techniques, future research will also focus on improving our understanding of the thresholds of pathogens in grapevine propagation material that lead to disease expression, and the influence of abiotic stresses during vineyard establishment.

Figure 8. Vine with stunted shoots on the left cordon emerging from high on the trunk above infected wood and healthy shoots on the right cordon emerging from the bottom of the trunk below infected wood

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Targeted manipulation of vine balance: does vine balance directly affect fruit composition?

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Abstract

Vine balance is a concept describing the relationship between carbon assimilation (usually estimated using a measure of canopy size, such as pruning weight) and utilisation of the resulting carbohydrates for fruit production (usually estimated using harvest yield). Manipulating vine balance through leaf area or crop load adjustments affects the proportion of the vine's carbohydrate production required to mature the fruit. It is commonly considered that composition of the berry, and resulting wine, is strongly affected by vine balance.

Field manipulations of vine balance were replicated in three contrasting regions, Hilltops, Murray Darling and Langhorne Creek, for three seasons. These were early defoliation (pre-capfall), late defoliation (pre-veraison), 50% crop removal (pre-veraison) and minimal pruning. Defoliation was also simulated in mature, fruiting, potted vines at Wagga Wagga, by enclosing whole vines in chambers and supplying them with low CO_2 air. This allowed the impact of defoliation on vine carbon assimilation to be separated from any impact on bunch exposure.

Changing vine balance affected the fruit maturation rate, but had a less consistent effect on fruit composition. Late defoliation (higher ratio of fruit load to canopy size) reduced total anthocyanin content, despite elongating the maturation period, whereas crop removal had little effect. Interestingly, early defoliation had a limited effect on vine balance, but resulted in increased total tannin content. In both cases, it is possible that the observed compositional effects were caused by changes in bunch environment. Reducing carbon assimilation in the chamber experiment also reduced maturation rate, but did not affect the relationship between sugar and anthocyanin concentrations in the berry. Overall, there was no conclusive evidence that the changes in vine balance achieved had a direct effect on fruit composition.

Webcast of this presentation available at http://bit.ly/16thEdwards.

Introduction

The importance of 'balanced' vines for the production of fruit with optimal quality is widely acknowledged and significant resources are expended in achieving a vine balance deemed appropriate for a specific vineyard and product. However, the concept of vine balance is an empirical one and defies a clear and specific definition. What this and other definitions have in common is an acknowledgement of the role of the canopy in providing carbon for the ripening of fruit and the volume of fruit in generating a demand for that carbon. In essence, vine balance is a source-sink relationship.

For a given climate or environment, carbon fixation by the vine depends on photosynthetic capacity and light absorption by the canopy, with canopy size being the major determinant for vines that are not subject to significant water or nutrient constraints. Consequently, indices of vine balance typically include canopy size, or a surrogate of it, as well as crop load. The most common example being the Ravaz Index (e.g. Bravdo et al. 1984), which is simply the ratio of yield to pruning weight. As both canopy size and yield (or potential yield) can be measured directly, such a definition of vine balance can be determined objectively. This assists in the reliable and repeatable manipulation of vine balance in the vineyard and an assessment of the effects of this. For the purposes of this study, where leaf area has been calculated from measurements of canopy light interception, we are defining vine balance as the ratio between crop load (kg) and canopy area (m^2); this also has the advantage of being a fairly widely used viticultural index, if not as common as the Ravaz Index.

Decades of research have demonstrated that the rate of sugar accumulation in the berry and the development of the secondary metabolites, such as anthocyanins, flavonols and condensed tannins (proanthocyanidins) are highly correlated in both field grown vines (e.g. Pirie and Mullins 1977) and artificial environments (e.g. Larronde et al. 1998). Furthermore, Dai and colleagues (2014) demonstrated that sugar supply to the berry can initiate anthocyanin production in the absence of additional hormonal signals. Manipulating vine balance, through canopy or yield adjustments, thereby altering carbohydrate supply to the fruit, clearly has the potential to alter berry composition, but the question is raised as to what extent, if any, the accumulation of sugar and those other metabolites can be separated. If not, then it could be argued that the main effect of vine balance is on harvest date; if so, then manipulation of vine balance may allow more targeted effects to drive different wine styles.

The photosynthetic supply of carbon is also not the sole driver of berry composition, and the potential influence of fruit exposure and canopy microclimate is widely recognized. The concentration and type of phenolic compounds in berry skins, for example, can be influenced by both temperature and light, with temperatures above 35°C detrimental to anthocyanin production, and light affecting both the type of anthocyanins and accumulation of flavonols (Spayd et al. 2002; Downey et al. 2004). Superimposed on the source-sink and carbohydrate supply component of vine balance is, therefore, an effect of the fruit microclimate, which may vary between vineyards according to specific management practices or differences in climate.

This study has combined replicated field manipulations of vine balance across three different viticultural regions with whole vine chambers that reduce vine carbon uptake through scrubbing of CO_2 from the air, to manipulate vine balance independently of fruit environment. This approach allowed direct (carbon availability to the crop) and indirect (e.g. changes in fruit light environment) effects of vine balance management to be separated and the relative importance of those effects investigated. We were particularly interested in understanding the mechanisms underlying vine balance effects on fruit composition and, from a practical vineyard perspective, what canopy or crop management techniques can be imposed for reliable and predictable wine quality responses.

Materials and methods

Field sites

Three trial sites were established in commercial vineyards prior to the start of the 2013/14 growing season: Cleggetts, Langhorne Creek, SA; Deakin Estate, Murray Darling, Vic.; and Barwang, Hilltops, NSW. Each site was part of an existing block, planted with Shiraz vines during the 1990s. At the SA site, vines were on their own roots, planted at 2 m spacing, with 3.2 m row spacing, trained to a bilateral cordon and spur-pruned. At the Vic. site, vines were on Schwarzman rootstock, planted at 2.44 m spacing, with 3 m row spacing, trained to a double, bilateral cordon and mechanically-hedge pruned with hand clean-up. At the NSW site, vines were on own roots, planted at 2.1 m spacing, with 3.3 m row spacing, trained to a bilateral cordon and spur pruned.

Each site utilised a different randomised complete block layout with four replicates, each of which encompassed three rows, providing a 'measurement' row with a buffer row on each side. At the SA and NSW sites, each replicate included fifteen vines per row, whereas at the Vic. sites each replicate included twenty vines per row.

Field treatments

Five management regimes were used to manipulate vine balance (ratio of yield to canopy size) and applied as similarly as possible at all three sites:

- T1: control – all vines in T1 replicates received the standard management practice for that site.
- T2: early defoliation – all fully expanded leaves (approximately the first eight leaves) were removed from each vine in the T2 measurement rows, pre-anthesis, E-L stage 19 (Coombe 1995). The treatment was also applied to all the buffer vines at the NSW site and either one or both buffer rows at the SA site, depending on the season.
- T3: crop thinning – all bunches were counted on at least eight vines per site, averaged and half this number of bunches removed from each vine in the T3 measurement rows pre-veraison, E-L stage 32.
- T4: late defoliation – all vines in the T4 measurement and buffer rows were mechanically hedged pre-veraison, E-L stage 32. The hedging cut the foliage at the edge of the fruiting zone, with approximately half the canopy removed where possible.
- T5: minimal pruning – all vines, measurement and buffer rows, were left un-pruned at the end of the first growing season, with low hanging canes cut about 30–50 cm above the ground prior to budburst.

The treatments were first applied during the 2013/14 growing season and re-applied to the same vines in the 2014/15 and 2015/16 growing seasons. Weather conditions at each site were monitored using matched weather stations placed adjacent to the trial block at each field site (Measurement Engineering Australia, Magill, SA, Australia).

Field sampling and measurements

Vine balance was assessed as the ratio between peak canopy size and harvest yield. Canopy size was estimated from leaf area index, measured using a LiCor Li2200 or Li2000 Plant Canopy Analyser (LiCor Nebraska, US) on at least two occasions from late December to early February in each season. Harvest yield was measured directly by weighing the hand-harvested fruit from multiple vines per replicate (number of vines varied by site and season).

Samples for fruit/juice composition and gene expression were taken fortnightly from flowering to harvest in all seasons. At each site, four entire bunches per replicate were collected, using different vines each time. The berries were removed from the rachis and split into four randomised 50 berry sub-samples to ensure all analyses used matched fruit. Samples were stored on ice and used within 24 hours (juice assessment), or immediately frozen in liquid nitrogen (gene expression and fruit composition).

Harvest date was defined as the point where juice total soluble solids (TSS), measured using a refractometer, reached 24°Brix and was assessed individually for each treatment at each site. Fruit taken for harvest yield estimates was sent by refrigerated transport for standardised winemaking at the NWGIC in Wagga Wagga. The wines were made in stainless steel variable capacity fermenters with an initial SO_2 addition and standard adjustments of acidity and yeast assimilable nitrogen. One wine was produced per field replicate from each site, a total of 48 per year in 2014 and 2015.

Chamber experiment design

To complement the field-based component of the project, an experimental system was developed at the NWGIC in Wagga Wagga to specifically investigate the impact of carbon supply on berry ripening and composition. The system used large mature potted grapevines, which were cordon trained to a comparable canopy size to field grown vines, and located in a bird-proof enclosure. A system consisting of six transparent whole vine chambers was constructed to enclose the canopy, and sodalime-based scrubbers used to reduce the CO_2 concentration of the supply air to approximately 200 ppm in three of the chambers. The remaining three chambers were supplied with air at ambient CO_2 concentrations (400 ppm), generating an estimated twofold difference in canopy photosynthesis between treatments. The aim of this system was to vary carbon supply to the fruit (i.e. vine balance) independently of light and temperature effects and, through the use of reduced CO_2 rather than canopy or crop adjustments, to be able to impose and reverse the changes to vine balance at different times through berry development. For the results presented here, the system was utilised during the 2014–15 season to reduce CO_2 availability for 27 days from veraison, with a scrubbing period from January 4 to January 30.

During the course of the CO_2 scrubbing, 8 berry samplings were undertaken to monitor fruit composition and gene expression responses to differing carbon supply. Two more samplings were undertaken following the return of the 200 ppm treatment to ambient, and a final one at harvest. At each sampling, 50 berries were collected and weighed, and then separated into skin, juice and seed fractions. These were then frozen in liquid N and stored at −80°C for subsequent analysis. At 24°Brix, the fruit was harvested and yield parameters recorded.

Results and discussion

Climate measurements followed long-term trends, with mean January temperature (MJT) being highest at the Vic. site and lowest at the SA site (Table 1), with 2014 having the highest MJT and 2015 the lowest. Site differences were similar season to season, irrespective of the absolute MJT. However, MJT disguised commonalities in daily minima and maxima, with SA and NSW having the same growing season average daily maximum (approx. 28°C), but a different average daily minimum (approx. 11 and 13°C respectively) and Vic. and NSW having the same growing season average daily minimum, but a different average daily maximum (approximately 32°C at the Vic. site). Furthermore, SA had the lowest ET_o (data not shown).

Table 1. Mean January temperature (MJT; °C) of the three field sites in each of the three seasons during which field measurements were made.

	2014	2015	2016
SA	21.2	19.5	20.4
Vic.	25.5	23.4	24.7
NSW	23.5	21.0	22.2

The three sites were chosen to represent a range of climates and a range of vineyard management strategies and it was anticipated that this would result in different canopy sizes and yields between the sites. Averaged across the three seasons of measurements, this was indeed the case, with a near twofold range in canopy size (8–14 m^2) and a near fourfold range in yield (5.3–19 kg $vine^{-1}$) in the control plots (Table 2). As a result, the calculated vine balance (yield per unit canopy area) at the Vic. site was double that of the NSW site, which was 10% higher than that of the SA site.

Treatment effects on vine balance and its components

The T2 treatment was based on the work of Poni et al. (2013) who found that early-defoliation resulted in a 30–50% decrease in yield with little or no effect on canopy size, reducing the yield:canopy ratio. However, the work in Italy was carried on vertical shoot positioned vines (VSP) with in-season canopy adjustment, whereas our work was carried out on spur pruned vines that were allowed to 'sprawl'. To the best of our knowledge, this is the first time early-defoliation has been applied to vines managed in this way. The results of this work produced a contrasting result, with only an 8–13% reduction in yield, but also a 10–25% reduction in peak canopy size (Table 2). The effect on yield:canopy ratio was, therefore, much less than previously observed and, excluding the minimal prune treatment (see below) was the smallest adjustment of any of the management treatments applied at all three sites.

Of the four management strategies utilised, a mechanical form of the T3 treatment is, perhaps, the most likely to be implemented in Australian viticulture (Petrie and Clingeleffer 2006). The crop removal treatment, which was implemented by hand in our study, had no significant effect on canopy size, but was effective in reducing harvest yield, with reductions of between 30% and 45% (Table 2). Consequently, the yield:canopy ratio was reduced by the same proportion.

Summer pruning, equivalent to the T4 treatment used here, has been trialled in Europe as a mechanism to slow maturation (Stoll et al. 2010). Limited trials have previously been attempted in Australia, again on VSP managed vines (Whiting 2012; Savarino et al. 2013). As with the T2 treatment, to the best of our knowledge, the experiments presented here are the first attempt to implement this management strategy on 'sprawl' vines, noting that small adjustments to canopy size during the season is common practice in many vineyards, both cool and warm climate. The treatment reduced canopy size during the maturation period by 30–40% (Table 2) and had no effect on harvest yield at the SA and NSW sites, with a marginal effect at the Vic. site ($p=0.101$) due to accidental removal of some bunches during the treatment implementation. The yield:canopy ratio was, consequently, increased at all sites, with the effect being between 21% and 33%.

The effects of minimal pruning, T5, were only examined in the final season. Changing management from spur pruning to minimal pruning has a major effect on growth and the use of reserves in the first season of implementation, due to the large change in bud numbers per vine. Due to this, measurements were not made in that first season of adjustment (2014/15). Visual observation suggested that the extent of this adjustment process varied between sites and the canopy size data supports this, with the SA and NSW sites maintaining a much larger canopy on the T5 vines in 2015/16, but a smaller canopy being present on the T5 vines at the Vic. site (Table 2). Similarly, yield was increased at two sites, relative to control, and reduced at the third. The resulting yield:canopy ratio was close to control at all three sites.

In applying the treatments in a consistent manner across three trial sites that differed significantly in yield and climatic conditions, and doing this over three consecutive seasons, one of the original objectives of the project was to understand the extent of site or season specific responses to crop load or canopy manipulations. Where fruit or wine quality responses can be obtained in a predictable and reliable manner there is potentially much greater scope for widespread adoption than if practices have to be tailored for every specific situation. For T1 to T4, which were all applied over three seasons with as identical methodology as possible, the relative treatment responses have been remarkably similar across the three trial sites. It would be expected that some variation will arise as a result of the differing environments, but the findings suggest that across a common genotype, the physiological responses to changes in fruit exposure and source-sink relations are similar.

The impact on maturation rate of changing vine balance

Altering vine balance alters the source:sink relationship in the vine, with a higher yield:canopy size ratio requiring more carbohydrate to ripen the crop than a lower yield:canopy size ratio. Consequently, treatments that increase this ratio (T4, T2 at two sites, T5 at one site) would generate a greater demand on the canopy for photosynthate than controls and treatments that reduce this ratio (T3, T5 at one site) would reduce the demand on the canopy. In the former case, the additional carbohydrate could be supplied by an increase in photosynthetic rates, depletion of reserves (Smith and Holzapfel 2009), a longer maturation period, or a combination of these. In the latter case, the additional carbohydrate available could result in a down-regulation of photosynthetic rates, an increased allocation to reserves or a shorter maturation period.

As all treatments were harvested at a nominal total soluble solid content (TSS) of 24°Brix, the effect of altering vine balance on maturation period was assessed by determining the number of days difference between the harvest date of each treatment and control. This has been expressed as days in advance (positive numbers) or behind (negative numbers) the control harvest (Figure 1). In the T3 and T4 treatments, reducing the yield:canopy ratio advanced the date on which the nominal TSS content was reached and increasing the yield:canopy ratio delayed that date. For T5, the SA site was delayed, despite no difference in yield:canopy ratio and there was no effect on time of maturity at the Vic. site, despite a reduction in yield:canopy. However, in both cases, the growth of the vines was drastically changed by the minimal prune treatment and it is likely that the reserves were impacted and not yet in equilibrium with the canopy or

Table 2. Canopy size, harvest yield and their ratio (vine balance) averaged across three seasons of implementation of management treatments (T1–4), or for a single season (T5), at the three experimental sites, n=4.

		Canopy (m^2)	Yield (kg)	Vine Balance (kg m^{-2})
SA	T1: Control	9.7	5.5	0.58
	T2: Early Defoliation	7.3	4.8	0.67
	T3: Crop Thinning	9.7	3.1	0.31
	T4: Late Defoliation	5.9	5.7	0.99
	T5: Minimal Prune	17.2	10.5	0.61
Vic.	T1: Control	13.9	19.1	1.35
	T2: Early Defoliation	12.8	16.2	1.28
	T3: Crop Thinning	16.0	13.7	0.84
	T4: Late Defoliation	9.6	16.3	1.64
	T5: Minimal Prune	11.5	13.2	1.13
NSW	T1: Control	8.0	5.3	0.66
	T2: Early Defoliation	6.4	4.9	0.77
	T3: Crop Thinning	7.7	3.3	0.43
	T4: Late Defoliation	5.5	5.0	0.91
	T5: Minimal Prune	13.7	8.0	0.59

crop load. Another explanation is that the *efficiency* of canopy photosynthesis per unit area of leaf was altered in these vines, for example, by lower self-shading or reduced canopy density. Such an effect may also explain why the increase in yield:canopy ratio of the T2 treatment, albeit small, advanced rather than delayed maturation. The canopy structure of the T2 treated vines was affected as well as the canopy size, with greater porosity (data not shown) that may have increased photosynthetic efficiency of the canopy as a whole.

Whilst the direction of the treatment effect on maturation date largely matched predictions (T3, T4), the extent of the treatment effect on maturation date was more variable. For instance, T4 had a smaller numerical effect on the yield:canopy ratio than T3 at the SA site, but an equal or larger effect to T3 on maturation date. This suggests that some of the other factors noted above are also likely to be occurring, for example a potential increase in carbohydrate allocation to reserves in the T3 vines.

Effect of altering vine balance on wine composition

In general, T2 had a very limited effect on fruit composition (assessed as total anthocyanins and total tannins by UV-vis spectrophotometry) at harvest and T3 had no effect, whereas T4 significantly reduced anthocyanins (data not shown). When the wine was assessed for the same components, one month after bottling, the pattern of treatment effects was similar, but not identical (Figure 2). For example, the effect of T2 was greater, with a significant increase in wine colour density (WCD) and wine total tannin content across the three sites and limited effect of T3, with a small increase in WCD at two sites and an increase in tannins at one. The T4 treatment resulted in decreased WCD at all sites and decreased tannins at two sites (excluding NSW). Reduced fruit total anthocyanin concentration and WCD have been previously reported for late defoliation treatments (Whiting 2012); possibly due to the effect of increased bunch exposure to light and temperature during the anthocyanin accumulation period.

An overall impression of the results from the berry and wine analysis, while noting that data is still being finalized, is that treatment differences are more apparent in the wines than fruit; at least for the standard range of analysis conducted to date. This does not detract from the value of berry analysis, but raises a possibility that other factors such as tannin extractability or structure may vary in response to fruit exposure or photosynthetic carbon supply.

Manipulation of vine carbohydrate availability independently of bunch environment

The chamber system allowed carbohydrate availability within the vine to be manipulated independently of an environmental effect at the bunch level. Specifically, the system was used to replicate the effect of the T4 treatment through veraison, without increasing the sun exposure of the bunches; reducing whole vine photosynthesis by approximately half. This treatment (reduction in CO_2) reduced the *rate* of sugar accumulation in the berries and, consequently, the absolute amount of sugar in the berries (Figure 3a). Following the end of the CO_2 scrubbing period, juice sugar concentrations increased at a *rate* that matched the controls, but the absolute amount remained lower than the control fruit. The fruit did eventually reach the target TSS for harvest, but this was delayed by more than two weeks, relative to controls. To this extent, the effects of the CO_2 scrubbing treatment were indeed similar to those of the T4 treatment in the field.

In contrast to the field result, where fruit had greater light exposure and, presumably, a greater heat load (Haselgrove et al. 2000), the rate of anthocyanin accumulation matched the rate of sugar accumulation. Consequently, the ratio of sugar to anthocyanins was identical in the treatment and the controls (Figure 3b). The similarity in effect of the scrubbing treatment to T4 on sugar accumulation, but not anthocyanin accumulation suggests that the difference is due to some other factor in the field, with the effect of the late defoliation on the exposure of the fruit, and in particular temperature (Spayd et al. 2002) being the most obvious candidate.

Conclusions

Taken as a whole, the results presented here suggest that maturation rate can be manipulated successfully through adjusting vine balance and that such manipulations are effective across a wide range of climate and viticultural management. However, there is little evidence of maturation rate or vine balance directly impacting fruit composition, with the influence of the early and late defoliation treatments on inflorescence and bunch environment being more likely candidates for the treatment effects on composition. Despite the lack of effect of crop thinning on fruit composition, there appeared to be

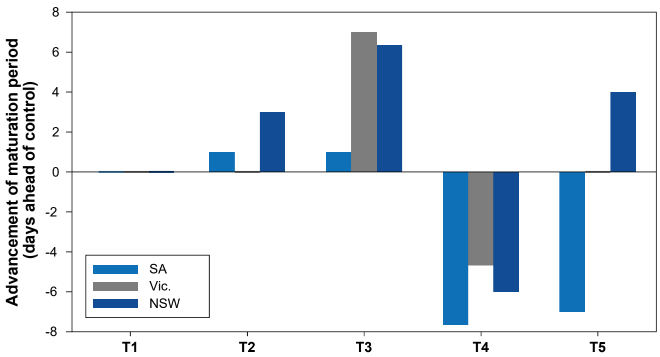

Figure 1. Days in advance (positive) of control or days delayed (negative) that fruit reached a TSS concentration of 24°Brix, averaged across three seasons of implementation of management treatments (T1–4), or for a single season (T5), at the three experimental sites, n=4.

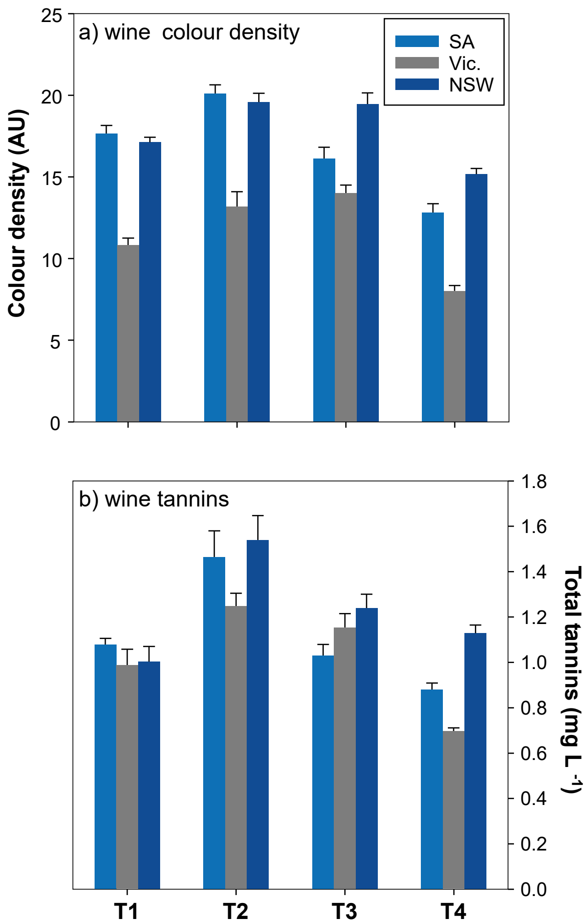

Figure 2. Wine colour density (top) and wine total tannins (bottom) one month after bottling, averaged across two vintages, where the fruit used was from vines subjected to a range of management strategies (T1-T4)

some benefit on wine colour density, although this was dependent on site and season (data not shown). It is expected that further, more in-depth, analysis of the fruits samples collected and wine produced will provide a greater insight into such effects.

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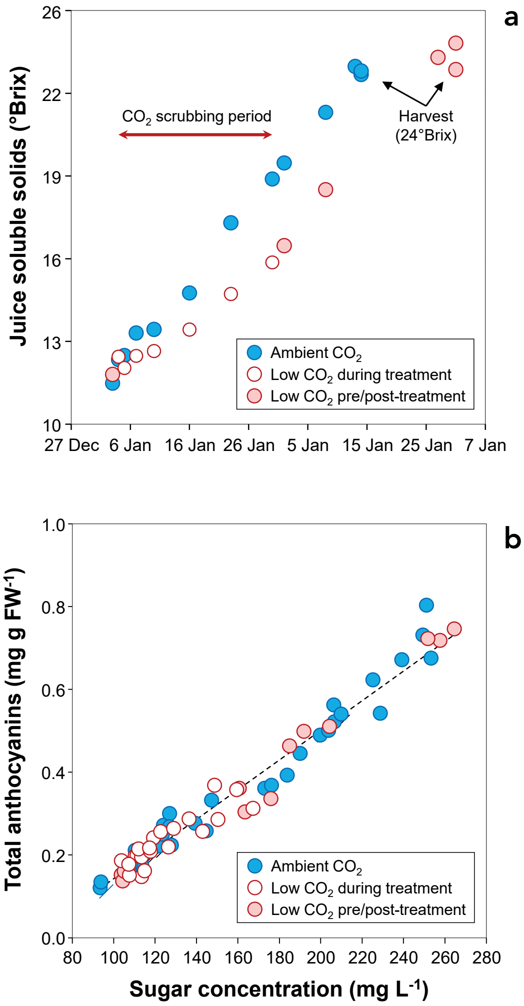

Figure 3. Juice total soluble solids (a: top) and anthocyanin concentration (b: bottom) of fruit from vines grown in ambient (blue) or reduced (red) atmospheric CO_2 concentrations for 26 days

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Assessing the impact of winter drought on vine balance and root activity

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Abstract

In regions such as the Barossa Valley, restricted water for irrigation combined with a decline in winter rainfall due to climate change is likely to present management challenges. We investigated the effects of winter rainfall exclusion and replenishment on vine phenology, yield components, berry quality parameters and root development.

Five treatments were established: vines exposed to natural winter rainfall (control); rainfall exclusion with irrigation to match natural rainfall using sprinklers (sprinkler-rain) or drippers (dripper-rain), rainfall exclusion with reduced winter irrigation (30% of control) applied throughout the winter (reduced-rain) and rainfall exclusion with irrigation (50% of control) applied at budburst to refill the soil profile (spring-rain).

Compared to the control, the top-up winter irrigation treatments (sprinkler-rain and dripper-rain) advanced budburst by approximately 2 days. This difference persisted at anthesis (1.9 days) and during ripening (approximately 1.1°Brix). In contrast spring-rain delayed development before (1.9–5.5 days) and during ripening (0.2–0.6°Brix). Reduced-rain reduced yield by 42%, relative to the control vines, whereas top-up irrigation treatments (sprinkler, dripper and spring-rain) reduced the yield by approximately 20%. Yield differences were driven mainly by the lower bunch and berry weight of reduced-rain, and the higher bunch number in control. At budburst, the treatments affected the fine root (<2 mm diameter) development; with the control and spring-rain showing the greatest root length per surface area.

Understanding the impact of reduced winter rainfall on vine performance and the extent to which replacement irrigation can modify this will improve industry resilience to climate change as well as optimise the use of scarce water resources.

No paper available, please view this presentation at http://bit.ly/16thBonada.

Boosting varietal thiols in white and rosé wines through foliar nitrogen and sulfur spraying

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Abstract

Between 2005 and 2011, the impact of foliar application of nitrogen alone or with sulfur on grape and wine characteristics was assessed on six French grape varieties (Sauvignon Blanc, Colombard, Gros Manseng, Négrette, Fer and Melon) grown in the south-west of France and the Loire Valley, and on Carignan in northern Spain. Nitrogen applications were carried out at veraison with the use of a formulated form of urea at different rates (from 5 to 20 kg nitrogen unit/ha) combined with elemental soluble sulfur applications at reduced doses (from 5 to 10 kg/ha). On average, the spraying of 20 kg of nitrogen combined with 10 kg of sulfur induced a twofold increase in nitrogen content of the grapes at harvest and a threefold increase in varietal thiols (3-mercapto-hexanol and 3-mercaptohexylacetate) in rosé and white wines at pilot scale (30 L tanks). These results were confirmed by wine tasting. The same conclusions could not be drawn when making red wines. The technique induced a small loss in phenolic compounds, had a limited effect on the volatile aroma composition of red wines, but often produced reductive flavours even if nitrogen sprayings were not combined with sulfur applications. In most cases, no secondary effects were noticed on vines (e.g. yield, maturity, sanitary status) and wines (e.g. protein instability). This technique is now routinely implemented by French winegrowers to improve the aromatic composition of white and rosé wines.

Webcast of this presentation available at http://bit.ly/16thSchneider.

Introduction

Varietal thiols are aroma compounds first discovered in Sauvignon Blanc wines (Tominaga 1998). They have been identified in white wines made from several cultivars such as Gewürztraminer, Riesling, Colombard, Petit Manseng and Semillon (Tominaga et al. 2000), and they also contribute to the aroma of rosé and red wines (Murat et al. 2001). Among the thiols with desirable flavours found in wines, 3-mercaptohexanol (3MH) with a zesty aroma of grapefruit and 3-mercaptohexylacetate (3MHA) which smells like boxtree and passionfruit are the most known and studied. These compounds are released in wine during alcoholic fermentation by the yeast enzymatic metabolism from odourless precursors identified in grapes as S-cysteine and S-glutathione conjugates (Peyros Des Gachons 2000).

It has been shown that high nitrogen status of grapevines enhances the synthesis of cysteinylated aroma precursor and glutathione in grapes (Choné et al. 2006). High nitrogen status can be obtained through soil fertilisation which can lead to increased vegetative growth, plant susceptibility to pathogens (i.e. downy mildew, bunch rot) and yield. Around veraison a large part of the nitrogen assimilated by the grapevine is found in bunches (Conradie 1986). Even if nitrogen is preferentially assimilated by the roots, grapevines, like many other plants such as maize, wheat and tomato (Genter et al. 1998), have the ability to uptake nitrogen in the form of urea through their leaves. Thus, foliar spraying of nitrogen could be used in viticulture to limit the dependency of fertilisation on soil composition and water status and reduce losses through nitrogen leaching. The combination of nitrogen and sulfur foliar spraying has been reported to enhance the production of glutathione and sulfur amino-acids on wheat crop (Téa 2004). Since varietal thiols are linked to the plant nitrogen uptake and sulfur metabolism by its S-cysteine and S-glutathione conjugates, described as aroma precursor, foliar applications of nitrogen and sulfur are likely to have an impact on the aroma composition of wines.

Between 2005 and 2008, a first set of experiments was conducted on several grape varieties and in different winegrowing regions to investigate the impact of applications of nitrogen combined with elemental soluble sulfur on must and wine composition of white and rosé wines. As the use of urea is not allowed in organic viticulture, a second experiment was conducted in 2010 with three commercial products mainly composed of amino acids and obtained from enzymatic digestion of animal, vegetal and marine proteins. When making red wines, nitrogen fertilisation cannot be combined with sulfur because of the high risk of developing reductive flavours. Thus, a third set of experiments was carried out in 2011 to answer this specific question. This paper summarises the main findings obtained from these experiments.

How were nitrogen and sulfur applied?

Experiments were undertaken using randomised complete block designs with three replications of 12 continuous vines per treatment. To allow sufficient dilution of the fertiliser and avoid toxicity, foliar sprayings were carried out at 400L/ha using Stihl SR 340 pneumatic spraying equipment (Stihl, Waiblingen, Germany), divided into two applications: the first one at the onset of veraison; the second one about one week later, at full veraison. Quantities of nitrogen and sulfur units per hectare mentioned throughout the manuscript refer to the sum of these two applications.

For the first experiment on white and rosé wines, a formulated form of urea (Folur, Tradecorp, Belgium) whose concentration in nitrogen was 220 g/L, was applied between 2005 and 2008 to foliage at the rate of 10, 15 and 20 kg/nitrogen unit per hectare. These applications were combined or not with elemental soluble sulfur (Microthiol, Cerexagri, France) at reduced doses (from 5 to 10 kg of sulfur per hectare). Sprayings were tested on Colombard, Gros Manseng, Négrette and Sauvignon Blanc in the south-west of France, and Melon and Sauvignon Blanc in the Loire Valley. Average plant density of the experimental plots was 4,200 vines per hectare.

For the second experiment on organic nitrogen fertilisers, three commercial products obtained from enzymatic digestion of animal (Aminovital, Biofa, Germany), vegetal (Diaglutin, Biofa, Germany) and marine (Liquoplant B336, Plantin, France) proteins were tested in 2010 on Sauvignon Blanc grown in the south-west of France. Total nitrogen concentrations varied from 39 g/L for Liquoplant B336 to 108 g/L for Aminovital. Due to the high cost of these products containing mainly peptides, amino acids and ammonium, the quantity of nitrogen sprayed was limited to 10 kg of nitrogen per hectare.

The third experiment conducted in 2011 focused on the impact of a formulated form of urea containing 355 g/L of nitrogen (Azofol SR, Agronutrition, France) applied at 20 kg of nitrogen per hectare, on the composition of red wines made from Fer and Carignan grown in the south-west of France and northern Spain respectively. Due to the high risk of developing reductive off-flavours during alcoholic fermentation (AF), nitrogen fertilisation was not combined with sulfur.

For all the experiments, wines were elaborated at pilot scale (30 L). Grapes were processed under a strict non-oxidative protocol for white and rosé wines with AF taking place at 18°C. Specific yeast strains were used to enhance the release of varietal thiols from their precursors. For red wines, AF and maceration took place at 25°C for eight days. A Konelab Arena 20 sequential analyser using enzyme kits (Thermo Electron Corporation, Waltham, USA) was used to determine nitrogen in must as the sum of ammonia and amino-acid concentrations. For the first and second experiments, varietal thiols (3MH and 3MHA) were analysed in bottled wines according to the protocol described by Schneider et al. (2003). In 2011, 78 aroma compounds (major, trace and volatile sulfur compounds, polyfunctional mercaptans) were quantified in red wines according to the methods proposed by Ortega et al. (2001), Lopez et al. (2002), Lopez et al. (2007) and Mateo-Vivaracho et al. (2010). Glutathione and cysteine precursors of 3-mercaptohexanol were also analysed for Carignan and Fer.

Sensory analysis was conducted in each region with local experts six to eight months after the harvest.

The technique had a large impact on the nitrogen content of the must and the aroma composition of white and rosé wines

In most cases, nitrogen in urea form sprayed at veraison on grapevine induced an increase in the nitrogen concentration of the must (Figure 1A). The gain in nitrogen was linear: 50% and 100% increases were noticed for 10 and 20 kg/ha applications respectively. In some cases, no effect was observed and no simple explanation could be proposed probably because the spraying is strongly dependent on crop management, period and time of application, weather conditions and urea formulation. In comparison with a nitrogen-only application, the nitrogen and sulfur combination had no additional effect on nitrogen content of the must (Figure 1B). A gain would have been expected as previous results obtained on wheat grain suggested a synergy between nitrogen and sulfur (Tea 2004).

As 3MHA is formed by yeast during AF through esterification of 3MH (Swiegers and Pretorius 2007), we used the molar sum of these two compounds as an indicator of the varietal thiol potential. In comparison with controls, nitrogen and sulfur sprayings induced a three- to fourfold increase in varietal thiols in wines (Figure 2). This increase was observed even for control wines with high concentrations of thiols (from 10 to 40 nM/L).

In most cases when performing sensory analysis, both rosé and white wines from the nitrogen and sulfur treatment were judged more intense and presented higher scores for 'grapefruit' and 'tropical fruit' attributes. No undesirable sulfur/reductive notes were perceived in these wines at tasting.

Measurements of bunch rot severity conducted at harvest did not show significant differences between the treatments. Control and nitrogen-sprayed wines had the same level of protein instability and presented the same concentration in ethyl carbamate. As ethyl carbamate is produced in most alcoholic beverages, from urea or other nitrogen sources (i.e. glutamine or asparagine), the nitrogen spraying could have provoked a clear increase in this contaminant in bottled wines (Zhao et al. 2013).

Spraying of nitrogen and sulfur appears to be a powerful viticultural technique to produce white and rosé wines with enhanced concentration of varietal thiols. Applied to grapevines experiencing nitrogen deficiency and producing inexpressive wines, it could help to maintain grass cover, a soil management strategy which is known to lower nitrogen in must and induce off-flavours in wine (Spring and Lorenzini 2006). In other situations, it may promote over-expression of varietal thiols in wines.

Can the technique be adapted to organic viticulture?

In organic viticulture, copper-based fungicides are mainly applied in vineyards until veraison which contributes to increased copper content of the grapes at harvest in comparison with conventional farming. It has been shown that grapes and musts with high levels of copper led to wines with low concentrations of varietal thiols (Dufourcq et al. 2010). Therefore, the adaptation of the technique to organic viticulture could help to mitigate the detrimental effect induced by copper on wine aroma. Our results showed that fertilisers obtained from enzymatic digestion of proteins and containing peptides, amino acids and ammonium can be assimilated by grapevine via foliage (Table 1). The increase obtained for Aminovital was 48%, equivalent to that expected for a urea-based application (50%). These conclusions were unexpected as it has been reported on peach trees that the chemical form had a large influence on foliar-applied nitrogen absorption (Furuya and Umumiya 2002) with absorption rates being the highest for urea and nitrate, followed by ammonium and amino acids. Concentrations in varietal thiols measured in wines were very low and foliar sprayings had a detrimental impact on the quantity of molecules. No differences were found between the four wines at tasting.

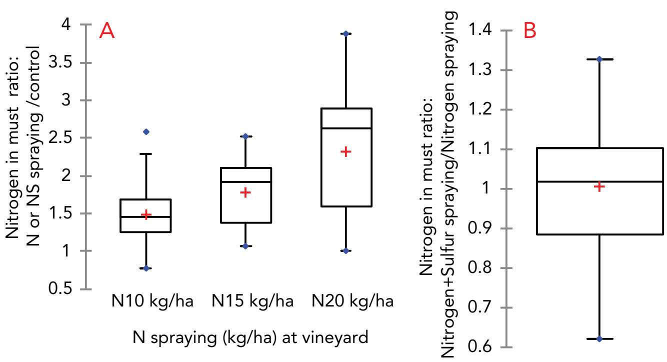

Figure 1. Effect of foliar applications of A) nitrogen combined or not with sulfur compared with a control treatment (n = 101) and B) nitrogen and sulfur compared with a nitrogen-only application (n = 42) on nitrogen status of the must. Nitrogen content of the control varied from 59 and 187 mg/L. Data were collected between 2005 and 2008.

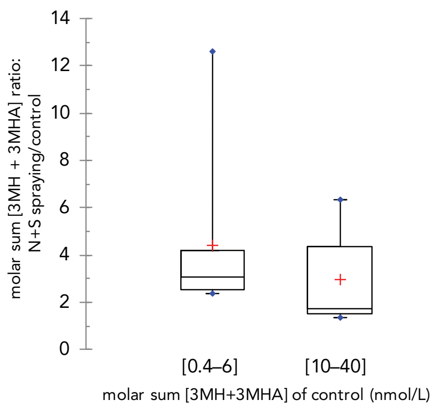

Figure 2. Gains in varietal thiols induced by the application of nitrogen and sulfur compared with a non-sprayed control treatment according to the concentration in molecules found in the control wines (n = 15). Data were collected between 2005 and 2008.

Forty-eight hours after the second application, a heatwave occurred in the south-west of France with temperatures surpassing 40°C. Severe burn damage, particularly marked for the Liquoplant B336 and Diaglutin treatments, was noticed on the foliage at harvest (Figure 3). The sugar concentration of the grapes at harvest was slightly impacted by this loss of functionality (results not shown). This phytotoxicity can be mainly explained by the concentration in nitrogen of the organic fertilisers which does not allow sufficient dilution.

Although some organic fertilisers showed good absorption rate and improved the nitrogen status of the must, their application provoked severe burn damage and had a negative impact on the aroma composition of wines. Additionally, their cost poses a serious threat to the development of the technique in organic viticulture. Prices of the tested fertilisers at the time of the study varied from 3 to 6 euros per litre; for 10 kg of nitrogen unit spraying, this represents a cost of 450 to 820 euros per hectare. For comparison purposes, the cost of a urea-based fertiliser at the same rate averaged 115 euros per hectare.

In order to optimise the absorption of nitrogen by the plant and benefit from a synergistic effect with the aim of reducing the quantities applied per hectare and the cost of the treatment, nitrogen applications were combined in 2011 with sulfur and nettle manure at 10%. None of the studied treatments stimulated the absorption of nitrogen by the plant. An economic study was also carried out using Viticoût – an online cost calculator developed by IFV (Viticoût) – to assess the impact of spraying equipment with recovery systems on the cost of the treatment. The use of this technology only allowed a 6% reduction in the cost of the spraying for a 10 kg/ha application.

Is the technique also suitable for the production of red wines?

To answer this question, foliar applications were carried out at 20 kg nitrogen unit per hectare without sulfur. In addition to the gain in nitrogen content, the foliar spraying had a significant impact on the acidity of the grapes (Table 2). Increases in pH and malic acid were observed for the foliar treatment. These changes which have been previously described for soil fertilisation (Bell and Henschke 2005) were more pronounced for Carignan in Spain, a vineyard experiencing a larger water deficit. For this cultivar, a gain in sugar concentration was also observed. By keeping the foliage greener and in a more functional condition, the foliar spraying might have contributed to a larger production of malic acid in the leaves and a better assimilation of potassium which stimulated the sugar production. For the two cultivars, wines from the foliar treatment had a lower Total Phenol Index (TPI) which is not completely unexpected as it has been shown that biosynthesis pathways of proanthocyanidins were down-regulated by an excessive nitrogen uptake (Lillo et al 2008).

Among the 78 aroma compounds analysed in bottled wines, 18 were significantly impacted by the treatment (Table 3). The changes were low in intensity (less than 50% of variation in concentration in comparison with the control) for most and only affected compounds found at low concentrations and/or known to have a low impact on wine sensory properties. Surprisingly, even if the foliar treatment induced average gains of 25% and 30% in glutathione and cysteine precursors of 3-mercaptohexanol respectively (results not shown), concentrations of 3-mercaptohexanol were not impacted in finished wines. Red winemaking conditions (higher temperature and turbidity) are less favourable to the release of 3-mercaptohexanol (Masneuf-Pomarède et al. 2006) from its precursors and the small gain in precursors might not have been sufficient to observe a real impact in bottled wines. The absence of nitrogen/sulfur combination in this experiment might also have played a role. Lacroux et al. (2008) showed that foliar applications of nitrogen without sulfur had no impact on 3-mercaptohexanol in wine. In the same way, the technique had little effect on fermentative aroma compounds (i.e. ethyl esters, acetates and fatty acids) whose production during AF is strongly dependent on temperature and turbidity (Moreno et al. 1988). From a sensory point of view, the technique increased slightly floral notes as a likely consequence of the gain observed in ethyl cinnamate and β-damascenone. Marked reductive off-flavours were also noticed for the nitrogen treatment which can be explained by the higher concentration in hydrogen sulfide found in wines.

Table 1. Effect of the foliar application of organic nitrogen fertilisers on nitrogen in must and varietal thiols in finished wines made from Sauvignon Blanc in 2010.

Treatment	Nitrogen in must		3MH (ng/L)	3MHA (ng/L)	Molar sum 3MH +3MHA (nM/L)
	Content (mg/L)	Gain in comparison with the control (%)			
Control	115	–	439	44	3.53
Liquoplant B336	136	18%	128	11	1.02
Diaglutin	153	33%	174	37	1.30
Aminovital	170	48%	128	15	1.04

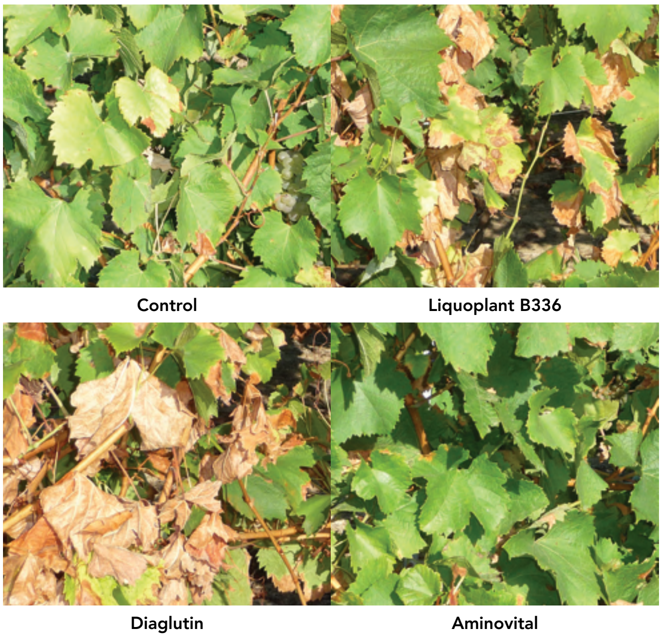

Figure 3. Appearance of the foliage at harvest according to the different organic fertilisers applied at veraison.

Table 2. Effect of foliar application of urea at 20 kg/ha of nitrogen unit on grape characteristics for Fer and Carignan in 2011. Anthocyanins and Total Phenol Index were measured in finished wines

Cultivar	Treatment	Sugar (°Brix)	Total acidity (g/L tartaric acid)	pH	Tartaric acid (g/L)	Malic acid (g/L)	Nitrogen (mg/L)	Anthocyanins in wine (mg/L)	TPI wine
Fer N	Control	21.1[a] a[b]	3.46 a	3.24 b	3.61 a	3.03 b	156 b	385 a	45 a
	Nitrogen (20 kg/ha)	21.2 a	3.46 a	3.32 a	3.66 a	3.16 a	247 a	375 a	41 b
Carignan N	Control	23.3 b	2.33 a	3.72 b	3.74 a	1.87 b	258 b	285 a	45 a
	Nitrogen (20 kg/ha)	23.7 a	2.37 a	3.83 a	3.77 a	2.35 a	342 a	293 a	40 b

[a]Mean of 8 replicates. [b]Different letters within a column and cultivar indicate means different at p≤0.05 by Fisher's test.

Conclusion

The foliar application of sulfur and nitrogen at veraison is a powerful viticultural technique to over-express the varietal character of white and rosé wines without undesirable side effects. It is routinely implemented by French winegrowers on thousands of hectares and a Decision Support System (DSS) dedicated to the technique is available online at www.vignevin-sudouest.com. At present, due to the high cost of the products and the foliar toxicity observed under our conditions of application due to less dilution, the technique cannot yet be adapted to organic viticulture. The interest of the technique was limited when making red wines. It had a low impact on the aroma composition, induced a small loss in phenolic compounds and produced reductive off-flavours.

Acknowledgements

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Table 3. Aroma compounds in wine impacted significantly by the foliar fertilisation.

Chemical family	Compound	Impact of the nitrogen spraying	Level of concentration in wines
Ester	Diethyl succinate	+a	>c
	Ethyl butanoate	+	>
Acetate	Butyl acetate	+	>
	Ethyl acetate	+	>
Alcohol	1-butanol	+	>
	2-phenylethanol	-b	<d
	Metionol	-	=e
	Benzyl alcohol	+	<
Carbonyl compound	Diacetyl	+	>
	γ-decalactone	+	<
Terpenol and norisoprenoid	Geraniol	+	=
	β-damascenone	+	>
Phenol	2,6-dimethoxyphenol	-	<
	4-vinylphenol	+	<
	Eugenol	+	<
	Guaiacol	-	<
Cinnamates	Ethyl dihydrocinnamate	+	=
Mercaptans	Hydrogen sulfide	+	=

[a]significant gain. [b]significant loss. [c]above the aroma threshold. [d]below the aroma threshold. [e]equivalent to the aroma threshold.

A step forward in unravelling the uniqueness of Australian Cabernet Sauvignon wine aroma

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Abstract

Australian Cabernet Sauvignon wines are often appreciated for distinctive fruit aromas and specific elegant herbal attributes such as 'eucalyptus', 'mint', 'bay leaf' and 'dried herbs'. While the occurrence of 'eucalyptus' character in Australian red wines has been associated with the presence of 1,8-cineole (eucalyptol), the origin of these typical herbaceous notes remains poorly understood. The compound 1,4-cineole, which has a very similar structure and odour to 1,8-cineole, has been recently identified in Australian red wines, but quantitative data and sensory characterisation have not been reported.

This work investigated the contribution of 1,4-cineole and 1,8-cineole to Cabernet Sauvignon wine aromas. Detectable levels of 1,4-cineole were found in 104 commercially available Australian red wines, with concentrations ranging from 0.023 to 1.6 μg/L, with higher concentrations measured in Cabernet Sauvignon than in Shiraz and Pinot Noir wines. A comparison between 51 Australian and 26 French predominantly Cabernet Sauvignon wines revealed that 1,4-cineole and 1,8-cineole concentrations were 4- and 18-fold higher in Australian than French wines respectively. Two-thirds of Australian Cabernet Sauvignon wines had 1,4-cineole concentrations of ≥ 0.5 μg/L, a level that sensory studies indicated can be detected by the majority of panel members. Descriptive analyses revealed that 1,4-cineole, both on its own and in association with 1,8-cineole, may contribute to 'hay' and 'dried herb' aromas in Cabernet Sauvignon wines. The presence of 1,8-cineole has also been found to enhance 'blackcurrant' aroma in Cabernet Sauvignon wines. These results demonstrate that cineole isomers may be valuable aromatic contributors to Australian Cabernet Sauvignon wine typicality and potential markers of regionality.

Webcast of this presentation available at http://bit.ly/16thAntalick.

Introduction

The Bordeaux wine region of France is both a key reference point and benchmark of quality for Cabernet Sauvignon wines, despite the undisputed excellence of other regions such as Margaret River or Coonawarra in Australia. Whilst comparisons between Australian and Bordeaux Cabernet Sauvignon wines are common, it may be misleading as Australian Cabernet Sauvignon wines are as inimitable as Bordeaux wines.

Wine aromatic typicality is often referred to in the concepts of uniqueness and provenance. The understanding of regional typicality is important as it provides a reference standard for winemakers and can lead to an increased knowledge and appreciation of regional potential, important in a highly competitive international market context. Typicality studies increasingly utilise objective compositional measures, which increase the capacity to define discrete styles (Schuttler et al. 2015).

While Australian Cabernet Sauvignon wines are often appreciated for their distinctive fruit aromas, they are also described using attributes such as 'eucalyptus', 'mint', 'bay leaf' and 'dried herbs' (Halliday 2011). These differ from the descriptions commonly ascribed to the predominantly Cabernet Sauvignon wines from Bordeaux. Studies have demonstrated that these herbal characters are generally positively perceived by consumers (Saliba et al. 2009; Lattey et al. 2010), with the ability to provide balance and complexity to the overall wine aromatic spectrum. Besides the occurrence of eucalyptus notes reported to be associated with the presence of the terpenoid 1,8-cineole (eucalyptol) in red wine (Figure 1) (Capone et al. 2011), the origin of these specific aromas remains poorly understood. 1,4-cineole (Figure 1), another monoterpene with a similar structure and natural occurrence to that of 1,8-cineole, has also been reported in Australian red wines (Robinson et al. 2011). However, to our knowledge, 1,4-cineole has not been previously quantified in wines and the sensory impact of this compound in wines has not been reported.

This study investigated the occurrence of 1,4-cineole in Australian Cabernet Sauvignon and Bordeaux red wine. It also examined its contribution, both independently and in combination with 1,8-cineole, to wine aroma. In addition, the potential role of cineole isomers to the regionality of Australian Cabernet Sauvignon wines and the origin of 1,4-cineole in red wines was investigated.

Material and methods

A quantitative method was developed and validated in order to perform a survey of 1,4-cineole and 1,8-cineole in the following 104 Australian red wines; 51 Cabernet Sauvignon (mean age 3.5 years), 4 Cabernet Sauvignon/Merlot blends (mean age 7 years), 27 Shiraz (mean age 3.5 years) and 22 Pinot Noir wines (mean age 2 years). The wines originated from different Australian wine regions, including important regions for Cabernet Sauvignon wine production such as the Barossa Valley, Coonawarra, McLaren Vale and Margaret River. Importantly, the Pinot Noir wines were not sourced from the same regions as the Cabernet Sauvignon and Shiraz wines. 26 Bordeaux wines (mean age 11 years), which were predominantly Cabernet Sauvignon wines, were also investigated. Discriminative and descriptive sensory methods were used to characterise the contribution of 1,4-cineole and 1,8-cineole to Cabernet Sauvignon wine aroma.

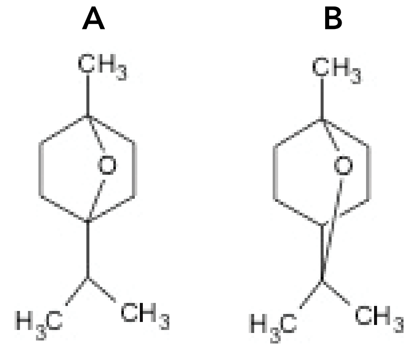

Figure 1. Chemical structure of 1,4-cineole (A) and 1,8-cineole (B)

Results and discussion

Survey of cineole isomers in red wines

1,4-cineole was detected in all wines analysed, with concentrations ranging from 0.023 to 1.6 μg/L. An important varietal effect was observed with average concentrations 8.4 and 2.7-fold higher in Cabernet Sauvignon than in Shiraz and Pinot Noir wines respectively. All Shiraz wines exhibited concentrations below 0.2 μg/L and 87% of Pinot Noir wines showed concentrations below 0.4 μg/L. Conversely, 1,4-cineole concentrations were above 0.4 μg/L in 68% of the Cabernet Sauvignon wines analysed, including 7 wines with concentrations above 1 μg/L (Figure 2A). The concentrations of 1,8-cineole were also cultivar dependent, with a higher proportion of Cabernet Sauvignon wines (87%) having concentrations above 1 μg/L, corresponding to the 1,8-cineole perception threshold (Hervé et al. 2003), compared to Shiraz (54 %) and Pinot Noir wines (44 %) (Figure 2B). Similar results have been previously reported for Australian red wines (Capone et al. 2011). The presence of a high concentration (23 μg/L) of 1,8-cineole in one of the Pinot Noir wines suggests that further investigation into influence of grape variety, in addition to region, on 1,8-cineole occurrence in Australian red wines would be valuable.

A comparison between 51 Australian Cabernet Sauvignon and 26 Bordeaux wines revealed that 1,4-cineole concentrations were significantly higher in the Australian (0.59 ± 0.33 μg/L) wines compared to those from Bordeaux (0.25 ± 0.15 μg/L). Bordeaux wines were older than Australian Cabernet Sauvignon wines and 1,4-cineole concentration was overall higher in aged wines (Figure 3). The influence of wine age on 1,4-cineole concentration will be discussed further. Despite the older age of Bordeaux wines, 1,4-cineole concentrations were significantly higher in Australian Cabernet Sauvignon wines. To emphasize the relative importance of 1,4-cineole in Australian wines in comparison to those from Bordeaux, the same comparison was also completed with consideration to wine age (Figure 4). The concentration of 1,8-cineole was on average 18-fold higher in Australian Cabernet Sauvignon (2.8 ± 3.2 μg/L) than in Bordeaux wines (0.16 ± 0.09 μg/L). Capone et al. (2011) previously reported that 1,8-cineole concentration was not influenced by wine age. These results suggest that both 1,4-cineole and 1,8-cineole are potential markers of Australian Cabernet Sauvignon aromatic typicality.

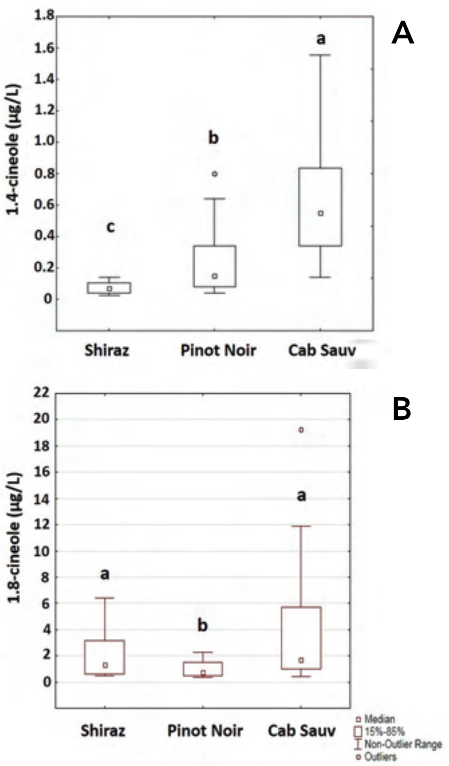

Figure 2. Distribution of 1,4-cineole (A) and 1,8-cineole (B) concentrations in Australian red wines represented as box plots with the minimum, maximum, median and quartiles. Different letters represent significant (p ≤ 0.05) differences

Contribution of 1,4-cineole to Cabernet Sauvignon wine aroma

The sensory impact level of 1,4-cineole was assessed in Cabernet Sauvignon wine using a series of triangle tests. An addition of 0.54 μg/L of 1,4-cineole in a Bordeaux Cabernet Sauvignon wine, to produce a final concentration of 0.63 μg/L, was required before it was detected by the panel (n = 18). 60% of Australian Cabernet Sauvignon wines exhibited higher concentrations of 1,4-cineole than this sensory threshold level, whereas only 9% of Pinot Noir wines and no Shiraz wines exceeded the level of 1,4-cineole required for sensory impact.

Descriptive analysis was also undertaken to characterise the effect of cineole isomers on Cabernet Sauvignon aromatic profile. The main sensory attributes used to describe the wines spiked to achieve final concentrations of 1,4-cineole and 1,8-cineole of 1.6 μg/L and 2.5 μg/L respectively were 'hay', 'dried herbs', 'fresh', 'mint' and 'blackcurrant'. The 'fresh' and 'minty' aromas are common characteristics of numerous Australian Cabernet Sauvignon wines with which 1,8-cineole has been previously associated (Capone et al. 2011). Therefore, the descriptive analysis focused on the investigation of 'hay', 'bay leaf' and 'blackcurrant' attributes. The tests were performed by comparisons of aromatic perception of the 'hay', 'bay leaf' and 'blackcurrant' reference standards. These standards were prepared in a Cabernet Sauvignon base wine and compared to the control base wine and wines that had been spiked with 1,4-cineole (1.6 μg/L) and/or 1,8-cineole (2.5 μg/L) (Table 1). For each test, the panel assessed the olfactory similarity of the samples against the standard presented. Statistical analysis showed the addition of 1,4-cineole, both independently and in combination with 1,8-cineole, enhanced 'hay' aromas in

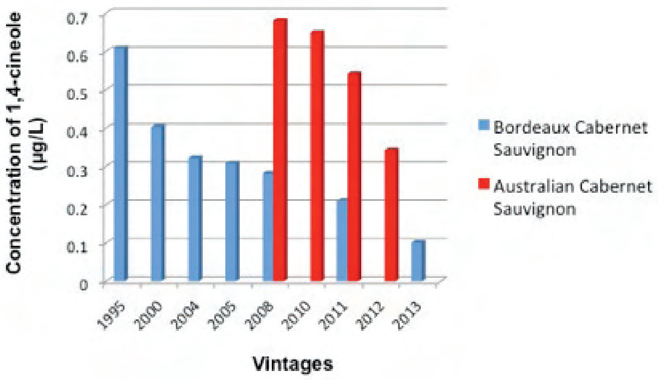

Figure 3. 1,4-cineole concentrations in an Australian (single cultivar) and a French (blend) Cabernet Sauvignon wine from different vintages

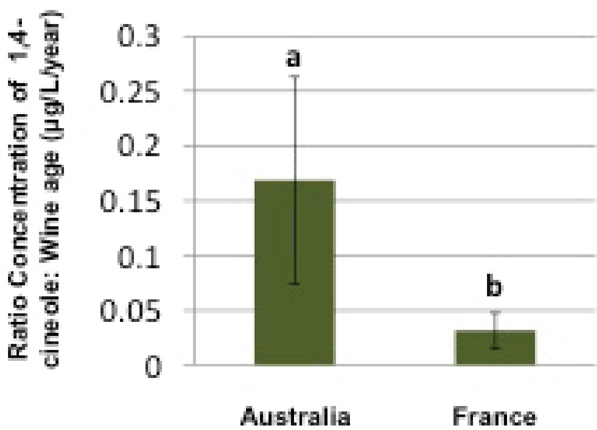

Figure 4. Comparison of the 1,4-cineole concentration: wine age ratio between Australian and French Cabernet Sauvignon wines. Different letters represent significant (p ≤ 0.05) differences

comparison to the control wine ($p<0.05$) (Figure 5A). The intensity of 'bay leaf' notes was also significantly enhanced by the association of 1,4-cineole and 1,8-cineole ($p<0.05$) (Figure 5B). In contrast, the panel was not able to significantly separate the different wines according to 'blackcurrant' aromas, even though the addition of 1,8-cineole only tended to be perceived with more pronounced notes of 'blackcurrant' (data not shown). To check the veracity of these observations, a final comparison between the control wine and the same wine spiked with high concentrations of 1,8-cineole (10 μg/L) was undertaken. The wine spiked with 1,8-cineole was perceived significantly higher for 'blackcurrant' aromas than the control wine, confirming the previous trend ($p<0.05$).

These findings indicate that 1,4-cineole, both in isolation and in combination with 1,8-cineole, may contribute to the 'hay' and 'dried herbs' aromas that have been reported in Australian Cabernet Sauvignon wines. These compounds have both been reported in different aromatic herbs such as thyme (Amarti et al. 2011), sage (Muller and Muller 1964) and bay leaf (Hogg et al. 1974) and the contribution of 1,8-cineole to the sensory perception of aromatic herbs has been reported in bay leaf and rosemary essential oils (Marzouki et al. 2009; Moss and Oliver 2012). While several studies have identified 1,8-cineole as a potent aroma of blackcurrant (Latrasse et al. 1982; Mikkelsen and Poll 2002), the current study suggests only a tentative relationship between 1,8-cineole and 'blackcurrant' aroma in some Australian Cabernet Sauvignon wines, possibly in combination with other compounds such as dimethyl sulfide (Lytra et al. 2014), is plausible.

Table 1. Code of samples used for sensory analysis

Code	Description	Concentration
CS	Cabernet Sauvignon wine	–
CS+1,4c	1,4-cineole in CS wine	1.6 μg/L 1,4-cineole
CS+1,8c	1,8-cineole in CS wine	2.5 μg/L 1,8-cineole
CS+1,8c-10	1,8-cineole in CS wine	10 μg/L 1,8-cineole
CS+1,4c+1,8c	1,4-cineole + 1,8-cineole in CS wine	1.6 μg/L 1,4-cineole
		2.5 μg/L 1,8-cineole

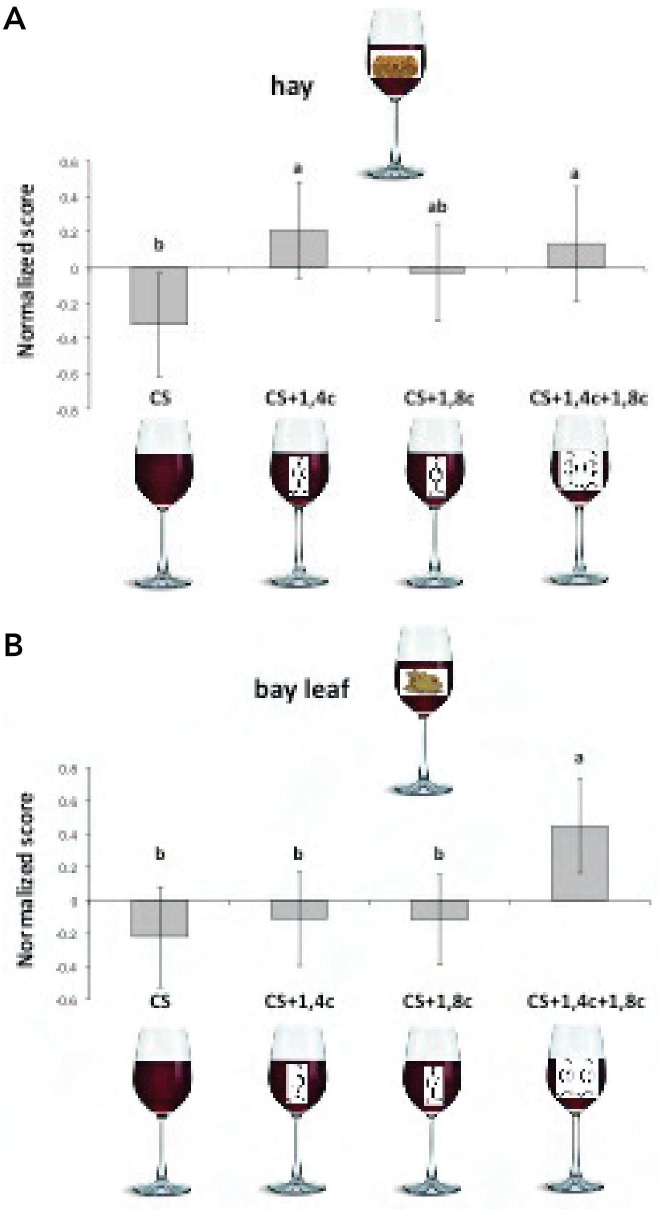

Figure 5. Descriptive analysis: Evaluation of the contribution of 1,4-cineole and 1,8-cineole to hay (A) and bay leaf (B) aromas in a Cabernet Sauvignon wine (CS) using the deviation from reference method (test of similarity). The number of panelists was 33. Different letters represent significant ($p \leq 0.05$) differences

Potential markers of Australian regionality

Significant variations in 1,4-cineole and 1,8-cineole concentrations were measured between wines originating from different regions of Australia (Figure 6). The Cabernet Sauvignon wines originating from Margaret River exhibited higher concentrations of 1,4-cineole than wines from Barossa/McLaren Vale ($p<0.05$) and to a lesser extent Coonawarra ($p = 0.08$) (Figure 6A). Conversely, higher concentrations of 1,8-cineole were found in Cabernet Sauvignon and Shiraz wines produced from Coonawarra compared to McLaren Vale/ Barossa ($p<0.05$) and Margaret River to a lesser extent ($p = 0.13$)

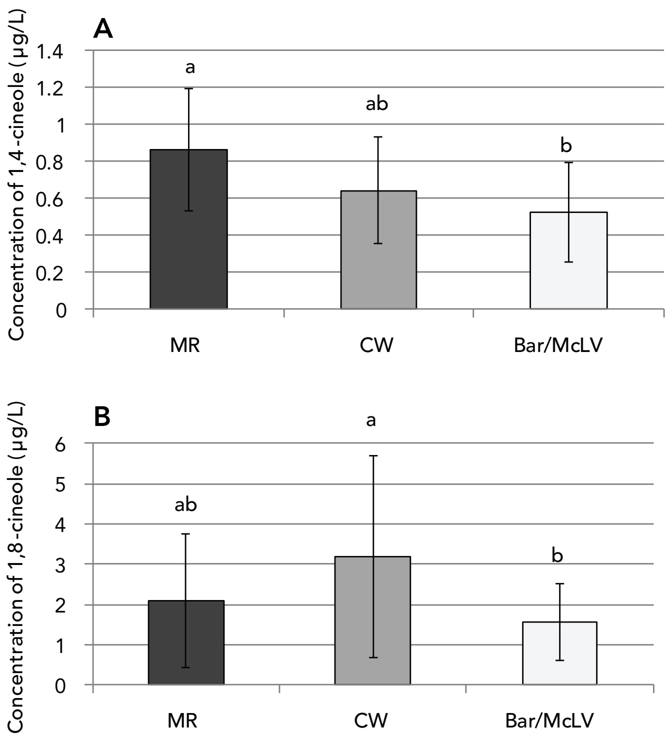

Figure 6. Effect of geographic origin on 1,4-cineole concentration in Australian Cabernet Sauvignon wines (A) and 1,8-cineole concentration in Australian Cabernet Sauvignon and Shiraz wines (B). One way ANOVA was used to compare data. Different letters on a column represent significantly ($p \leq 0.05$) different concentrations expressed in μg/L. Standard errors were used for the error bars. MR: Margaret River; Bar/McLV: Barossa/McLaren Vale; CW: Coonawarra. The wine distribution for Figure 5A is as following: MR: n = 13 with vintages as following: 2011: n = 9; 2010: n = 3; 2009: n = 1; CW: n = 12 as following: 2011: n = 3; 2010: n = 7; 2009: n = 2; Bar/McLV: n = 13 as following: 2011: n = 8; 2010: n = 4; 2009: n = 1. The wine distribution for Figure 5B is as following: MR: n = 17 as following: Cab. Sauv./Shiraz: n= 14/n = 3 and vintages: 2012: n = 1, 2011: n = 11, 2010: n = 4, 2009: n = 1; CW: n = 21 as following: Cab. Sauv./Shiraz: n= 16/n = 5 and vintages: 2012: n = 3, 2011: n = 5, 2010: n = 10, 2009: n = 2, 2005: n = 1; Bar/McLV: n = 22 as following: Cab. Sauv./Shiraz: n= 13/n = 9 and vintages: 2012: n = 1, 2011: n = 14, 2010: n = 6, 2009: n = 1

(Figure 6B). Regional variations of 1,8-cineole in Australian Cabernet Sauvignon have been previously reported (Robinson et al. 2012). The higher levels of 1,8-cineole found in the Coonawarra Cabernet Sauvignon wines agree with anecdotal sensory descriptions which include 'eucalyptus' and 'minty' aromas which are reminiscent of 1,8-cineole (Halliday 2011).

The potential contribution of 1,4-cineole and 1,8-cineole to the regional typicality of Australian Cabernet Sauvignon wines aroma was investigated by comparison of Coonawarra and Margaret River wines using a sorting method. Wines were grouped according to their geographic origin (Figure 7) with Coonawarra wines associated with 1,8-cineole and attributes such as 'eucalyptus', 'bay leaf', 'licorice' and 'black cherry'. These results indicate that 1,8-cineole might be an important marker of Coonawarra Cabernet Sauvignon and contribute to the 'eucalyptus', 'bay leaf' and 'fresh licorice' aromas that are often empirically reported in these wines. Margaret River wines were associated with a high 1,4-cineole:1,8-cineole ratio, 3-isobutyl-2-methoxypyrazine (IBMP) and descriptors such as 'hay', 'forest floor', 'capsicum', 'red fruit' and 'blackcurrant'. This region is generally known to produce wines with some elegant herbaceous aromas. IBMP has been reported to contribute to 'green' aromas perceived in Cabernet Sauvignon wines from Margaret River (Wilkinson et al. 2006). Even though IBMP was correlated more strongly to Margaret River wines than Coonawarra wines, the concentrations measured in the present study were considerably lower (< 13 ng/L) than reported in previous work. This suggests that compounds other than IBMP might contribute to Margaret River Cabernet Sauvignon typicality. The average concentrations of 1,4-cineole in Margaret River and Coonawarra wines selected for the sorting task were in the same range (0.74 and 0.69 µg/L respectively). However, the 1,4-cineole:1,8-cineole ratios were higher in Margaret River wines and correlated better with the herbaceous attributes perceived in these wines. These findings suggest that 1,4-cineole might contribute to the aromatic typicality of Margaret River Cabernet Sauvignon, when it is associated with moderate levels of IBMP and 1,8-cineole. In contrast, high concentrations of 1,8-cineole in combination with 1,4-cineole and IBMP seem to favour the expression of 'bay leaf' aromas. A 'bay leaf' aroma was found to be a more important descriptor for Coonawarra wines with an average concentration of 1,8-cineole of 7.7 µg/L, which was 2.9-fold higher than in the Margaret River wines.

Contrary to the descriptive analysis results, no relationships between 1,8-cineole and 'blackcurrant' aromas were identified when the Coonawarra and Margaret River wines were compared. This lack of consistency confirms that the perception of 'blackcurrant' aromas in red wines is complex and it is probably not due to only one or two compounds.

Overall, it appears that the relative concentrations of 1,4-cineole and 1,8-cineole might contribute, probably with other compounds, to the regional differentiation found between Margaret River and Coonawarra Cabernet Sauvignon wines.

Origin of cineole isomers in red wines

Preliminary investigations to help determine the origin of 1,4-cineole in red wines was also undertaken. The 1,4-cineole:1,8-cineole concentration ratios in the Australian Cabernet Sauvignon wines in this study ranged from 0.015 to 1.24, suggesting that the two compounds may have different origins. The presence of eucalyptus trees within the vicinity of vineyards has been reported to favour higher contents of 1,8-cineole in the corresponding wines (Capone et al. 2012). Other studies suggest that 1,8-cineole found in Australian wines could also be derived directly from grapes, particularly in the case of Cabernet Sauvignon (Kalua and Boss 2009). The varietal and regional effect on 1,8-cineole concentration highlighted in the present study add to the uncertainty regarding the origin of 1,8-cineole in red wines, and suggests factors other than proximity to eucalyptus trees may be important in determining the final concentration of this compound in wines. Additionally, analyses of different vintages of a unique Australian and French wine label demonstrated that the concentration of 1,4-cineole increased with vintage age (Figure 3). The analysis of an Australian Cabernet Sauvignon wine, artificially aged by heating at 40°C for 5 weeks showed an increase of 1,4-cineole concentration from 0.09 to 0.23 µg/L. Both these results suggest that 1,4-cineole is either partially or totally chemically synthesised during wine ageing.

The differences in 1,4-cineole concentrations measured between Australian and Bordeaux Cabernet Sauvignon probably indicates that Australian climatic conditions favour higher levels of 1,4-cineole precursors in grapes. Climatic indices for the Margaret River, Coonawarra and Bordeaux regions were calculated for the growing season from 1995 to 2014 (Table 2). Temperature-related indices (Huglin and Cold Night indices) are very similar in Margaret River and Bordeaux, while they are lower in Coonawarra. On the other hand, the frequency of heatwaves, assessed by counting the number of days with T max above 35°C, is 4 to 5 times higher in Coonawarra compared to the Bordeaux and Margaret River regions. Growing seasons are drier in Australia compared to Bordeaux, while winter rainfall is higher in the Margaret River. Global solar radiation is higher in Australia, particularly in Margaret River, and Australia is known to experience 12 to 15% higher UV radiation than Europe (Gies et al. 2004). Although the Coonawarra and Margaret River regions are often reported as having a similar climate to Bordeaux, the summary of climatic data (Table 2) illustrate important differences between these regions. Even though the effect of climatic factors

Table 2. Average climatic indices calculated for the growing season of the Coonawarra, Margaret River and Bordeaux regions from 1995 to 2014. The values were calculated for one site per region based on SILO database (Jeffrey et al. 2001) and Meteo France data for Australia and Bordeaux respectively.

Index/Region	Coonawarra	Margaret River	Bordeaux
Huglin index	2018	2154	2144
Cool Night index (°C)	11.5	14.9	15
Rain (mm)	192	159	361
Number of days with T max > 35°C	15	4	3
Global solar radiation (MJ/m^2)	4124	4693	3970

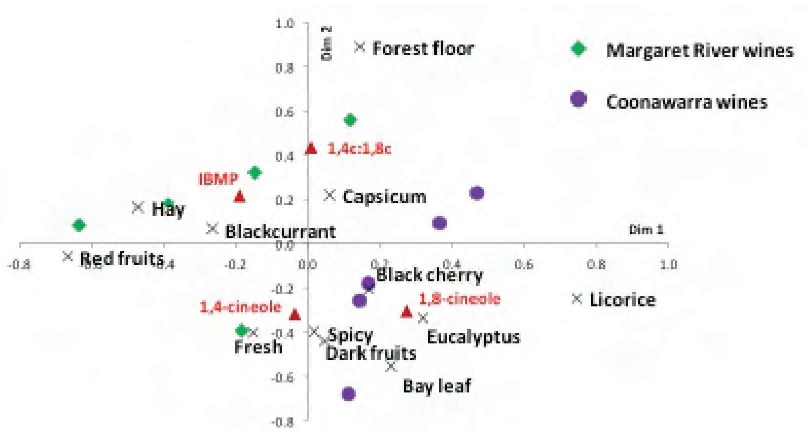

Figure 7. Two-dimensional multi-dimensional scaling (MDS) configuration of the 10 sorted Australian Cabernet Sauvignon wines from Margaret River (diamond) and Coonawarra (circle), and correlations of the sensory terms (cross) and chemical compounds (triangle) with the dimensions

on other terpenoids has been reported (Young et al. 2016) further investigations to determine the cause of cineole isomer differences in grapes and wine are required to more fully elucidate the reasons for the regional differences reported in this study.

Conclusion

Australian Cabernet Sauvignon wines are often compared to Bordeaux wines, a leading reference for this variety. Coonawarra and Margaret River, two of Australia's leading Cabernet Sauvignon regions, have also been described as having a similar climate to Bordeaux. As outlined in this paper, these regions are in fact quite different from a climatic perspective and produce significantly different Cabernet Sauvignon wine. Both 1,4-cineole and 1,8-cineole have been shown to contribute to aromatic typicality of Australian Cabernet Sauvignon wines.

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In-mouth flavour release from grape-derived precursors: unlocking hidden flavour during tasting

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Abstract

Glycosides are non-volatile compounds in grape berries, which can transfer into wine and break down during vinification to release free volatiles. It has been reported that smoke-related glycosides of volatile phenols can be degraded in-mouth, releasing smoky flavour from these bound precursors during consumption, most likely through the action of salivary bacterial enzymes. To test whether other types of odourless glycosides can contribute to flavour and aftertaste through breakdown in-mouth, glycosides were isolated from Riesling and Gewürztraminer grapes and wine, and were found to release important aroma compounds such as geraniol and linalool when incubated with saliva. Studies were conducted to assess the sensory significance of glycosides in model wine using time-intensity methodology. The mean sensory panel data showed that the glycosides gave a lingering fruit flavour at elevated concentration but had no statistically significant flavour effect at wine-like levels. However, individual responses were found to be highly variable. Some individuals consistently rated the glycosides as having significant flavour even at relatively low concentrations. Individual variability was further investigated using a panel of 39 subjects and several glycosides in water. Around three-quarters (77%) of the assessors were able to detect flavour from at least one of the glycosides, suggesting that the ability to experience flavour from grape-derived glycosides is widespread. Overall, this study found that breakdown of grape-derived glycosides in the mouth can play a role in wine flavour intensity and aftertaste, and the variation in sensory response could be a reason why different people appreciate different types of wines.

Webcast of this presentation available at http://bit.ly/16thParker.

Introduction

During wine tasting, the overall flavour impression and the persistence of flavour after swallowing or spitting are hallmarks of a high quality wine (Leske et al. 2013). Flavour is partly driven by volatile compounds, but also by non-volatiles such as sugars, salts, acids, phenolic compounds (including tannins) and polysaccharides, with aroma, taste and texture all contributing (Baker and Ross 2014b, a).

Many of the most important volatile flavour compounds in wine, including monoterpenes, norisoprenoids and volatile phenols, are also present in a bound form, joined to sugars as glycosides. Glycosides of volatiles are found in grape berries at concentrations much higher than their free volatile forms (Baumes 2009; Hjelmeland and Ebeler 2014). These glycosides, also called glycoconjugates, are non-volatile and have no aroma. Some of the locked volatile aroma molecules are known to be released quite rapidly during winemaking, by the action of yeast or bacterial enzymes, and more can slowly be released through ageing in bottle due to gradual breakdown in the acidic wine environment.

The type and abundance of the glycosides in grapes varies with the variety, contributing to differences in varietal flavour in the resulting wine (Williams et al. 1992). Grape berries of the floral varieties such as the Muscats, Gewürztraminer and Riesling contain particularly high concentrations of monoterpenes including geraniol, linalool, nerol and α-terpineol, which give floral or citrus aromas and flavours in wine, along with even higher concentrations of monoterpene glycosides (Black et al. 2015). Glycosidic precursors of other important wine compounds such as TDN (aged Riesling character, kerosene-like) and β-damascenone (enhancing fruitiness) can also be present, although the mechanisms of their formation are not well understood and probably involve a number of steps (Black et al. 2015).

Whether the non-volatile glycosides can act as important flavorants in wine during consumption is a fascinating question. Can glycosides release their locked flavour in the short time of wine consumption, considering the low pH of wine and presence of ethanol? A study published in 1999 demonstrated the hydrolysis of the simple glycoside compound hexyl glucoside in the mouth in one individual, with a perceptible flavour reported when a high concentration was tasted in water (Hemingway et al. 1999). A further study showed that another kind of flavour precursor, the non-volatile sulfur compounds in onions, can breakdown in-mouth, giving rise to perceptible flavour and contributing to lingering oniony aftertaste (Starkenmann et al. 2008). The same research group demonstrated that the precursor cysteinyl 3-mercapotohexanol, tasted in water at a relatively high concentration, was broken down during tasting to give the distinctive 'passionfruit'/'boxtree' character of 3-mercaptohexanol, which is an important compound in Sauvignon Blanc (Starkenmann et al. 2008). However, the concentration used was ten times higher than the concentration typically found in wine and these studies used water for the tasting matrix.

Recent work at the Australian Wine Research Institute has shown that smoke-related volatile phenols can be released from glycosides during tasting, most likely through the action of salivary bacterial enzymes (Parker et al. 2012; Mayr et al. 2014). The smoke-related glycosides led to a 'smoky'/'medicinal'/'ashy' flavour or aftertaste when presented to sensory panellists in model wine (a buffer system at wine pH with alcohol), creating a strong flavour within 30 seconds and lingering for as long as 15 minutes in sensitive individuals. However, in the case of smoke-affected grapes and wines, there is a large accumulation of volatile phenol glycosides following the absorption of volatile phenols from smoke in the air around the vine, with the concentration of phenol glycosides becoming unusually high (Hayasaka et al. 2010; Hayasaka et al. 2013) compared to levels of other flavour glycosides in grapes. The presence of ethanol, high acidity, low temperature and glucose were shown to reduce the amount of free guaiacol released from guaiacol glucoside in saliva assays in the laboratory (Mayr et al. 2014).

While glycoside release will be significantly inhibited by the ethanol and low pH of the wine matrix when a wine is in the mouth, the buffering capacity of saliva allows the mouth to recover quickly once the wine is swallowed or expectorated. The glycosides remaining in the mouth and throat can then be hydrolysed at body temperature and neutral pH, releasing volatiles. The hydrolysis occurs due to salivary bacterial enzyme action. This can be demonstrated by using antibacterial mouthwash such as chlorhexidine or Listerine®, which has a profound and lasting effect on the ability to hydrolyse glycosides in the mouth, with inhibition for as long as two to six hours after application (Walle et al. 2005; Mayr et al. 2014). In other words, using antibacterial mouthwash or toothpaste before tasting wine will stop flavour from being released from glycosides, rendering them flavourless.

'Retronasal' aroma is a very complex effect to study scientifically, as aroma volatiles during eating or drinking must reach the olfactory receptors at the back of the nose to be able to be perceived as flavour. This depends on the chemical properties of the volatiles; aroma compound-receptor interactions; the highly variable biological factors of saliva composition (including saliva pH, enzyme composition and diversity of bacteria); saliva flow-rate; temperature in the mouth; and physiological factors like breathing, swallowing and mouth movements during consumption (Buettner and Beauchamp 2010). Most people have experienced that when they are affected by a cold, food tastes bland, due to the lack of movement of air from the mouth to the olfactory receptors. Pinching the nose during eating or drinking has the same effect.

As wines of floral white varieties have high levels of monoterpene glycosides (Baumes 2009), investigations in this project have initially concentrated on these varieties. The floral varieties are commercially very important to the Australian wine industry. According to the Wine Australia Vintage Report 2016, Muscat Gordo Blanco, also known as Muscat of Alexandria, has a large contribution to the Australian winegrape crush, being ranked the eighth most important grape variety, red or white, by tonnes, with a crush of 56,710 tonnes and a value of $12.4 million (Wine Australia 2016). Riesling was ranked 11th at 28,224 tonnes and $21.7 million. Gewürztraminer was ranked 14th at 14,219 tonnes and value of $5.2 million.

Glycoside characterisation

Glycosides were isolated from Riesling and Gewürztraminer grape juice and wine, washed to remove bitter phenolic glycosides and free volatiles, and then characterised. Liquid chromatography–mass spectrometry confirmed the presence of a diverse range of monoterpene glycosides, including monoterpene glucosides and monoterpene disaccharides. Glycosides of the important rose-like 2-phenylethanol compound were also detected, but these were considered unlikely to contribute to flavour due to the very high sensory threshold of 2-phenylethanol (Ferreira et al. 2000). 2-Phenylethyl glycosides were similar in concentration in the Riesling and Gewürztraminer samples. The monoterpene glycosides were detected in the Riesling and Gewürztraminer extracts, with approximately 10 times more abundance in the Gewürztraminer extracts.

After confirming that the glycoside extract was free from volatiles that could impart aroma and confound the experiment, the glycosides were incubated with glycosidase enzyme, and the liberated volatiles were analysed using solid phase microextraction-GC-MS. As expected, the important 'floral'/'citrus' odorants geraniol, nerol, alpha-terpineol – and smaller amounts of linalool – were among the released volatiles, along with the less important hexanol, benzyl alcohol and 2-phenylethanol.

The glycosides were also incubated with saliva (Figure 1), and a similar pattern of volatiles was observed, with geraniol and linalool among the released volatiles.

Analysis of volatiles in the breath after tasting glycosides

To confirm the presence of free volatiles in the mouth after tasting glycosides, exhaled breath was sampled using a method based on a published procedure (Buettner 2004; Buettner and Welle 2004; Mayr et al. 2014). Gewürztraminer wine glycosides were taken into the mouth, swirled around the mouth then expectorated, and the volatiles in the mouth were then sampled by passing exhaled breath across a polymeric silicone adsorbent material for five minutes. The volatiles were analysed using GC-MS and compared to controls, including exhaled breath before placing glycosides in the mouth. The monoterpenes geraniol, nerol, and α-terpineol were clearly and reproducibly detected using this technique, showing that as well as in the test tube, glycosides can be broken down in the mouth.

Sensory time-intensity studies

The question remained whether the breakdown of glycosides is enough to provide a real flavour effect for a wine drinker. Glycosides were extracted from Gewürztraminer wine and juice and assessed in model wine at elevated concentration, approximately five times the concentration in the initial wine. A panel of eleven people were trained in the technique of continuously rating overall fruit flavour, with intensity recorded for a period of 120 seconds. Overall fruit flavour was defined as 'floral', 'citrus', 'stone fruit' or 'confectionery'. After a number of training and practice sessions, the panellists were presented with the samples, individually, covered, in a randomised order, in three-digit-coded, black wine glasses at 22–24°C. The assessments were made in isolated booths over several days, and data was collected on a computerised system. Synthetic geranyl glucoside was also included in the study. This glycoside gives the monoterpene geraniol.

As shown in Figure 2, fruit flavour was rated for each of the glycosides studied. The glycosides from juice had the highest maximum scores, after a delay in the start of flavour perception, and had the longest aftertaste. This was not surprising as aroma glycosides are at higher concentrations in juice than in finished wine. Geranyl glucoside had a more rapid increase in flavour. The overall flavour effect was similar for all three glycosides.

The samples were also assessed for aroma intensity and there was no significant difference in 'fruity'/'floral' aroma compared to the model wine, although there was a slightly higher rating for this attribute for the juice precursor sample.

On close inspection of the data for each panellist, it was revealed that of the 11 judges, only six consistently rated flavour from the glycosides.

The result was encouraging, showing that even with the short amount of time involved when tasting, with low pH and in the presence of ethanol, enough glycosides can be degraded to release flavour. The effect of the glycosides at wine-like concentrations needed to be tested, to better determine the relevance to a real wine tasting experience. Glycosides were extracted from Gewürztraminer and also Riesling wine, purified to remove phenolics and any free volatiles, and added to model wine at the equivalent concentration of the initial wine, with and without wine volatiles. Geranyl glucoside was also included at a concentration comparable to that found in wines.

For these more dilute samples there was no statistically significant flavour from the glycosides when assessing data from the panel as a whole. Again, individual responses were very variable, and it was found that five of the 12 panellists rated 'fruity'/'floral' flavour for the glycosides.

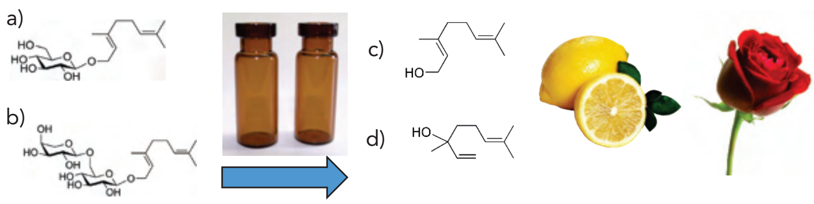

Figure 1. Examples of monoterpene glycosides geranyl glucoside (a) and geranyl vicianoside (geranyl α-L-arabinose-β-D-glucoside) (b) found in grape and wine extracts, and examples of hydrolysis products geraniol (c) and linalool (d) detected upon incubation with whole saliva.

Interestingly, the presence of glycosides enhanced the flavour intensity and duration compared to the volatiles alone for some judges (see Judge 1, Riesling, Figure 3), even at these lower, wine-like concentrations. Glycosides also gave rise to flavour when tasted alone without volatiles (for example Judge 1 in Gewürztraminer and Riesling, and Judge 2 in Riesling but not Gewürztraminer (Figure 3)). The glycosides did not enhance the flavour of the Gewürztraminer volatiles to the same extent, possibly because the volatiles for the Gewürztraminer were high in concentration, overwhelming the effect of the glycosides.

Examining individual variability in flavour response from glycosides

The time intensity results revealed that there was individual variability in the flavour response to glycosides, with approximately half of the panel consistently rating 'fruity'/'floral' flavour from the glycosides, but others not responding at all, or only weakly for one or two replicates. In order to investigate this further, a larger panel of 39 individuals was recruited. Three types of glycosides were tasted individually in water: geranyl glucoside and glycosides extracted from Gewürztraminer wine, as well as guaiacyl glucoside. Guaiacyl glucoside, which gives the smoky phenol guaiacol, was also included as an example of a quite different chemical compound, to better understand individual variability. The samples were assessed in triplicate presentations, with control samples of water also assessed. The panellists were asked to rate 'fruity'/'floral' and 'smoky' flavour.

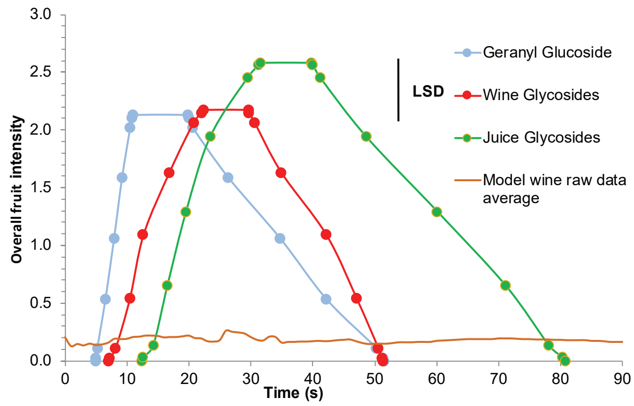

Figure 2. Mean time intensity curves for 'overall fruit' flavour intensity, from 11 judges x 3 replicates for samples with added glycosides, assessed in model wine. Juice glycosides are shown in green, wine glycosides are red, geranyl glucoside is light blue and model wine is brown.

Positive responses for each judge were determined by analysis of variance. Results showed that 54% (21 out of 39) of the panel consistently rated flavour (either 'fruity'/'floral' and 'smoky') over and above the water control for the Gewürztraminer glycosides. For the other glycosides, 46% (18 out of 39) consistently rated significant flavour in the geranyl glucoside, and 64% (25 of 39) detected significant flavour in the smoky guaiacyl glucoside. So it might at first appear that this phenomenon of being able to detect flavour from glycosides is restricted to approximately half of the population, but closer examination of the data revealed a more complex array of responses. Figure 4 shows that some people could perceive flavour from each of the glycosides, some for only two or one, and some for none. The largest group (28%) responded to all three types of glycosides. The second largest group (23%) did not consistently perceive flavour from any of the glycosides, although they may have given a flavour response for one or two of the replicate tests. Other people were able to detect flavour from only one or two of the three glycosides. For example, 13% rated flavour from the guaiacyl glucoside only. Overall, 77% of the judges were able to detect flavour from at least one of the glycosides, suggesting that the ability to experience flavour from glycosides is widespread.

In thinking about the reasons why people might have different responses to the glycosides, there are several factors involved. In order to be able to detect retronasal aroma from the glycosides, a number of steps must occur. The glycoside must be broken down in the mouth, presumably due to glycosidase enzymes from oral microbiota (Hemingway et al. 1999; Walle et al. 2005). Several studies have shown that different people can have different populations of microorganisms resident in their mouth (Avila et al. 2009; Lazarevic et al. 2010). The glycosidase enzyme activity of saliva from four individuals was shown in an earlier study at the AWRI to vary from 4% to 68% when tested using guaiacyl glucoside in laboratory assays (Mayr et al. 2014), so this could be a large source of variation. The panellists in these studies were asked to refrain from using antibacterial mouthwash or antibacterial toothpaste in the mornings of tastings, and winemakers could consider this while preparing for a tasting. Also, the volatile compound must be able to pass through the air from the mouth to the nose to be sensed by olfactory receptors in the olfactory bulb (Buettner and Beauchamp 2010). For some people, it

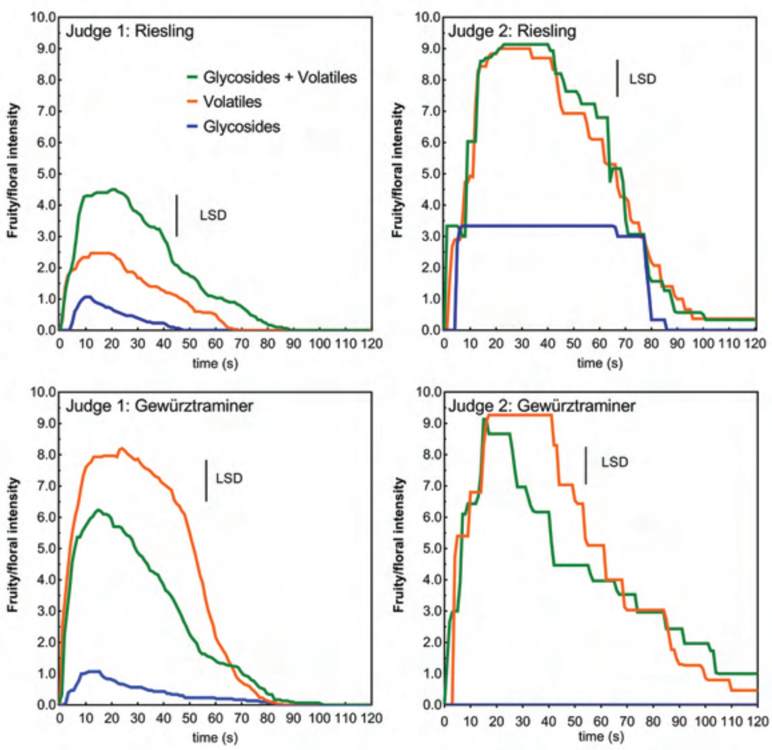

Figure 3. Example time intensity curves for two of the judges who perceived flavour from glycosides at wine-like concentrations. The least significant difference (LSD) is shown, P= 0.05

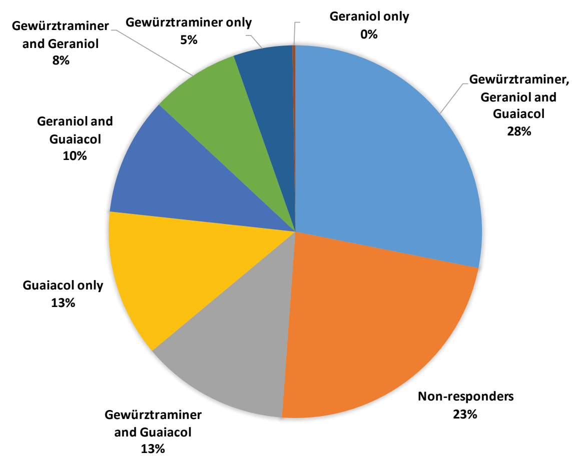

Figure 4. The proportion of judges who responded to three types of glycosides: geranyl glucoside, guaiacyl glucoside and Gewürztraminer wine glycosides, n=39 judges.

is likely that there are restrictions in air, and thus volatile compound, movement from the mouth to the nose. Variations in the anatomy of the panellists, and breathing/swallowing behaviour when tasting can also influence retronasal perception (Buettner and Beauchamp 2010). The ability to detect aromas by the olfactory receptors is also highly variable among individuals, with at least 14% variation in odour receptor genes reported to be typical between two human individuals (Zhang et al. 2007). These factors together might explain the variation in response to glycosides, and partially explain different sensory responses to wines, and therefore preferences.

Conclusion

This study has shown that breakdown of odourless grape-derived glycosides during wine consumption can play a role in wine flavour, contributing to fruity flavour intensity and lingering aftertaste during the wine tasting experience. Even with the short time these compounds are present in the mouth, and with the inhibitive effects of low pH and the presence of ethanol, it seems that hydrolysis occurs rapidly enough and strongly enough to give perceptible flavour. While the effect was strongest when glycosides were tasted at quite high concentration, even at typical wine-like levels close to 50% of the individuals reported a flavour effect. Whether the release of flavour from these glycosides adds to the flavour and persistence of aftertaste of a typical wine, with the presence of free volatiles, is still not certain, but for some individuals this seems to be the case.

The large variation observed across individuals provides a new insight into the reasons for differences in sensory and preference responses to wines. Further research is needed to better understand the factors underlying this variation in responses.

Enhancing the glycoside pool in wines is likely to have a positive effect on wine flavour, providing an opportunity to increase the intensity and duration of desirable grape-derived flavours. However, most methods of enhancing glycosides, such as extending skin contact time, will also enhance undesirable bitterness and astringency due to non-aroma-active phenolic glycosides, which make up a large proportion of the glycoside pool in grape skins. Understanding the effect of winemaking techniques on glycosides could produce wines with higher concentrations of glycosides, thereby creating wines with more lasting flavour on the palate rather than lots of volatiles creating a fleeting impression up-front. One way of preserving glycosides would be to avoid enzymes and yeasts with glycosidase activity. This could be particularly useful when creating low alcohol wines, providing a means to boost flavour. Current work at the AWRI involves assessing practical means of extracting flavour-active glycosides from grape skins without enhancing bitterness. If it were possible to extract non-bitter glycosides from marc on a large scale, these could be very valuable to enhance flavour, and a study to assess the potential application of this is currently being conducted at pilot scale.

So far this work has concentrated on floral white varieties high in monoterpene glycosides, but it would be exciting to investigate other types of glycosides, such as norisoprenoids and benzene derivatives, and to expand this research to other varieties, and to other types of precursors such as those involved in tropical fruit flavour.

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High-throughput phenotyping of malolactic bacteria

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Abstract

*A roboticised method has been developed to facilitate high-throughput systematic profiling of large numbers of malolactic bacteria (*Oenococcus oeni*) strains for malolactic fermentation (MLF) efficiency and response to wine stress factors. Using miniaturised wine fermentations in 96-well microplates, the robotic system can be used to prepare and inoculate multiple combinations of bacterial strains and stress factors in red or white wine, and then analyse malic acid in thousands of samples over the course of MLF. In any one run, up to 40 bacterial strains can be screened for MLF efficiency and response to wine stresses such as alcohol, low pH and low temperature, with approximately 5,000 or more individual L-malic acid analyses performed. This provides a highly efficient MLF screening capability compared to conventional testing methods. In one application of the method, large numbers of* O. oeni *strains from the Australian Wine Research Institute Wine Microorganism Culture Collection (AWMCC) and other sources were profiled for response to wine stress factors. Phenotypic profiles in a red and white wine are presented, revealing a wide spectrum of stress response characteristics.*

Webcast of this presentation available at http://bit.ly/16thCostello.

Introduction

Malolactic fermentation is well recognised as an important secondary fermentation in the winemaking process. It is carried out by some lactic acid bacteria (LAB), principally strains of *Oenococcus oeni*, and is essential in the production of red wines, as well as some white and sparkling base wines. Typically occurring after alcoholic fermentation, MLF is chiefly characterised by the conversion of L-malic acid to L-lactic acid and carbon dioxide, leading to a reduction in wine acidity. In addition, other metabolic activities associated with malolactic (ML) bacteria can bring about further modifications to a wine's sensory profile, including impacts on 'buttery', 'fruity' and 'vegetal' aromas and some mouth-feel attributes (Davis et al. 1985; Liu 2002; Bartowsky and Henschke 2004; Bartowsky and Pretorius 2009; Costantini et. al. 2009; Sumby et al. 2014). A third advantage of MLF is that the removal of malic acid increases microbial stability of wine; in red wine production, winemakers avoid bottling wines with residual L-malic acid content due to the risk of MLF occurring in the bottle, as this increases turbidity and leads to gas formation (Davis et. al. 1985).

Induction and control of MLF can be challenging and fickle. A major contributing factor in this regard is the difficult conditions that the bacterium is exposed to in wine including: low pH, sulfur dioxide, high ethanol concentrations and suboptimal temperatures. Extremes in any of these parameters can lead to a slow and protracted MLF, requiring many months for completion, or even MLF failure. Such delays can be detrimental to wine quality and lead to increased costs, for example through increased heating to maintain optimal temperatures (typically 18–22°C), and extended periods of wine storage exposing wine to oxidation and increasing the risk of spoilage.

Ongoing research efforts to improve winemaker control over MLF have provided valuable knowledge on the many factors that govern the growth and malolactic activity of wine LAB (Wibowo et. al. 1985, 1988; Bauer and Dicks 2004). Furthermore, a wider range of commercial malolactic starter cultures has become available in Australia over recent decades. However, despite such advances, MLF can still be problematic.

One factor that, to date, has not received a great deal of attention when considering reliability and efficiency of MLF is genetic diversity amongst *O. oeni*. Recent publications in this field (Borneman et. al. 2012; Campbell-Sills et. al. 2015; Sternes and Borneman 2016), however, are changing this situation. It is apparent from this work that there is enormous diversity, which will affect a range of important phenotypic traits that, in turn, will influence MLF efficiency and tolerance to wine stress factors.

Moreover, in an Australian context, it is important to note that none of the commercially available MLF starter cultures use bacteria isolated from Australian wine. Since European wine conditions are quite different to those from Australia, particularly in wine alcohol content, it is plausible that malolactic starter cultures prepared from *O. oeni* strains of European origin may not be optimal for induction of MLF in some Australian wine conditions. It is therefore pertinent to explore the potential of Australian isolates of *O. oeni* for MLF performance.

This has been a driver for the Australian Wine Research Institute (AWRI) to develop a phenotypic screening program with the aim of identifying robust strains from the many Australian isolates of *O. oeni* held in the AWMCC. This collection is a highly valuable resource and contains genomically sequenced, genetically diverse bacterial strains isolated from numerous Australian wines over many decades. Candidate test strains for screening were selected from the collection that were representative of phylogenetically distinct sub-groups of *O. oeni.*

The phenotypic screening of malolactic bacteria for MLF performance and robustness requires assessment of growth and/or malic acid degrading capabilities in wine or other suitable test media. Importantly, stress factors can also be applied, including: low pH, high alcohol content, presence of SO_2, low temperature, or combinations of these parameters (Vaillant et. al. 1995; Solieri et. al. 2010; Torriani et al. 2011).

However, due to the large number of fermentations required for such a screen, the use of time-consuming traditional microbiological methods is impractical; the number of test fermentations quickly escalates into the thousands when even a modest number of strains (e.g. 70) are assessed in several different wine types, with multiple stresses and the need for replicates. Further, an even larger number of malic acid analyses is required to monitor the progress of MLF in each fermentation, rendering such a study beyond the scope of traditional techniques. Consequently, screening a large pool of strains for robustness requires a more efficient and automated approach to process a vast number of fermentations.

To this end, the use of multiwell plates (microplates) has enabled adoption of high-throughput methods in a variety of microbiological applications, including fermentation research (Samorski et. al. 2005; Duetz 2007; Licciolli et. al. 2011). Each multiwell plate has discrete microwells (typically 96) that can be used as an array of 150–200 µL micro-fermentations. A robotic liquid handling workstation is used to dispense test wines and inoculate bacterial starter cultures into multiple 96-well microplates. Over the course of MLF, microplates are harvested and analysed for malic acid using a roboticised enzyme assay, enabling systematic monitoring of each micro-fermentation, with approximately 5,000 samples tested in each study.

Results and discussion

An example of the results obtained from the high-throughput system for screening malolactic bacterial strains is shown in Figure 1. In this study, the response to stresses of high alcohol and low pH of 38 *O. oeni* strains from the AWMCC were tested in a Shiraz wine. The majority of strains were found to complete MLF in the reference wine within 6 to 14 days, whereas application of either low pH or alcohol stress increased the time to complete MLF from less than 10 days to greater than 30 days.

The stress tolerance of each strain is more clearly depicted in the plot of the time length to complete MLF in the reference wine compared to each stress condition. In the case of alcohol stress (Figure 2), the range of stress responses were categorised as either sensitive (13 strains), intermediate (17 strains) or tolerant (8 strains). Under the stress condition, tolerant strains completed MLF within 10 days, with several strains completing MLF in 7 days or fewer. It is also noteworthy that a number of intermediate and sensitive strains which performed well in the reference wine exhibited very poor MLF efficiency under alcohol stress. While potentially suited to low alcohol conditions, such strains would be considered highly inefficient for MLF induction in wines with any level of alcohol stress, and are therefore unsuitable for further testing.

The efficacy of the high-throughput system is further demonstrated in Figure 3, which shows the responses of 39 *O. oeni* strains to singular and combined stresses of low pH, high alcohol and low temperature in Chardonnay wine. The tolerant strains are again easily identifiable, exhibiting the least time to complete MLF under the stress conditions.

Overall, the screening studies in each wine required up to 3 months to undertake using the high-throughput system, whereas traditional methods would have required at least 12 months.

Following the screening studies, *O. oeni* strains identified as tolerant to wine stress factors were chosen as candidates for further testing in laboratory- and pilot-scale trials. In these trials, the candidate strains

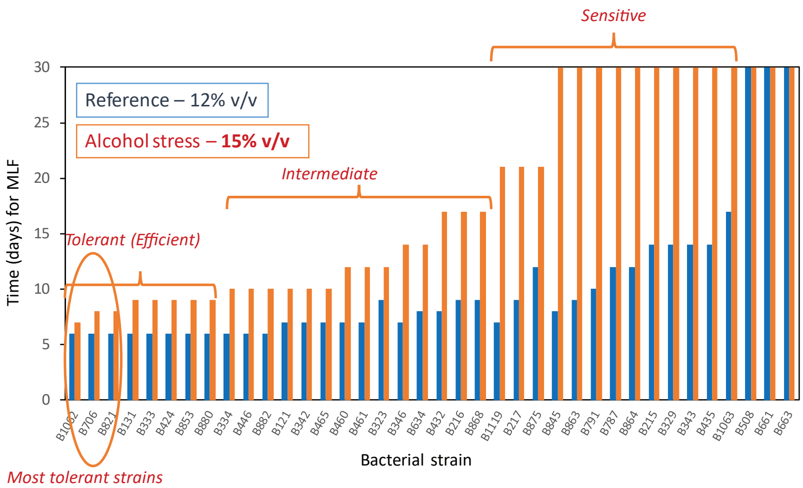

Figure 2. Effect of alcohol stress on time to complete MLF by 38 *O. oeni* strains in Shiraz wine. For each strain, the time to complete MLF in reference wine (12% v/v ethanol, blue bars) is plotted next to the time to complete MLF under the stress condition (15% v/v ethanol, red bars). Alcohol stress response categories (sensitive, intermediate, tolerant) were assigned to strains according to the time to complete MLF under the stress condition compared to the reference wine. Where vertical bars extend to the axis maximum, MLF was either complete, partially complete or not initiated.

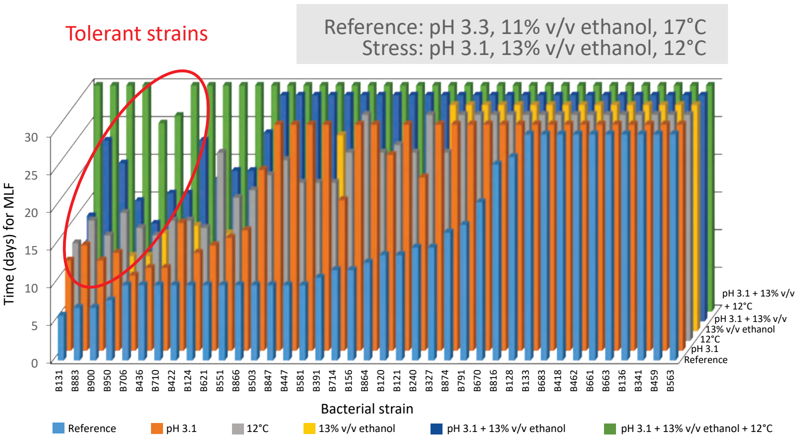

Figure 3. Effect of single and combinations of stress factors (low pH, low temperature and high alcohol) on time to complete MLF for 39 *O. oeni* strains in Chardonnay wine. For each strain, the time to complete MLF in the reference wine (pH 3.3, 11% v/v ethanol; 17°C; blue bars) is plotted next to the single stress (pH 3.1, brown bars; 12°C, green bars; 13% v/v ethanol, purple bars) and combined stresses (pH 3.1 and 13% v/v alcohol, light blue bars; pH 3.1, 13% v/v alcohol and 12°C, orange bars). Stress tolerant strains (circled) were identified as those with a comparatively efficient MLF response under the stress condition compared to that of the reference wine. Where vertical bars extend to the axis maximum, MLF was either complete, partially complete or not initiated.

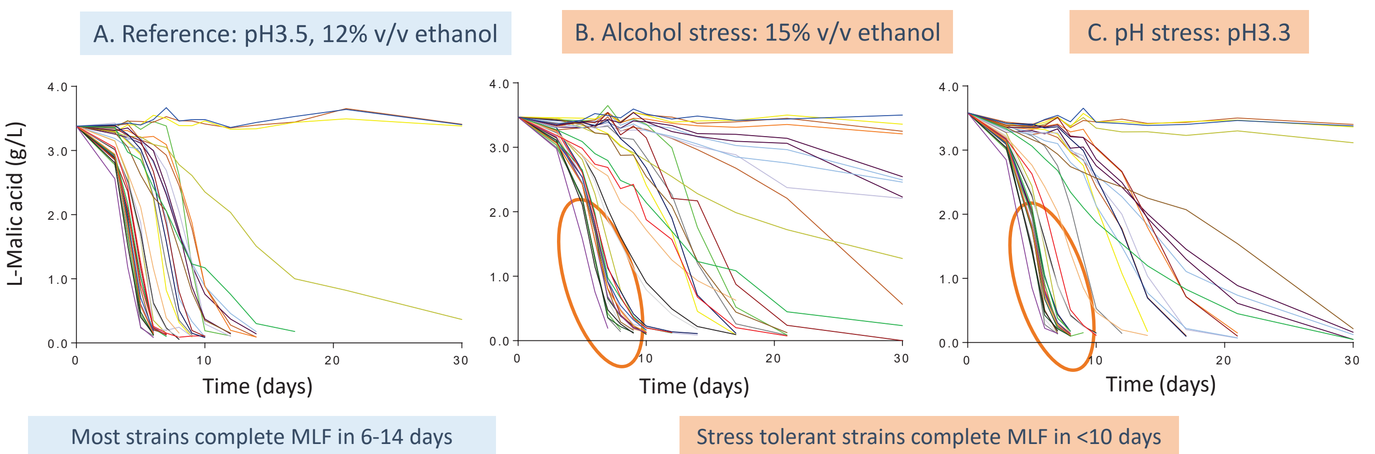

Figure 1. High-throughput MLF screening of 38 *O. oeni* strains for responses to alcohol and pH stresses in Shiraz wine. Each line represents the progress of MLF by a bacterial strain in (A) reference wine, (B) with alcohol stress (15% v/v), or (C) pH stress (pH 3.3). Stress-tolerant strains (circled) are those which completed MLF within 10 days under either stress condition.

were tested for MLF performance, as well as sensory impacts (i.e. absence of faults) and diacetyl production in red, white and sparkling base wines on a 2 to 20 L scale. From this, two strains were subsequently chosen as preliminary selections for winery-scale testing, one strain each for red and white wine. Commercial-scale evaluation of these two strains for MLF induction in the 2016 vintage has shown promising results, with further winery trials planned for additional strains during the 2017 vintage.

Conclusions

A high-throughput robotic system has been developed to enable screening of large numbers of *O. oeni* isolates for robustness and MLF efficiency in red, white and sparkling base wines. Using microfermentations in a multiwell plate format, this methodology facilitates testing multi-factorial combinations of bacterial strains and stress factors, and the analysis of L-malic acid in many thousands of samples.

Using this high-throughput platform, a wide range of phenotypic diversity in stress tolerance and MLF efficiency has been demonstrated amongst Australian isolates of *O. oeni* strains housed in the AWRI Wine Microorganism Culture Collection. Significantly, a number of uniquely robust Australian isolates have been identified. Laboratory- and pilot-scale testing of these robust strains has led to successful trialling and evaluation of two selected strains at winery scale.

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Wine perception and identification

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Abstract

Among the tasks a sommelier must perform in order to become a master sommelier is the identification of six wines in 25 minutes. The level of detail is daunting: vintage, grape variety, country of origin, appellation and quality designation must be precisely identified to succeed. Sommeliers attempt this feat by a system of analysis called 'deductive tasting', learned over years of practice. An examination of this deductive tasting reveals details of how the sensory system, working with memory, creates odour images. This paper will discuss recent research on the psychophysics of wine odorant mixture perception and how the deductive tasting process allows a sommelier to identify wine with such accuracy.

Webcast of this presentation available at http://bit.ly/16thAcree.

Introduction

In the 2013 documentary film *Somm* (Wise 2013), Ian Cauble is shown practicing for the final exam for a Master Sommelier competition. With a colleague, he is attempting to identify a wine using deductive tasting techniques. As he holds the glass at a 45-degree angle analysing the appearance, he begins a 'rap' not unlike the beginning of the play *Hamilton*. It begins, 'Wine 1 is a white wine, clear star bright, no evidence of gas or flocculation, the wine has a light straw core consistent to green in the edge…' and he continues to identify subsets of features that come from his grand set of all wine properties.

Figure 1 lists the odour labels used by Ian after his orthonasal sniffs: 'lime' (candy), 'lime' (zest), 'apples' (crushed). These labels were associated with his memories of lime and apple. The binary pairs of odorants shown in Figure 1 in the red and green ovals may have stimulated the choice of these labels. After swilling the wine the odour labels, 'lilies' (white), 'slate' (crushed), 'chalk' (crushed), 'hillside' (crushed), and 'tennis balls' may be represented by the perception of the binary odorant pairs in the black, yellow and white ovals. Taken together with visual, taste and chemesthetic sensations Ian concludes correctly the wine is a 2009 Riesling from the Clare Valley (a high value producer). Assigning the five pairs of five odorants known to be in Riesling wines, as shown in Figure 2, to the labels reported by Ian during this analysis is speculative but on the surface believable and using simulations testable.

The hypothesis is that during the deductive tasting process Ian is attempting to detect subsets of odorants that form familiar odour images to him. He is experiencing the wine smell as a series of overlapping sensations that come from his past experience with other recognisable odour objects. To test this hypothesis, we need an experimental procedure that is rapid and precise enough to measure the interaction between odorants, odours and labels. To do this we have developed a sniff olfactometer (SO) (Acree et al. 2014; Wyckoff and Acree 2016), shown in Figure 3, to determine response probabilities to mixtures of binary and tertiary odorants and compare them to simulant mixtures or actual wines. The SO delivers 15 mL pulse of headspace released from a sample to the olfactory nares of a subject

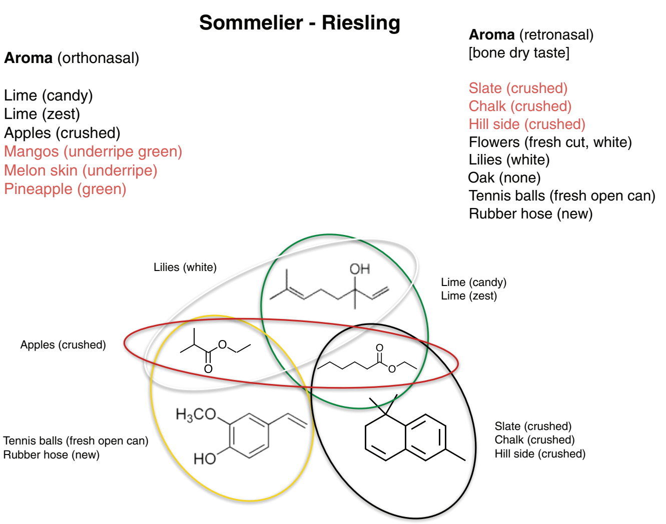

Figure 1. Aroma descriptors used by Ian Caudle to identify a 2009 Clare Valley Riesling and the odorants that may have generated his perceptions (Wise 2013)

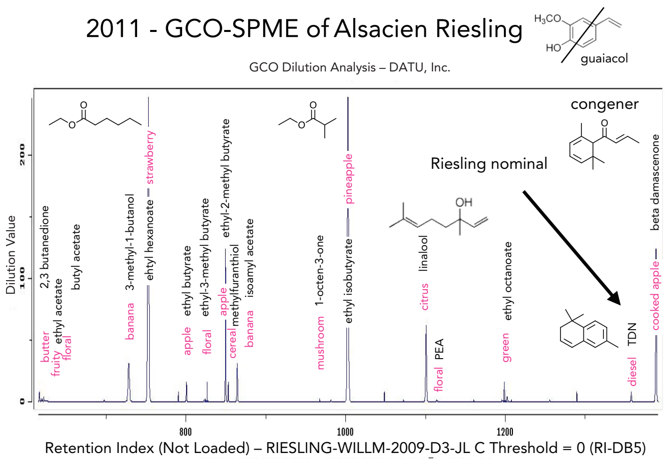

Figure 2. A GCO chromatogram of an Alsacien Riesling showing four of the five compounds in Figure 1. Missing is guaiacol not detected in the sample but showing damascenone that is recognisable at low levels in mixtures (Acree et al. 2013; Sacks et al. 2012)

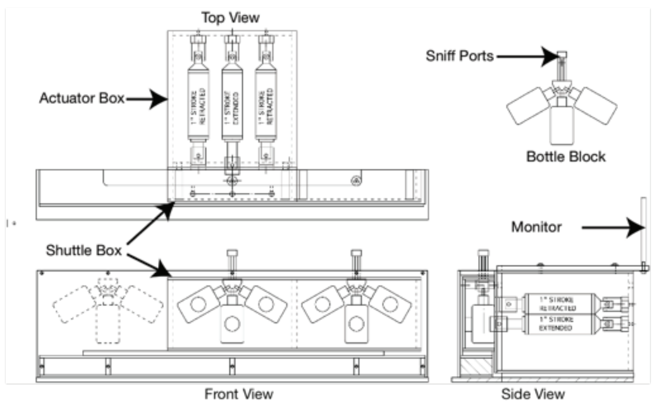

Figure 3. Sniff olfactometer designed to deliver 15 mL of headspace in 70 milliseconds during a two second inhale from above a 50 mL sample in a 250 mL PFA squeeze bottle. The subjects are visually and audibly cued to inhale by a program written in PsychoPy (Peirce 2007; Peirce 2008)

after being cued to inhale lasting 70 milliseconds. Instructions appear on a monitor to motivate a choice of labels or to scale intensity. The experiments are double blind with trials less than 10 seconds' duration (Acree et al. 2015b).

In a typical experiment with Sauvignon Blanc odorants, the subject was exposed to binary mixtures of odorants and queried to make a forced choice between two labels, here 'apple' or 'passionfruit' (Figure 4). It summarises a single trial of the protocol repeated 10 times on seven ratios to yield the probabilities plotted in Figure 5 for two compounds reported in Sauvignon Blanc.

Figure 5 shows the logit function that predicts the probability the subject detected 'passionfruit' odour as a function of the log of the ratio of 3-mercaptohexyl acetate to ethylhexanoate. Also shown is the inverse probability that 'apple' was selected. The dotted line indicates the LOG of the ratio at which there are equal odds (EO) of choosing 'apple' or 'passionfruit' and that point on the x-axis, the log of the ratio at the EO, is the equal odds ratio (EOR). The triangles indicate the range of binary ratios in which both odorants are recognisable. This plot was normalized to set the EOR = 1. Although outside this range it is unlikely a subject will recognise one of the odorants. This does not mean that the weaker odorant does not have an effect on other odorants even though they are not recognisable – this remains

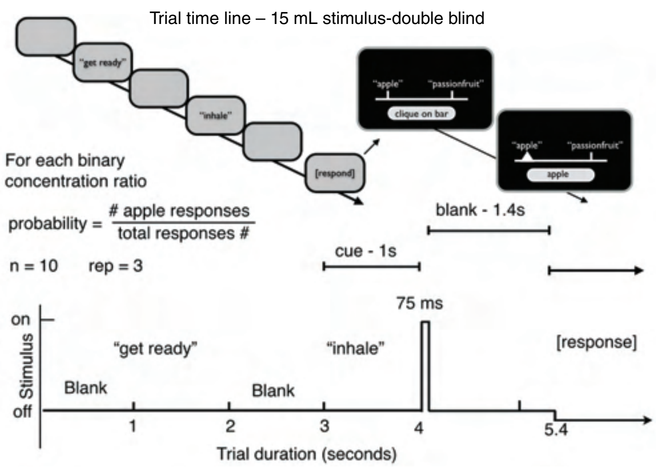

Figure 4. A typical time line for a single trial designed to determine a 'forced choice' response to a brief encounter with a mixture of two stimulants ethyl butyrate and 3-mercaptohexyl acetate. The two choices were 'apple' and 'passionfruit' (Peirce 2007; Acree et al. 2015a)

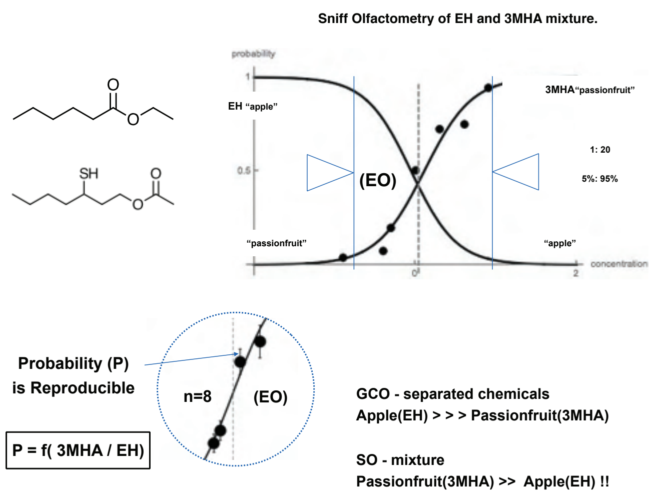

Figure 5. The logit model plot of the probability of choosing the label 'apple' or 'passionfruit' when smelling mixtures of ethyl hexanoate and 3-mercaptohexyl acetate as a function of their concentration ratio. In the circle are shown the error bars (standard error for eight replications). A single plot produces a sufficiently precise estimate of the equal odds ratio (EOR) (Acree et al. 2015a).

to be determined. The dotted circle shows a blow-up of the data near the EOR including the mean scores and standard errors for eight replications. The high degree of reproducibility of the responses is remarkable.

Unlike Sauvignon Blanc aroma, 'potato chip' aroma has only three odorants at high potency in the headspace. We studied this simpler model using binary and tertiary mixtures of the 3 key odorants in potato chips (crisps): methanethiol, methional, and 2-ethyl-3,5-dimethylpyrazine (Rochelle et al. 2017). Using a protocol similar to that shown in Figure 4, we determined the probability a subject would choose a label e.g. 'potato' or 'cabbage' when they encountered a very brief puff (70 milliseconds) of the binary mixture of methional and methanethiol as a function of the concentration ratios. When these ratios were greater than 20, or less than .05, the subjects reported smelling only one compound but when they were in the range 20 to 0.05-fold they could clearly detect both compounds simultaneously when the bottles were squeezed by hand slowly. A logit model fit of the data yielded a precise estimate of the EORs for each of three pairs.

When the three key odorants found in potato chips were combined at the ratios predicted by their binary behavior, the probability of the subject to choose the label 'toast', 'potato', or 'cabbage' is shown by the bar labelled 1 in Figure 6. An iterative change in these concentrations, 2 through 6, shows the changes in the three response probabilities. Only solution 6 showed equal response to all three labels (tertiary EOR). This indicates the difficulty of predicting the response of more complicated mixtures of odorant from their individual or binary properties. This mixture does not smell like potato chips to the subject. In antidotal odour comparisons of ratios used to determine the EOR, the bars labelled 5 in Figure 6 produced mixtures that smelled closer to potato chips. Sensory tests are ongoing to determine the ratios that produce potato odour images.

In addition, Figure 6 lists the composition of the EOR formulation for two subjects, indicating a striking difference between their EORs. For these two subjects it is remarkable how different their sensation of potato chips is, even though they both have no problem visually recognising potato chips. It is likely that we form our images not in an attempt to recognise chemicals but to recognise objects labelled by other humans. The chemistry is just an accession number used to find the label.

Sauvignon Blanc (SB) aroma is more complicated than potato chips and requires the analysis of more binary mixtures. Recent work comparing SB wine from two sources shows the complexity of the problem was evidenced by the difficulty of analysing more than three odorants simultaneously. Here four of the known odorants reported in SB wine were tested in binary, tertiary and quaternary mixtures

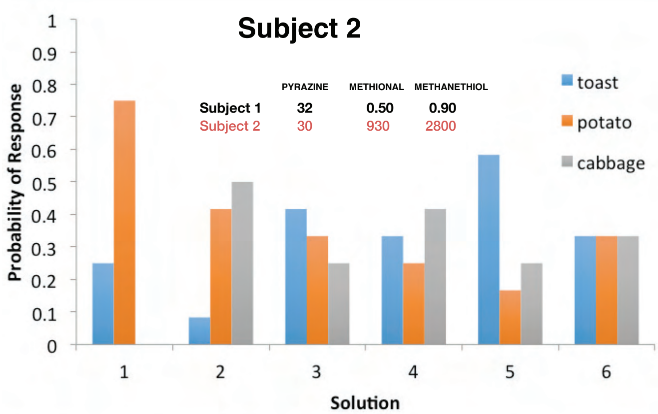

Figure 6. The response probabilities of the three labels 'toast', 'potato' and 'cabbage' to different concentrations of 2-ethyl-3,5-dimethyl pyrazine and methional. Concentration 1 was predicted to produce equal odds in a tertiary mixture from its behaviour in binary mixtures. Solution 6 had the concentrations that produced equal odds responses to all three compounds for subject 1. Note the difference from the concentrations found for subject 2.

of ethyl butyrate (EB): 'apple', iso-butyl-2-methoxypyrazine (IBMP): 'green pepper', 3-mercapto-hexanol (3MH): 'sulfur' and iso-amyl alcohol (3M1B): 'whisky'. Figure 7 compares response probabilities for each of the odorants in mixtures of different composition.

The black bar on the left is the composition predicted from the six binary combinations. The 5th bar to the right is the composition that yielded the odds closest to 0.25. We do not expect the EOR composition to smell like SB wine but the odour image inducing composition should have binary EORs between 20 and .05 of the normalised EOR to be recognisable. Although none of these formulations produced convincing SB simulants, some showed SB odour elements. Either more components are required or a different four would perform better.

There are several other odorants that could play a role in creation of SB odour image as revealed by Gas Chromatography–Olfactometry. It isn't always possible to predict the behaviour of odorants in mixtures from their behaviour in other mixtures or in isolation. Still there is evidence that one subject could detect all four compounds in a mixture and if the limit of four predicted by Laing (Laing and Francis 1989; Rochelle et al. 2017) is correct then we may need to find another four. The next challenge is to determine what components are required for SB odour image using binary tests of the most potent odorants detected analytically and demonstrate their importance in sensory tests.

We are just beginning our study of Sauvignon Blanc, which has more than four odorants – but which ones actively contribute remains to be determined. It is, however, likely that the perception of complex sensory mixtures of stimulants must involve both analytical (components) and configural (odour image) processes. Average wine drinkers are not sommeliers but their experiences modulate their perceptions in the same way that training enhances what sommeliers can do. Although individual differences play a confounding role in consumer behavior (Thomas-Danguin et al. 2014) it is tractable because there are many common experiences that form our perceptions. For the wine scientist it is clear that a small number of compounds drive perceptions and it is the ratio of these compounds that determines their individual importance.

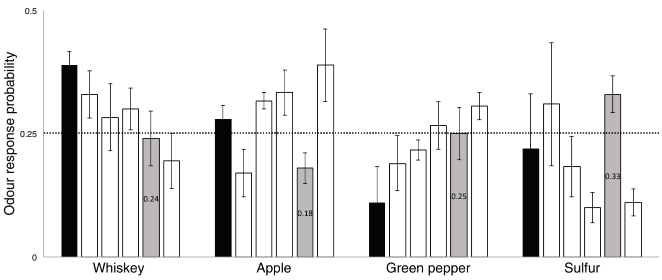

Figure 7. The response probabilities for each odour in the six different mixtures of the four Sauvignon Blanc components 3M1B-EB-IBMP-3MH. The mixture on the left was the predicted EOR for the four components from binary tests.

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Biologically driven differences in sensation: implications for the wine industry

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Abstract

Quantitative sensory data is typically collected with descriptive panels using participants who have undergone tens or hundreds of hours of training. Likewise, expert wine tasters spend years learning and perfecting their craft. However, it has also been known for over 80 years that individuals differ in their ability to taste certain chemicals, a phenomenon classically illustrated with the phenylthiocarbamide (PTC) taster/non-taster phenotype. In 2003, the biology behind this difference was revealed, and subsequent research has shown that this is only one of many examples. Innate differences in sensation driven by biology have the potential to influence how wine is perceived. This paper provides a brief primer on a few of the mechanisms behind these differences, along with an overview of recent research directly relevant to wine. It will conclude with a brief discussion of implications for wine research and marketing, including biologically driven market segmentation.

Webcast of this presentation available at http://bit.ly/16thHayes.

Defining flavour

Even among scientists, flavour is a deceptively simple term that can mean starkly different things to different speakers (Delwiche 2003). For many decades, flavour chemistry focused almost exclusively on the detection and identification of the volatile compounds found in foods or beverages, with or without a human assessor. Likewise, the flavour industry sells billions of dollars worth of compounded flavours that consist (almost exclusively) of volatile compounds that are included in finished products for their odour activity. Conversely, among, psychologists, neuroscientists, behavioural scientists, and sensory scientists, flavour is commonly defined as the collective, integrated perceptual experience that arises from stimulation of multiple, anatomically distinct sensory systems including taste, smell and oral touch (Lawless 1996; Small and Prescott 2005). For the remainder of this manuscript, we use the later definition throughout – that is, we consider flavour to be a singular percept that results from simultaneous activation of gustatory nerves, olfactory nerves, and somatosensory nerves located in the oral cavity and nose. Perhaps less obviously, if we accept this definition, there is an implicit corollary: if flavour is a perceptual phenomenon, then a human assessor is required for its measurement, as perceptions cannot be quantified solely with an instrument. The next section discusses how flavour can be measured objectively and reproducibly with humans.

Measuring flavour: descriptive analysis and its use in wine evaluation

Descriptive analysis was originally developed to provide quantitative data on the sensory profiles of packaged foods as part of the product development process at large food companies (e.g. Stone et al. 1974; Szczesniak et al. 1975). However, the utility of these sensory fingerprints was quickly recognised by the wine community, at least for research purposes (e.g., Schmidt and Noble 1983; Aiken and Noble 1984). Although it is rarely stated explicitly, descriptive analysis fundamentally rejects the idea of the expert taster, as it uses mean ratings from a *group* of participants (rather than one expert), and the data generated are subject to statistical analysis to characterise the product. Additionally, descriptive analysis methods focus exclusively on the sensory attributes of the product, and do not attempt to quantify affective or hedonic responses, or to provide any measure of quality, in contrast to various other scoring systems.

While many different variations have been developed (see Murray et al. 2001), almost all descriptive analysis approaches share the same core steps: panellist screening and selection, term generation, concept formation and training, and blind product evaluation. Panellists are first screened for their perceptual ability to sense certain compounds found in the product, their verbal ability to describe the percepts these compounds evoke, and their ability to interact with other panellists. Next, relevant descriptors for the products are identified by the panel through a consensus building process (or the panel leader), and the panellists are trained to identify these sensations, and to use intensity scales consistently. The training process typically includes spiked samples and/or exemplars, which aid with concept alignment around the chosen descriptors, and allows the panel leader to confirm panel performance. Finally, the panellists evaluate the products blind, and the data are collected and analysed statistically to generate a profile of the product (see OMahony 1991; Lawless and Heymann 2010).

Individuals who do not perform on par with the other panellists are retrained, or may eventually be removed from the panel. The assumption here is that poor performance is a result of inadequate training or perhaps effort. However, this assumption ignores a key point – namely, that not everyone perceives taste and smell stimuli in the same way, and these differences have a biological basis (i.e. Blakeslee 1932; Wooding 2006). Across individuals, there are substantial differences in sensitivity to various chemical stimuli, including many found in wine. Historically, existence of these biological differences has typically been ignored in descriptive analysis, although this is largely understandable, as the wide extent of these differences has only become apparent over the last decade (see Hayes et al. 2013 for a review). More broadly, and of special relevance for the winemaker, the existence of biological differences in sensation also implies that the same product can taste very different for different consumers, and that such differences are not merely the result of training and experience, or the lack thereof. Below, we will discuss wine relevant examples of individual differences in chemosensation that occur across taste, smell and touch, and how these might influence purchasing behaviour.

Mechanisms behind the sensations evoked by wine and wine constituents

Wine contains numerous taste active compounds, including sugars, tannins, and ethanol. Although the sweetness from sugars, and in the case of red wines, the bitterness from tannins are certainly critical components of the overall sensory experience from wine, with regard to biologically driven differences in perception, the single most relevant stimulus may be ethanol itself.

Taste sensations, including sweetness and bitterness, occur when taste receptors located in the oral cavity (i.e. the tongue, palate, epiglottis, and oropharynx) are activated by specific ligands. These receptors – hT1Rs and hT2Rs – are encoded by several families of genes (*TAS1Rs* and *TAS2Rs*, respectively). For sweetness, two different taste receptor proteins, T1R2 and T1R3, come together to form a single receptor complex with multiple binding sites. This explains how a single receptor can respond to a varied range of structurally diverse compounds that are all perceived as being sweet by humans (Hayes 2008; DuBois 2016). Meanwhile, the system for sensing bitterness in humans is much more complex, as the *TAS2R* family contains 25 functional genes and 11 (non-functional) pseudogenes (Meyerhof et al. 2010; Risso et al. 2014). This large family of receptors allows us to sense an extremely wide range of compounds as being bitter. Many but not all of the compounds humans describe as being bitter are secondary plant metabolites: for example, catechin and epicatechin (e.g. Thorngate and Noble 1995; Kielhorn and Thorngate 1999). The biological complexity of the bitter taste system also explains why many of the earlier attempts to systematically describe a chemical structure for bitterness were unsuccessful: with 25 different receptors, there is no *one* bitter structure. Within the context of this broad receptor repertoire, substantial effort has been undertaken to deorphanise the bitter taste receptors – that is, to identify the specific compounds that activate them (Meyerhof et al. 2010; Thalmann et al. 2013; Lossow et al. 2016). Somewhat ironically then, we still do not have direct evidence of which bitter receptor(s) are activated by the most abundant sensory active compound in wine, namely ethanol.

Even by itself, ethanol is a perceptually complex stimulus – it elicits bitterness and sweetness, as well as chemesthetic sensations like warmth, burning, or irritation (Berg et al. 1955; Green 1988; Scinska et al. 2000), sensations which are frequently referred to as heat in the wine literature (e.g. Pickering and Gordon 2006). The burn from ethanol appears to be mediated by the TRPV1 receptor (Trevisani et al. 2002; Blednov and Harris 2009), which is better known for its role in the response to capsaicin from chilli peppers (Hayes 2016). Notably, the perceptual quality of ethanol changes as a function of concentration. Near threshold, it is bitter (Mattes and DiMeglio 2001), and bitterness tends to be the predominant sensation at lower concentrations (~4 to 16% v/v), followed by burning, drying and sweetness, with burn outpacing bitterness and sweetness at higher concentrations (~16 to 48% v/v) (Nolden and Hayes 2015). These patterns are shown in Figure 1.

Drying sensations caused by ethanol presumably result from delubrication caused by precipitation of salivary proteins, although within wine, tannins are presumably much more important for this sensation. In 1991, the American Society for Testing and Materials

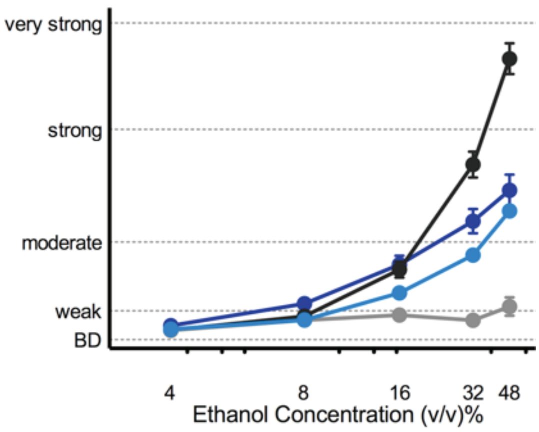

Figure 1. Quality specific functions for ethanol in water. Ratings from 100 participants were obtained using a general labelled magnitude scale (Hayes et al. 2013). Black lines represent burning, dark blue is bitterness, light blue is drying, and gray is sweetness. Data are replotted from (Nolden and Hayes 2015).

(now ASTM International) defined astringency as the complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins; however, other evidence suggests drying, roughing, and puckering are distinct sub-qualities which are not interchangeable (Lee and Lawless 1991; Bajec and Pickering 2008; Fleming et al. 2016a). Although Bate-Smith is widely credited with observing that astringency was a touch sensation and not a taste in 1954, the idea that astringency is really a touch sensation is actually much older. In 1909, Charles Samuel Myers noted weak acids cause both astringency and sourness, and the difference between the two sensations could be clearly observed by first painting the tongue with a solution of 5–10% cocaine! Because the taste nerves and touch nerves were each numbed by the cocaine but recovered at different rates, the observer could use this approach to differentiate between the true taste character of sour and astringency (Myers 1909).

Today, it is widely accepted that polyphenols cause astringent sensations via a three-step process (Jobstl et al. 2004; Bajec and Pickering 2008). First, randomly coiled proline rich proteins found in saliva bind to polyphenols and become more compact. Next, these compacted complexes cross-link, forming larger dimers. Finally, aggregation of these dimers causes the proteins to precipitate. This precipitation then causes a loss of lubricity in the mouth which is perceived as drying and roughing. Similarly, the interactions of protein and tannins is the basis of the widely known Harbertson-Adams assay (Harbertson et al. 2003), and *in vitro* methods appear to have some utility in predicting perceived astringency, at least at the group level (e.g. Mercurio and Smith 2008; Rinaldi et al. 2012).

However, recent work suggests there may be other mechanisms besides simple delubrication that also play a role in astringency. For example, epigallocatechin-gallate (EGCG) and epicatechin (EC) are both astringent, but EGCG reduces salivary lubricity, while EC does not (Rossetti et al. 2009). Other data suggest tannic acid/malic acid and tannic acid/alum mixtures show superadditive responses in regard to drying and roughing sensations, which is consistent with multiple mechanisms for astringency (Fleming et al. 2016a). Finally, there is evidence that compounds with a galloyl ring may directly act on a still unidentified receptor on trigeminal neurons (Schobel et al. 2014). Even if we accept that delubrication is the primary mechanism by which polyphenols, organic acids and ethanol cause astringency, salivary flow and protein content are highly variable across people; the potential influence of these individual differences on wine perception will be discussed in a later section.

Without its unique and subtle aromas, wine is merely an alcoholic fruit juice. Present understanding of the human olfactory system is based largely on the seminal work of Linda Buck and Richard Axel which earned them the Nobel Prize for Physiology or Medicine in 2004. When a volatile chemical reaches the olfactory epithelium at the top of the nasal cavity, it may interact with narrowly tuned olfactory receptors (ORs) expressed on olfactory neurons. Each olfactory neuron only expresses a single type of OR, and each OR is narrowly tuned for a specific chemical structure. These ORs are G-protein coupled receptors (GPCRs) encoded by a very large gene family; in humans, we have about 350–400 different OR genes that are functional (Shepherd 2004; Mainland et al. 2014). Collections of neurons that express a specific OR project to small distinct regions in the olfactory bulb which are known as glomeruli. Critically, the pattern of activation across glomeruli is what gives rise to a specific sensation. That is, we are able to perceive thousands of different odours with only ~400 receptors because they emerge from the unique pattern of activation across glomeruli. This combinatorial code is what allows us to rapidly distinguish between a Coonawarra Cabernet Sauvignon and a Fingerlakes Seyval Blanc with just a quick sniff.

Having covered these mechanisms, we can now discuss how individual differences in perception for taste, smell and astringency can be explained by biological variation across people, and how these differences relate to wine.

Differences in bitterness perception across people can be explained by genetic variation

Individual differences in bitterness perception have been studied for over 80 years (Blakeslee 1932). These differences were discovered accidentally in the early 1930s when Fox, a chemist at DuPont, noticed he could not taste the bitterness of phenyl-thio-carbamide (PTC) while his coworker Noller could (Wooding 2006; Herreid et al. 2014). This trait is genetically linked (Blakeslee and Fox 1932), and the molecular basis of this trait was discovered in 2003 (Kim et al. 2003). Much of the variability in response to PTC, and a structurally similar compound propylthiouracil (PROP), is due to three single nucleotide polymorphisms (SNPs) in *TAS2R38*, a bitter taste receptor gene (Kim et al. 2003; Duffy et al. 2004a; Bufe et al. 2005). This change in amino acid sequence alters the functionality of the receptor (Tan et al. 2012). These SNPs (A49P, V262A, and I296V) are in linkage disequilibrium (LD) and are often inherited together, resulting in two common (Garneau et al. 2014) and four rare (Boxer and Garneau 2015) haplotypes. At concentrations above threshold, the lower functioning haplotype is the AVI variant, while the PAV variant is more functional (Hayes et al. 2008). Since this discovery, the gene sequences of other bitter taste receptors have been determined, leading the identification of other functional SNPs within bitter taste receptors (e.g. Soranzo et al. 2005; Hayes et al. 2011; Roudnitzky et al. 2011; Allen et al. 2013; Hayes et al. 2015). Several of these genetic variants are associated with individual differences relevant for the perception of wine, and will be discussed below.

Genetic variation in taste receptor genes associated with differential sensations, liking and intake of alcohol and alcoholic beverages

Numerous reports suggest overall intake of alcohol beverages associates with variants located within *TAS2R* bitter receptor genes (Duffy et al. 2004a; Wang et al. 2007; Hayes et al. 2011; Dotson et al. 2012). Such associations implicitly or explicitly assume a multistep causal chain from sensation to liking which subsequently leads to differences in intake (see Hayes 2015). Recent data helps fill in this putative causal chain, showing that the perceived bitterness of ethanol systematically differs across people as a function of variation in *TAS2Rs*. Consistent with the intake data, individuals who are homozygous for the PAV allele for *TAS2R38* report more bitterness from sampled ethanol than AVI/PAV heterozygotes, or AVI homozygotes, at least on the posterior tongue (Nolden et al. 2016).

Pilot data from our laboratory suggests reported liking of alcoholic beverages varies as a function of TAS2R genotype, at least for unsweetened beverages. In a pilot study, we asked 146 adults of mixed ancestry to rate liking for 20 different alcohol beverages, 27 foods, and 16 non-food items on a questionnaire (Byrnes and Hayes 2013; Hayes et al. 2015) using a generalised hedonic scale; they were also genotyped for two SNPs in the *TAS2R38* gene (A49P, and V262A). After excluding those with rare haplotypes, data from 133 adults (43 men; mean age of 26.6±7.6 SD) were analysed. Beverages were grouped into sweet (n=9) and not-sweet (n=7) groups after excluding four beverages due to low response rates (missing values >30%). For the sweet group (Figure 2, top), the main effect of beverage was significant [$F(8,835) = 20.15$; $p < 0.0001$], as mean liking differed across the individual beverages. However, the effect of genotype [$F(1,121) = 0.21$; $p = 0.65$] and the genotype by beverage interaction [$F(8,835) = 0.97$; $p = 0.46$] were not significant. Conversely, for the non-sweet beverage group, we observed main effects of genotype [$F(1,123) = 6.34$; $p = 0.013$], and beverage [$F(6,634) = 13.6$; $p < 0.0001$]. As shown in Figure 2 (bottom), the PA/* individuals (those 1 or 2 copies of the more functional allele) generally reported lower liking ratings to the individual beverages. When beverages are sweetened, this presumably causes mixture suppression which reduces bitterness and increases liking (Lawless 1977; 1979); thus, addition of a sweetener appear to blunt or attenuate any effect of genetics on liking.

The apparent congruence between the perceptual data, liking data, and intake data reported here and elsewhere is predicated on the idea that more bitterness leads to lower liking and thus lower intake. While logical and valid on its face, this interpretation is somewhat complicated by the observation that wine experts (relative to regular wine consumers) appear to be more responsive (not less responsive) to bitterness, at least for some stimuli (Hayes and Pickering 2012). This apparent discrepancy can be explained by distinguishing between bitterness as a quality that influences liking per se, and the widespread use of bitter taste compounds as a phenotypic markers of overall chemosensory function (see Hayes and Keast 2011).

Phenotypic markers of taste function and sensations from wine

The suprathreshold bitterness of PROP – a synthetic pharmaceutical not found wine – associates with multiple sensations from commercial red wines: those who report greater bitterness from PROP also report greater bitterness, astringency and acidity from sampled wine compared to individuals who report less bitterness from PROP (Pickering et al. 2004). Similar associations are observed with sampled ethanol and PROP (Prescott and Swain-Campbell 2000; Duffy et al. 2004b), and these effects persist even across repeated exposure (Prescott and Swain-Campbell 2000). Subsequently, Pickering and

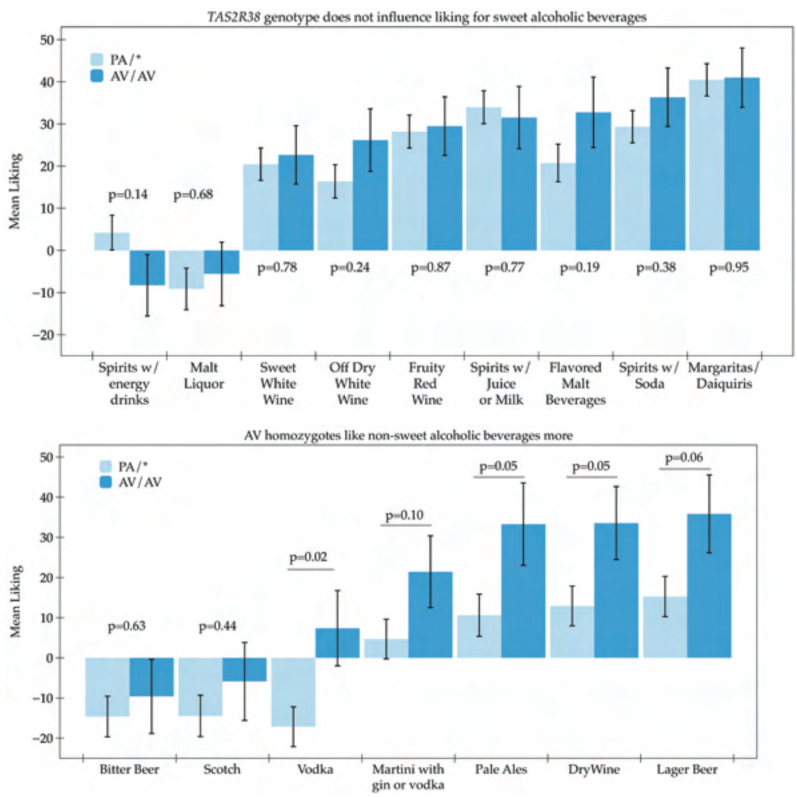

Figure 2. Data showing that the effects of *TAS2R38* genotype on liking for alcoholic beverages differs depending on whether or not the beverages are sweetened. See text for details.

colleagues (Pickering and Gordon 2006) observed associations between PROP bitterness and multiple characteristics of 16 commercial wines. Participants underwent training to understand various wine attributes and were familiarised using reference compounds. During testing, all participants wore nose clips to prevent retronasal and orthonasal olfaction, and they rated 11 different qualities on line scales with appropriate anchors on each. Of 11 qualities, 9 (acidity, saltiness, heat, tingle, particulate, smoothness, grippy, mouth-coat, and overall astringency) were significantly different across phenotypic groups: those who reported more bitterness from PROP rated 8 of the 9 qualities significantly higher than those who report less bitterness from PROP. Unexpectedly, effects for overall astringency went in the opposite direction, in direct contradiction with prior work (Pickering et al. 2004). This discrepancy may merely be an artifact of panel training: in the 2004 study, astringency was a holistic descriptor whereas the 2006 study trained participants extensively on various astringent subqualities (grippy, mouth-coat, etc.). That is, the training process may have altered participants conceptualisation of overall astringency relative to those in the 2004 study. Other data suggest retronasal aromas may also be greater in those who experience more bitterness from PROP (Pickering et al. 2006). Collectively, these data suggest that those who experience PROP as being more intense at suprathreshold concentrations may also experience increased taste, mouth-feel and aroma sensations from wine. Similar to the observation that the distribution of PROP responses is elevated among chefs (Bartoshuk et al. 2004), it seems possible that increased taste acuity and sensory response may provide some competitive advantage (Hayes and Pickering 2012; Pickering et al. 2013), although this is *not* a replacement for skill, expertise or training. Indeed, these data primarily reinforce the main thesis of this manuscript: that individuals differ widely in how they perceive wine.

Individual differences in perceived astringency

Individual differences in the perception of astringency have been repeatedly associated with multiple characteristics of saliva, including flow rate and protein content, both in model systems and in wine (Fischer et al. 1994; Horne et al. 2002; Monteleone et al. 2004). Consistent with the delubrication mechanism described above, when tannic acid is reacted with human saliva *in vitro*, the tannin-protein interaction causes the solution to become cloudy (similar to the chill haze found in beer), and this haziness can be quantified instrumentally (Horne et al. 2002). The original hypothesis was that more haze would track greater astringency across concentration. However, observed data indicated the opposite: high haze individuals perceive less astringency from tannic acid, which suggests increased protein content better protects some individuals from astringent agents (Horne et al. 2002). Subsequent work refined this idea by showing it isnt the total amount of salivary protein that matters per se, but rather the ability to replenish salivary protein after initial exposure (Dinnella et al. 2009). Separate studies in North America and Europe each suggest around 1 in 4 individuals are especially susceptible to oral astringency due to a reduced ability to replenish protein in their saliva, and these differences influence liking (Monteleone et al. 2011; Fleming et al. 2016b). Whether this susceptibility might have a genetic basis is presently unknown, but data from a recent Finnish twin study (Tornwall et al. 2011) shows salivary protein measures – specifically total protein, mucins, and proline rich protein levels – are more similar in monozygotic twins than dizygotic twins, suggesting these may be heritable, and this might differ between individuals due to genetic differences. More work is needed to confirm these findings and to explore them further, especially in relation to different wine styles, and the corresponding different concentrations and composition of astringent stimuli they contain.

Differences in odour perception can be explained by genetic variation across people

Human odour receptor (OR) genes show a high degree of genetic variability, so it seems likely that this variability may systematically influence the perception of a wide range of odorants. However, research in this area has been slow, as specific stimuli (ligands) have only been identified for 10 to 12% of ~400 intact human ORs (Mainland et al. 2014; Noe et al. 2017). Nonetheless, perceptual differences have been successfully identified for a handful of stimuli and OR gene polymorphisms, including isovaleric acid (cheesy, sweaty), androstenone (sweaty, urinous), beta-ionone (floral), cis-3-hexen-1-ol (green, grassy), and guaiacol (smoky) (Keller et al. 2007; Menashe et al. 2007; Jaeger et al. 2012; Lunde et al. 2012; Jaeger et al. 2013; Mainland et al. 2014). Functional assays *in vitro* have confirmed some of these (e.g. Jaeger et al. 2013; Menashe et al. 2007; Mainland et al. 2014), while others show discordant results between human phenotypes and *in vitro* receptor activation data (e.g. Jaeger et al. 2012). This is a highly active area of research and it seems very likely our understanding will be much more comprehensive within the next five to ten years.

In regard to wine, the two most interesting candidates for genetic variation in odour perception are probably the *OR2J3* gene and cis-3-hexen-1-ol (C3HEX), and the *OR10G4* gene and guaiacol. C3HEX is widely known for the characteristic grassy green character it imparts to a wide range of foods (including white wine), and variability in sensitivity to C3HEX can influence liking for various foods (Jaeger et al. 2012), although wine was not tested. Subsequent work identified three SNPs near *OR2J3* that associated with differences in detection thresholds for C3HEX, but only one (a nonsynonymous T113A substitution) fell within the functional gene (McRae et al. 2012). Whether this gene variant is sufficient to influence the sensations from or liking for wine is currently unknown. Turning to guaiacol, there are four common variants of the *OR10G4* gene, and these variants explain differential activation by guaiacol *in vitro*, and associate with differences in both intensity and liking in human volunteers (Mainland et al. 2014). Notably, these differences appear to be highly specific, as they did not associate with intensity or liking for 67 other odorants, including structurally similar vanillin and ethyl vanillin. In red wines, the best estimated thresholds (BETs) for guaiacol are approximately 23–37 ug/L; critically, wines made from grapes exposed to smoke may contain concentrations above this threshold (Parker et al. 2013). Whether genetic differences in the *OR10G4* are sufficient to influence the detection and acceptability of smoke tainted wines is unknown, although it seems possible. Also, it is currently unknown whether 4-ethylguaiacol or 4-ethylphenol might activate *OR10G4.* If they do, this raises the question of whether differential responses (perceptual or affective) to *Brettanomyces*-associated odours may differ with genetics.

Finally, no discussion of individual differences in wine aromas is complete without mentioning rotundone. This sesquiterpene is found in peppercorns (*Piper nigrum*) and some wine varieties like Shiraz and Noiret, and is responsible for the characteristic peppery aroma they share (Wood et al. 2008). While most individuals are able to detect rotundone at very low concentrations, approximately 1 in 5 individuals are unable to smell it, even at very high concentrations. Presumably, these individual differences may have a genetic basis, but this remains to be tested.

Closing thoughts

At their 1932 exhibit on taste perception for the American Association for the Advancement of Science (AAAS) meeting, Blakeslee and Fox (1932) stated:

> Thomas Jefferson said all men are created equal, but he had not tried [PTC] crystals. Taste tests show people are different. Our world is what our senses tell us. Each [of us] lives in a different world.

By quoting them here, we do not mean to suggest that PTC or PROP phenotypes are uniquely predictive of wine sensations, liking, or preferences: they are not. Rather, this trait is merely one example of how individuals may differ in their perception. Indeed, a substantial body of evidence collected over the last eight decades supports the notion that everyone experiences their own unique flavor world (McRae et al. 2013). (For more detailed reviews, see Hayes et al. 2013; Running and Hayes 2016). Blakeslee and Fox (1932) claimed PTC tasting ability was the most effective method we know of for demonstrating innate but unsuspected differences between people in physiological response. In our own demonstrations of such individual differences, we have switched from using thiourea compounds like PTC or PROP to the sulfonyl amide sweeteners saccharin or AcesulfameK, both because they are more readily available, and because they are actually found in the food supply, unlike PTC or PROP. For odour, beta-ionone may be the best exemplar of differential response, given that rotundone is not widely available.

More broadly, we have long maintained that individual differences are not merely an academic curiosity or student demonstration, but rather may be the basis for systematic, biologically driven market segmentation. This idea is becoming more widespread, with multiple research groups focusing their efforts on biological differences in the perception and/or liking of real foods (e.g. Haryono et al. 2014; Pickering et al. 2016; Shen et al. 2016). As more evidence accumulates, it is not surprising that awareness of the need to engage other specialists (like geneticists and neuroscientists) to understand the mechanisms behind individual variation in perception is growing among sensory scientists, as was highlighted at the recent Pangborn Sensory Science Symposium in Sweden (Jaeger et al. 2017).

For all foods, but especially for wine, biologically driven differences in sensation interact with other determinants of consumer behaviour and use, including familiarity, expertise, expectations, culture, prior experience, gender, age, personality, price, and availability. Given the web of complex interactions among these factors, interdisciplinary research that attempts to integrate multiple aspects are required to avoid reductionism. It may be that sensory phenotypes are largely irrelevant in light of these other factors, at least as predictors of behaviour. Nonetheless, if Blakeslee and Fox are right that we each live in our own sensory worlds, my choice to drink an off-dry Riesling over a big Cabernet Sauvignon may not be a sign that I am an uneducated philistine, but rather could be a function of my inability to replenish salivary proteins in the presence of tannins. Likewise, if I am unable to smell rotundone due to my biology, I am unlikely to pay $100+ for a premium Shiraz.

Such innate differences also call into question the role of experts and writers as potential arbiters of quality, if they do not experience wine in the same way as a specific consumer due to biological differences that cannot be overcome with experience or training. This is not to imply that experts do not have a critical role within the wine industry, but rather that winemakers and marketers need to be very clear about who their target consumer is. The sales volume of what another 16th AWITC speaker called good, gluggable, easy drinking wines suggests there is a strong consumer demand for these products. This may also be due to biology, rather than a lack of sophistication, a fact that should be kept in mind when making products for different segments.

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Minerality in wine: the case of Chasselas wines in Switzerland

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Abstract

The terms 'minerality' and 'mineral' are increasingly used by both wine professionals and consumers. In the last two decades, they have been omnipresent in wine tasting notes and often used as a positive selling point. Yet, those terms are absent from general dictionaries, and lack formal definition. The aim of this paper is to explore step by step several aspects of the concept of minerality.

This work first showed that professionals and consumers do not have the same understanding of the term 'minerality' and that it is often used as a substitute for the term 'terroir'. Secondly, it was not possible to find a perfect consensus among all tasters to identify a good example of a mineral wine. However, by combining all tasters' judgements across the 80 tested wines, a mineral and a non-mineral category could be identified. Thirdly, it was possible to highlight that mineral wines are characterised by a higher aromatic freshness and acidity with 'gunflint' odours, and by a higher concentration of malic acid and free sulfur dioxide which certainly protect some volatile molecules. Finally, it was found that mineral wines were more appreciated by consumers who described themselves as 'wine lovers'.

Webcast of this presentation available at http://bit.ly/16thDeneulin.

Introduction

The concept of 'minerality' has been increasingly used in tasting notes and other media to describe wines. Although it seems to be used in a positive way, no clear definition has ever been provided. The beginning of its popularity appeared around 1988 in French with Renouil (1988). It is difficult to know precisely the starting point of its use in English, but it seems to have been a little later, starting around 2000. In 2012, 'minerality' appeared twice as often as the term 'fruity' in 258,000 tasting notes from the Wine Spectator website. Most often, minerality seems to be used to point out the quality of wine or the character of wine aromatics in varieties such as Riesling (Schüttler et al. 2015)the descriptors involved, as well as the chemical composition which leads to typicality perception. In total, 30 wines were tasted by wine experts and rated for Riesling wine aroma typicality. Then, descriptive analysis was undertaken by a Frequented Free Comment Profiling (FFCP). Minerality has been associated with a range of different sensory characteristics. It can refer to a family of odours such as 'flint', 'chalk', 'wet stones', 'graphite' and 'oyster shell' or 'smoke' (Casamayor 1998; Green et al. 2011; Ross 2012). It can also refer to a lack of flavours (Parr et al. 2015) or to a palate perception. For some authors, mineral elements were claimed to confer a kind of tastiness to the wine (Silvestre 2010) and could modify mouth-feel and taste (Caillé et al. 2011; Vignon et al. 2012). But Maltman (2013) totally disproved this hypothesis, arguing that mineral concentrations are below their sensory threshold. Beyond a sensory perception, minerality also appears as a global term to describe all elements coming from the soil or the terroir (Dawson 2009; Molesworth 2009). Given that minerality is not currently well-defined and carries various meanings (Deneulin et al. 2014b; Deneulin and Bavaud 2016), it was decided to carry out a research project to better understand the meaning of the term 'minerality' in wine today. The study focused on the most typical variety of Switzerland, the white Chasselas variety.

This paper summaries the major results for the different aspects of minerality that have been studied in this project and it has been structured around the main questions that have been answered:

1. What comes to mind for (a) consumers and (b) professionals when they hear the term minerality associated with wine?
2. Is it possible for wine professionals to find a sensory consensus on what is a good example of a mineral wine?
3. What are the sensory and analytical characteristics discriminating a good and a poor example of mineral wines?
4. And finally, do consumers appreciate mineral wines?

What comes to mind for (a) consumers and (b) professionals when they hear the term minerality associated with wine?

Experimental protocol

A survey aimed at French-speaking wine professionals and consumers from Switzerland and France was distributed online between 2011 and 2013. It consisted of two parts: the first contained three open-ended questions and the second, several close-ended questions characterising the sociodemographic profile of each respondent. This paper only considers analysis of the answers to the open-ended question: 'If I speak to you about minerality in wine, what comes to your mind?' A total of 1,898 and 1,697 answers were collecting from wine professionals and consumers respectively. After several pre-processing steps consisting of correcting mistakes, grouping words into standardised forms and removing all words which did not carry any information, vocabulary was analysed and compared between the two groups. The number of citations and specific characteristics were highlighted to understand what came to mind for each group of respondents. In this first part, the participants were not asked to taste any wine, the goal was simply to discover their concept and understanding of minerality.

Results

Diversity of answers

Globally the number and the diversity of words available to wine professionals to describe their understanding of minerality were greater than those available to consumers. As expected, professionals also seemed more familiar with the concept. Table 1 presents a comparison of lexical diversity between consumers and wine professionals. 'Tokens' is the total number of words, 'types' is the number of different words and 'hapax' is words with just a single occurrence. Lexical diversity was greater for professionals than for consumers: in plain text, professionals used 28 different words to describe minerality on average against just 17 for consumers. This difference was the same after pre-processing, with 14 meaningful words for professionals versus 9 for consumers.

Common and specific vocabulary used by professionals and consumers

Among the ten most frequent terms, five were common to both consumers and professionals. The two terms 'wine' and 'minerality', with respectively 1,440 and 607 citations for professionals and 1,078 and 258 for consumers, were repeated from the question. The three other terms were 'terroir' (637 times for professionals and 394 for consumers), 'stone' (837 for professionals and 392 for consumers) and 'aroma' (371 times for professionals and 294 for consumers).

The specificity of vocabulary used was also an important feature distinguishing professionals and consumers. To analyse specific types used by each group, a chi-squared test was conducted on the 196 words used more than 30 times. The analysis revealed two significant semantic profiles, given in Table 2.

Professionals associated minerality with sensory perceptions, both aroma and palate perceptions. The most relevant type was 'fusil' for 'pierre à fusil' in French (meaning 'gunflint' in English). About 30% of professionals thought that minerality was a 'flint' odour, whereas it was mentioned spontaneously by only 8% of consumers. Professionals formed a clear response around an aromatic expression of 'flint' and 'smoke' associated with 'chalk', 'freshness', 'salinity', 'acidity' and 'tenseness'. Minerality also seems to be a qualitative perception of subtlety, balance and aromatic length (Deneulin and Bavaud 2016).

Terms used by consumers were completely different. Many of them did not know what minerality was and could not answer the question (13% of them signalled their uncertainty with marks such as ? or …). The sensory dimension appeared only with the word 'taste' but no particular taste (or odour) was mentioned. They associated minerality with the taste of wine given by the soil where the vine has grown but they did not know how it expressed itself in wine. They also associated it with the composition of wine and compared minerality with mineral water and the various minerals ions it contains (Deneulin et al. 2014a).

Table 1. Lexical diversity between consumers and wine professionals

	1,898 Professionals		1,697 Consumers	
	Number	Average per professional	Number	Average per consumer
		Raw data		
Tokens	52,316	27.6	29,371	17.3
Types	4,353	2.3	2,767	1.6
		After pre-processing		
Tokens	25,908	13.7	14,293	8.6
Types	2,634	1.4	1,667	1
Hapax	1,302	0.7	838	0.5

Partial conclusion

This first stage of the study showed the difficulty of providing a clear and shared definition of minerality in wine. The concept appears to have multiple meanings and be quite ambiguous. Indeed, it is not surprising that the available vocabulary appears too poor to describe the sensory perception. The second stage of the project aims to discover if, behind vocabulary, wine professionals share a common sensory perception of minerality in wine (sensory without verbal description).

Is it possible for wine professionals to find a sensory consensus on what is a good example of a mineral wine?

Experimental protocol

Eighty Chasselas wines (vintage 2012) were equally selected from the four French-speaking cantons of Switzerland (Vaud, Valais, Genève, Neuchâtel). Based on tasting notes, half of them were reputed to have mineral character and the second half were reputed to be non-mineral. Wines were coded by their canton (VD, VS, GE, NE) and a number from 01 to 80. All of the wines were evaluated by 62 Swiss wine professionals from the same four cantons, plus 19 wine professionals from Burgundy. Tasters were asked to evaluate the level of minerality by answering the following question, as mentioned in Loison et al. (2015):

> Imagine that you want to explain to someone what the minerality in wine is. For that, you can suggest they taste a wine. For each wine presented, you have to answer the following question: **Do you think that this wine is a good example or a poor example to illustrate to this person what minerality in wine is?**

The professionals were asked to answer on an unstructured scale anchored with 'very poor example' at the left end (corresponding to score 0) and 'very good example' at the right (corresponding to score 10). The 80 wines were divided into two blocks and the professionals evaluated 40 wines per session. Samples of 30 mL of wine were poured into INAO glasses labelled with three-digit codes. The glasses were presented in random order.

Results

This stage of the study had three main goals:

- to quantify the sensory consensus among Swiss professionals
- to identify two categories of wine (independent of the pre-selection which had been made): one recognised as having a high level of minerality and one recognised as having a poor level of minerality
- to compare the results based on where the tasters came from.

Table 2. Comparison of the most frequent words used by professionals (left) and consumers (right). Percentages represent the number of occurrences divided by the number of individual responses (1,898 for professionals and 1,697 for consumers).

Types		professionals %	consumers %	Types		professionals %	consumers %
fusil	gun (flint)	29.56	8.19	goût	taste	13.28	34.65
fraîcheur	freshness	17.97	3.65	indétermination	lack of knowledge	0.26	13.14
note	note	14.17	2.47	minéraux	mineral ions	3.64	14.14
silex	flint	19.60	5.01	terre	earthy	4.74	14.50
salinité	salinity	8.06	0.77	comme	like/as	0.00	3.59
aromatique	aromatic	7.90	1.18	penser	to think	10.70	16.91
expression	expression	8.01	1.24	vigne	vine	4.06	9.13
équilibre	balance	4.00	0.00	pousser	to grow	0.63	4.24
finesse	subtlety	5.85	0.59	eau	water	1.84	4.89
acidité	acidity	15.49	4.71	sol	soil	15.75	18.15
craie	chalk	8.17	1.71	terrain	ground	0.63	2.77
tension	tenseness	4.32	0.41	différent	different	1.26	3.54
longueur	length	4.06	0.35	composition	composition	0.58	2.47
Riesling	Riesling	5.64	0.94	rapport	link	0.90	2.36
fumé	smoky/toasted	2.69	0.00				
complexité	complexity	3.74	0.41				

Consensus among Swiss professionals

Each professional used the whole scale, classifying wine from 0 to 10. Thus, each of them found at least one poor example of mineral wine and one good example of mineral wine. No perfect consensus was found, so no wine was identified as the best example of mineral wine by all professionals. For the majority of individual wines, scores ranged from 0 to 10 as illustrated by box-plot in Figure 1. However, some wines received a majority of high scores (two examples shown in red) and some others received a majority of low scores (shown in blue).

Identification of two categories: poor and good examples of mineral wines

The second goal of this stage of the study consisted of identifying two categories of wine, one recognised with a high level of minerality and one recognised with a poor level of minerality. The wines were arranged based on the mean of their scores given by Swiss professionals only. In Figure 2, a continuum can be observed from the poorest example (mean score of 1.6 out of 10) to the best example of mineral wines (mean score of 6.6 out of 10). Limited agreement between professionals did not allow a mean score beyond 6.6. However, an ANOVA and a Least Significant Difference (LSD post-hoc) test permitted this continuum to be broken down and two significantly different categories of wines to be identified. Thus, seven wines (VD54, VS72, VS63, NE26, VD43, VS68 and GE05) can be considered as good examples of mineral wines (in red in Figure 2). Their mean scores ranged between 5.70 and 6.58. The seven poorest examples of mineral wines were also selected (in blue in Figure 2), excluding wines with off-flavours or specific characteristics (VS79, VD60, GE04, VD53, GE14, NE21 and VD52). These wines had mean scores between 3.14 and 4.

Minerality and origin of wine professionals

To fulfil the third objective of this stage of the study, the data were analysed to investigate if the concept of minerality was dependent on the origin of professionals. Professionals were divided into five categories based on their geographic origin (four Swiss cantons and Burgundy) and new means were calculated for each category.

For the ten professionals from the Canton of Geneva, the five most mineral wines came from the Valais area (including VS68 and VS63). A discussion with these professionals after the tasting sessions confirmed that they do not consider wines from Geneva to be mineral but they think that mineral wines come from the Valais area.

For the sixteen professionals from the Canton of Neuchâtel, the four best examples of mineral wines were in agreement with the general classification (NE26, VS72, VS63 and VD54), with the most mineral one coming from the Neuchâtel area.

For the fourteen professionals from the Canton of Vaud, the six best examples of mineral wines came from the Canton of Vaud (including VD43) and more specifically from an area east of Lausanne, which is recognised by many local wine professionals as making mineral wines.

For the twenty-two professionals from the Canton of Valais, good examples were selected from the four different cantons (including VD54, VS72 and VS68), thus they did not associate minerality with a specific area.

These results suggest that the concept of minerality can differ depending where professionals come from, and that their sensory perceptions match their conception of minerality. Indeed, under blind tasting conditions, they selected, as good examples of mineral the wines, those corresponding to what they declared or recognised as mineral.

Figure 3 illustrates the mean of the minerality scores given by nineteen professionals from Burgundy. Here again there is a continuum of scores, but this time it stretches from 2.4 to only 6.2. The professionals from Burgundy did not use the whole scale, probably

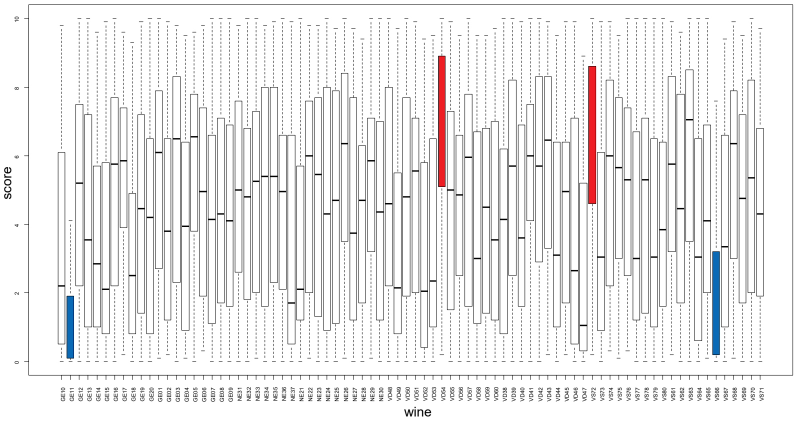

Figure 1. Diversity of scores for minerality provided by professionals and illustrated by box-plots (in red, two wines with a majority of high scores and in blue two wines with a majority of low scores).

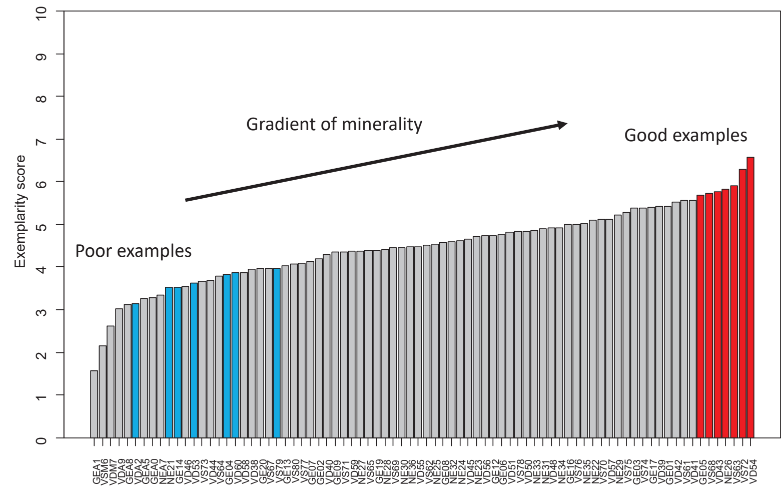

Figure 2. Gradient of minerality scores provided by Swiss professionals. In red, seven good examples of minerality and in blue, seven poor examples of minerality after excluding wines with off-flavours.

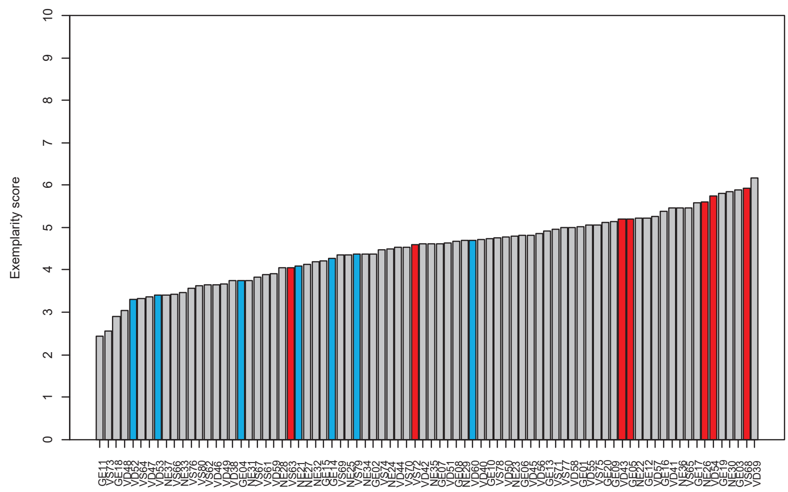

Figure 3. Mean minerality scores provided by professionals from Burgundy. In red, seven good examples of minerality and in blue, seven poor examples of minerality, as identified by the Swiss professionals.

due to their lack of knowledge of Chasselas wines. The few high scores did not exceed 6 out of 10. Amongst the seven wines identified as most 'mineral' by Swiss professionals, five were also considered as mineral (mean above 5) by Burgundy professionals, whereas two wines, VS72 and VS63, were classified with non-mineral wines, with mean scores of 4.59 and 4.04 respectively. The seven wines considered as 'non-mineral' were all scored below 5 out of 10.

Partial conclusion

In this study, all wine professionals found one good example of minerality amongst the 80 Chasselas tasted, but it was not the same for everyone. No single wine was consistently judged as a good example of a mineral wine. Even though the sensory concept of minerality was related to the geographic origin of the taster, it was possible to distinguish two categories of wines, 'mineral' and 'non-mineral', based on the mean of all scores. The objective of the third stage of this study was to evaluate how these two categories differ in sensory and analytical characteristics.

What are the sensory and analytical characteristics discriminating a good and a poor example of mineral wines?

Experimental protocol

Sensory description

Thirteen trained panellists from the expert panel of Changins participated in the sensory descriptive study. They are all well-trained, and have participated in tastings once a week for more than four years, describing wines and becoming familiar with sensory methodologies. They specifically worked once a week over seven months on the minerality project (including training sessions). Panellists used holistic methods such as projective mapping (or Napping®) and Free Sorting Task, as well as verbal-based methods such as Check-All-That-Apply and Quantitative Descriptive Analysis (QDA) (Valentin et al. 2012). Only the results from QDA are presented in this article. The 14 wines previously selected (7 mineral, 7 non-mineral) were evaluated based on 25 descriptors by 11 panellists. The letter M for mineral wines and the letter O for non-mineral wines (meaning 'other') were added to their original code (e.g. VDM54). Among the 25 descriptors, 12 were aromatic ('aroma intensity', 'vegetal', 'fruity', 'floral', 'lactic', 'dust', 'chalk', 'gunflint', 'matchstick', 'oxidised', 'aromatic freshness', 'aroma complexity') and 13 concerned taste and mouthfeel ('carbonic', 'acid', 'bitter', 'sugar', 'salt', 'volume', 'astringency', 'tight', 'structure', 'balance', 'freshness', 'aromatic length', 'mouth-watering'). For each descriptor, panellists had to evaluate the intensity they perceived on a 10-centimetre linear scale anchored by 'absent' on the left-hand side and 'strong' on the right-hand side.

Chemical analyses

Common wine analyses were conducted on the fourteen wines. These included alcohol, sugars, acids, sulfur dioxide (free and bound), metals and colour elements measured by different methods such as titration with bromothymol, enzymatic method, manual iodometry or visible spectophotometry. A subset of eight wines was then selected (four mineral and four non-mineral) based on their sensory characteristics and analysed by GC-Olfactometry (HS-ITEx: T= 50°C; $t_{extraction}$= 15 min (60 strokes), microtrap: Tenax TA 80/100 mesh). Eight trained panellists from Agroscope (two at a time on a 2W-GC-O-set-up) described the perceived odours and rated their intensity on a five-point-scale according to the VIDEO-Sniff-method (Fuchsmann et al. 2015). Data were processed taking into account detection frequency and odour intensity (mean aroma signal by classes ($OSC_{Int \times Det}$)) as well as the descriptive vocabulary used, and sorted into ten odour classes using the Acquisniff® software.

Results

Sensory description

ANOVA results showed significant differences among the fourteen wines tested for the following ten sensory descriptors out of twenty-five: 'aroma intensity', 'fruity', 'chalk', 'gunflint', 'matchstick', 'aromatic freshness', 'carbonic', 'acid', 'sugar' and 'volume', as shown in Figure 4. In general, the five mineral wines (in red) appeared to have a higher 'aroma intensity' with 'gunflint' and 'matchstick' odours.

Based on the scores for the ten descriptors, the fourteen wines were clustered into six groups of four, three and four wines, with three other groups of a single wine. The first group contained three mineral wines (NEM26, VSM72 and VDM54) and can be described as having higher aromatic freshness and acidity. In this group, minerality seemed mainly to be perceived on the palate. The second group contained three mineral wines (VSM63, VDM43 and VSM68) and one non-mineral wine (VSO79). These were described as having 'gunflint' and 'matchstick' odours and volume on the palate. In this second group, minerality seemed to be mainly related to an aroma dimension. The last group of four wines was composed of three non-mineral wines (GEO14, VDO52 and VDO53) and one mineral wine (GEM05). In this group minerality was associated with slightly oxidised odours. The three other wines differed greatly from each other. VDO60 appeared sweeter, NEO21 possessed lactic notes and GEO04 showed more 'vegetal' odours and astringency on the palate.

Chemical analyses

Only a few chemical characteristics were able to be used to discriminate mineral and non-mineral wines. Mineral wines had a higher level (200 mg/L more in average) of carbon dioxide. Moreover, free sulfur dioxide was higher by 10 mg/L in mineral wines than in non-mineral wines. However, the best chemical characteristic to distinguish mineral and non-mineral wines appeared to be malic acid which was higher in mineral wines. Indeed, five out of seven mineral wines had not completed malolactic fermentation (MLF), which explains the higher level of malic acid, but also of free sulfur dioxide (used to maintain microbiological stability) observed in these wines.

While more than 200 volatile compounds were detected in Chasselas white wine, only 60 odorants were perceived by the panellists during the GC-O. Each odorant was classified into one of ten pre-defined classes: buttery-cheesy, burnt, floral-fruity, green-fatty, malty-chemical, meaty, spicy, nutty, sulfur and earthy-undergrowth. They also associated with each odorant an intensity and a duration

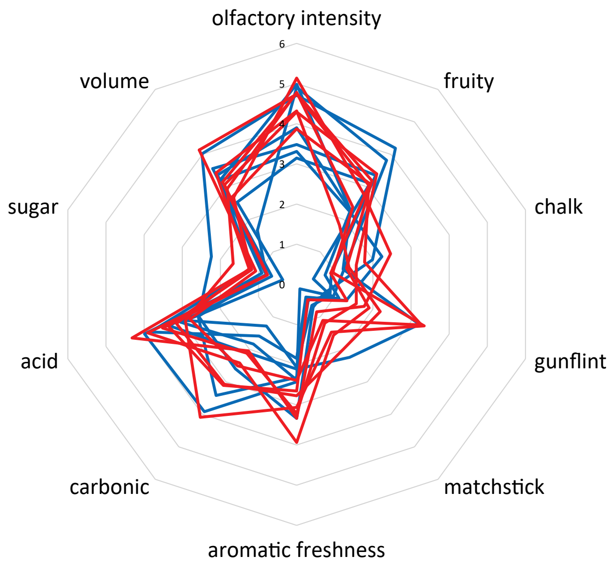

Figure 4. Spider plot of the 14 wines and 10 significant sensory descriptors (5%). Mean of the 11 evaluations for each wine. Mineral wines are shown in red and non-mineral wines are shown in blue.

of perception, according to the sniffing methodology (VIDEO-Sniff). The results indicate that the overall 'mineral' sensory perception might not be determined by a single molecule, but rather by the general aromatic balance of the wine. This is illustrated by the aromagrams of one mineral and one non-mineral wine (Figure 5) which show different patterns. The mineral wine is less influenced by the perception of carboxylic acids (described by the panelists as 'rancid' and 'fermented') and 3-methylbutan-1-ol ('pineapple', 'malty', 'cooked') than the wine classified as 'fruity' or non-mineral. Oct-1-en-3-one, on the other hand, a trace odour compound known for its 'earthy', 'metallic', and 'mushroom' aroma in combination with a low odour threshold, was perceived more intensely in the mineral wine VSM63. These differences could contribute to the often described 'freshness' of the mineral wines.

Partial conclusion

It is possible to distinguish mineral and non-mineral wines based on their sensory properties. Two kinds of minerality have been identified, one mostly characterised by in-mouth perceptions with high aromatic freshness and acidity, and a second one mostly characterised by odours of 'gunflint' and 'matchstick' with volume on the palate. Non-mineral wines were found to be sweeter, astringent, vegetal, with lactic odours or slightly oxidised. Malic acid appears to be an important driver for minerality in Chasselas wines, which is also often associated with a higher concentration of free sulfur dioxide. Finally, no single volatile compound was found to characterise minerality, which may rather result from a balance of different aromatic compounds.

The last stage of the study addressed a crucial question: do consumers appreciate mineral wines?

Do consumers appreciate mineral wines?

Experimental protocol

A panel of consumers tasted ten Chasselas wines (2012 and 2013 vintage), selected for their diversity of expression. These wines were not previously used in this project except the two mineral wines (NEM26 and VSM68). The diversity of the tasted wines was verified by Quantitative Descriptive Analyses (as summarised in Table 3). The two mineral wines were characterised by high intensity of 'gunflint' odours in comparison to the eight other wines.

Nearly 200 consumers were invited to give a liking score for eight (out of the ten) wines. Thus, each wine was evaluated by 155 to 166 consumers. Wines were allocated to consumers using an incomplete block design. The liking scores were ranked on a 9–point scale anchored with 'extremely unpleasant' and 'extremely pleasant'. Consumers were also asked to answer a few questions regarding their demographic profile.

Results

Means of liking scores ranged from 5.3 to 6.5 for the ten Chasselas wines. Means of the mineral wines were 6.3 and 6.1 for NEM26 and VSM68, respectively. The most appreciated wine possessed 'floral' odours and the second one was considered as 'fruity'. Mineral wines arrived in the third and fourth positions. They were generally appreciated but were not considered as the best wines by the consumers.

The consumers were then clustered into groups with similar likings. Six groups were identified, among which there were two groups who appreciated mineral wines:

- The first group was composed of 34 consumers who were mainly wine lovers coming from the Canton of Vaud who mainly drank Chasselas when drinking white wine.
- The second group was composed of 21 consumers who were wine lovers.

Two groups did not appreciate mineral wines:

- The first group appreciated the 'woody' wine and rejected mineral wines. These consumers were >50 years old and they mainly consume Chardonnay wines.
- The second group also appreciated 'woody', 'fruity' and 'floral' wines. They were younger and had a lower level of wine knowledge.

The two last groups were relatively neutral towards mineral wines.

Partial conclusion

The results of this clustering showed that the main characteristics of consumers who appreciated mineral Chasselas wines was their high level of knowledge and interest about wines (wine lovers). Moreover, many of those consumers were already used to consuming Chasselas wines. The oldest consumers interviewed preferred 'woody' wines and the youngest tended to prefer aromatic wines ('woody', 'fruity' or 'floral').

Thus, even though minerality is often used to sell wine as a marketing tool, all consumers do not appreciate minerality in wine.

Conclusion

This project tried to establish if the multi-faceted and controversial concept of minerality in wine could translate into a tangible reality. First, it was demonstrated that consumers and wine professionals do not have exactly the same understanding of minerality. Wine professionals refer to a sensory description whereas consumers associate the concept of minerality with the soil, demonstrating the

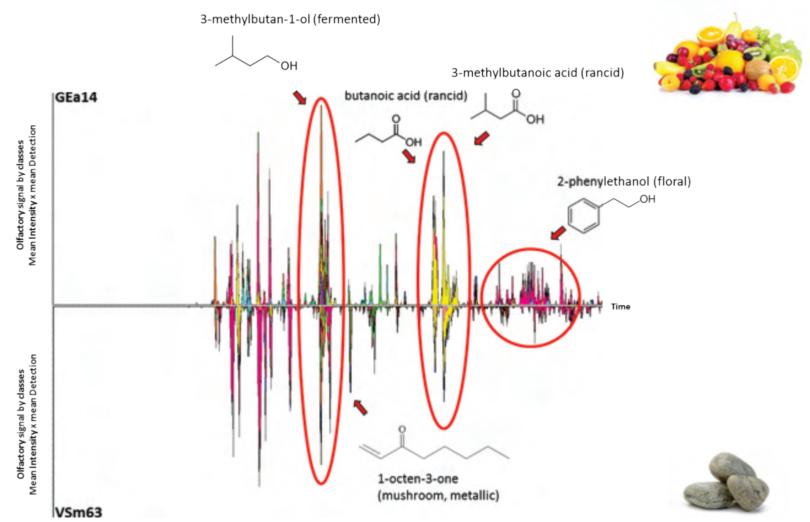

Figure 5. Mean olfactory signal by classes ($OSC_{Int \times Det}$) in one mineral wine (top) and one non-mineral wine (bottom) over eight panellists. Each odorant was classified into one of ten pre-defined classes: buttery-cheesy (yellow), burnt (black), floral-fruity (pink), green-fatty (light green), malty-chemical (dark brown), meaty (red), spicy (purple), nutty (light brown), sulfur (blue) and earthy-undergrowth (khaki).

Table 3. List of wines included in consumer tests, with the main sensory characteristics of each wine. NEM26 and VSM68 were used in the previous stage of the project

Wine number	Sensory characteristics
CH1	light and floral
CH2	citrus fruit
CH3	low aroma intensity
CH4	woody
CH5	gunflint and carbonic
CH6	milk aroma
CH7	oxidised
NEM26	light and mineral (gunflint)
VSM68	gunflint and volume
CH10	freshness and carbonic

belief that the soil on which the vines grow is responsible for the minerality in the wine. The results also suggest that the term 'minerality' is used, by both professionals and consumers, as a substitute for the term 'terroir'. Secondly, based on a tasting task, it was shown that there is no one single example of Chasselas wine recognised as a good example of mineral wine by all professionals. Rather, the selection of a wine as a good example of minerality is mostly dependent on the origin of the professional conducting the task. However, it was possible to distinguish two groups of wines, one recognised as good examples of minerality and one recognised as poor examples of minerality. These two groups were then characterised by sensory descriptors. Mineral wines were described by 'aromatic freshness' and 'acidity' or with 'gunflint' and 'matchstick' odours, whereas non-mineral wines were more diversely characterised. Malic acid and free sulfur dioxide appear as the main drivers of mineral Chasselas wines. Finally, it was demonstrated that mineral wines are appreciated by consumers with a high level of knowledge (considered as wine lovers).

This project focused on Swiss Chasselas wines, but the methodology could be applied to all other varieties. To conclude, minerality in wine should be considered as a wine style such as 'fruity' or 'floral' wines. Moreover, it could be an interesting selling point especially for wine lovers.

Acknowledgements

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Enhancing the experience – the science of food and wine flavour

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Abstract

Through many years of flavour creation and product development, a common thread in successful products has emerged: synergy. This paper will focus on our understanding of how synergies are created in wine and food, how they are measured, and their underlying relationship to consumer liking. In addition, a new consumer engagement strategy will be proposed based on the relationship of aroma synergies.

Webcast of this presentation available at http://bit.ly/16thNorris.

Introduction

The best art provokes. If you can combine human creativity, imagination, and skill in just the right way, you can elicit all kinds of emotional reactions. The creation of food and wine is a kind of art, and science is a tool used by artists to develop new insights into their craft. Flavour chemists, winemakers, and chefs are passionate 'scientific artists' and the desire to create 'living art' for the consumer to experience is shared by all three disciplines. The intersection between art and science can be difficult to define, as the disciplines speak very different languages. When they overlap, however, innovations emerge and new art is born.

Like art, a successful product evokes an emotion. We store the emotions as memories, and then we search for new stimuli, hoping that we might experience these emotions all over again. This leads us to seek pleasing foods and drinks to nourish our bodies and our minds.

Olfaction plays a fundamental role in human cognition, as it is important to emotion memory and social cognition (Zucco et al. 2012; Rouby et al. 2002). Humans can discriminate over one trillion olfactory stimuli (Bushdid et al. 2014). Lucia Jacobs (Jacobs et al. 2015) has recently shown that humans can map an arbitrary location using only aromas. Perhaps discrimination is only a secondary function of olfaction; the primary function may in fact be navigation.

I have spent many years in the flavour industry, and I have frequently witnessed the power of aromas to conjure a sense of time and place. For example, when well-known US baker Mike Kalanty smelled the single aroma compound known as benzaldehyde, his face immediately lit up. He was transported to the moment he first encountered the compound: when he was training as a chef in France, learning how to make marzipan. We all have the ability to map odour and re-orient to a learned location, but this fascinating ability has not been studied often. Our recent work with Minerva College students to create city smells allowed the students to share their history, connect at a deeper level, and create a common language with which to communicate. Smelling aroma compounds transported them 'home'.

Tasting rooms are an effective way to create a wine 'memory,' as they can conjure a sense of place and evoke an emotional response. Consumers will associate the beautiful surroundings, the aromas, and the time spent tasting with the specific wine/vineyard. When conducting wine tastings, we have found that it is beneficial to explain the differences in wines using three aromas, as it helps to classify the 'essence' of a given wine. This technique gave participants a new vocabulary with which to describe the wine, a positive emotional experience linked to location, and a tool they could use to remember and differentiate each wine.

Designing products

Traditionally, in order to design a new food or beverage product, a product developer assembles an array of ingredients representing attributes of interest and tries to make them meld into a 'whole', just like a winemaker will mix blending components to achieve an integrated wine. In everyday product development, creating a 'wow, knock your socks off' product is elusive.

One approach to new product development is to understand the attributes that drive liking/quality of successful food products. One source of such data is the use of experts to evaluate products. For example, ChefsBest is an organisation that uses Executive-level chefs to evaluate leading brands to determine their eligibility for food marketing 'best taste' awards. The ChefsBest judging process involves rigorous training and requires judges to pass an exam to qualify them as experts. The chefs work diligently in each session to produce a definition of quality, where quality is defined as a numerical sum of the attributes and intensity of the attributes that the product should exhibit. The product is then scored on the presence and intensity of these attributes.

Throughout the many years of judgings, ChefsBest has created a vast database of products, and their definitions for quality. In the example below, experts judged the leading graham crackers. The graham cracker with the code 'HMGCI' was found to be the highest in quality due to the overall flavour balance, mouth-feel (lack of grittiness), soft-crisp texture, sweetness and flavour intensity (Figure 1).

So, if we were designing a new food to compete with a graham cracker, we would know that mouth-feel, texture and overall balance were the most important attributes to focus on.

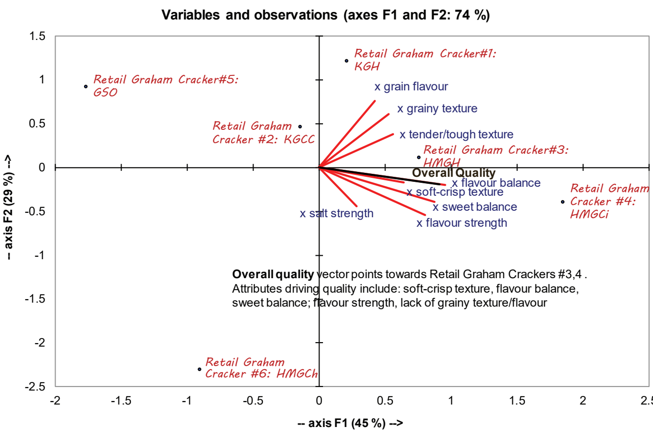

Figure 1. PCA plot showing the attributes that drive quality for graham crackers

Similar to food, wine quality perception has been measured to correlate with consumer purchase intent (Marin et al. 2007). Unlike ChefsBest, the wine sensory groups have not defined wine quality as the summation of attributes, but rather as a 'judgment about a product's overall excellence'. Given this, 'it is not surprising then that experts and consumers do not necessarily agree on what makes a high quality wine' (Saenz-Navajas et al. 2013).

In work that we did with Yaelle Saltman (pers. comm. 2016) where we added desirable flavour attributes to wine, we were not successful in increasing liking of the wines. We did not understand the attributes that drove liking, nor were we able to manipulate them. I would contend that we need to uncover the attributes that drive consumer liking and provide tools to winemakers so that they can manipulate these attributes. Our hypothesis is that we need to optimise mouthfeel (not too astringent), minimise off-notes, and provide an overall balanced wine. Charles Shaw demonstrated the likeability of 'two buck chuck,' a no fault wine that is slightly astringent, has minimal off-notes, and overall is balanced.

The goal then for the winemaker, product developer, flavour chemist or chef is to gain an understanding of how to create and manipulate the attributes of a successful product. This requires a fundamental understanding of the interactions of the matrix with flavour and basic tastes, also expressed as the interactions of volatiles and non-volatiles. For flavours and food, this requires a study of the detection threshold of compounds in a given matrix. For wine, the study is more difficult due to the presence of alcohol and the sheer number of non-volatile and volatile compounds. However, the overall goal is still to create a desirable set of attributes by determining the interactions between compounds (antagonistic or synergistic) and manipulating them throughout the winemaking process.

From a product development standpoint, creating a product with a desirable set of attributes often means using ingredients (or blending components for winemakers) that go together in such a way that experts and consumers find it difficult to break it into its parts. This is 'the wow factor', the immediate understanding that it 'tastes really good' followed by the recognition that you're not quite sure what you're tasting. The sum is greater than the parts. For example, the Snickers bar is one of the most popular chocolate bars on the market due to its combination of textures (crunchy peanuts, silky caramel, chewy nougat, and smooth chocolate), basic tastes, and flavour. These factors combine to make it 'interesting' and satisfying.

From a chef's point of view, the complex mixtures found in culinary dishes require synergistic interactions defined as the 'lifting of one or more aromas/flavours'. Balance and integration are sought-after attributes. Interactions between food ingredients that boost the positive notes are synergistic. For example, chefs know well the effect of garlic used at the correct level in cooking, as it provides the base/middle body of a food and gives lift to the volatiles:

> Garlic used as it should be used is the soul, the divine essence, of cookery. The cook who can employ it successfully will be found to possess the delicacy of perception, the accuracy of judgment, and the dexterity of hand which go to the formation of a great artist' (Waters 1920).

Synergy

When creating flavours, foods and beverages, synergy is a sought-after but difficult to measure effect. As the quote above suggests, synergy is about balance. The right amount of an ingredient can be the difference between merely consuming a product and 'having a memorable experience'. Synergy is often expressed as a mixture of flavour stimuli in which the mixture is something perceptually and experientially different from its individual components.

Science on its own cannot predict synergy. Flavourists, chefs, and winemakers undergo apprenticeships where they rigorously taste many products in many combinations in order to learn how synergy works. Perhaps the best example of flavour synergy is the cola flavour: oils of orange, distilled lime, and cinnamon; extracts of vanilla and kola nut; water, acid, and caramel color. If you combine these ingredients in the appropriate ratios, something emerges that is distinctly different from the sum of its parts.

Determining the types of compounds that create synergy would be advantageous. Sean LaFond discusses the concept that biologically relevant compounds may be predisposed to result in forming synergies (LaFond 2016). An example of such a synergistic system exists. There are specific neurological pathways that allow humans to process separate facial features (eyes, ears, nose, and mouth) into the cohesive and biologically relevant visual concept of a 'face' (Nelson 2001). Hence, perhaps blending occurs in the presence of biologically relevant molecules.

To test this hypothesis, let's look at what we know from practical experience about compounds that 'create synergy.' For Coke, our hypothesis is that despite its soapy flavour, it is the kola nut extract that is responsible for 'blending' the vanilla, cinnamon, lime, and orange flavours. Our hypothesis that biologically relevant molecules induce synergy would hold true, as kola nuts could be considered biologically relevant given their psychoactive potential.

Another example of compounds that can create synergy include monosodium glutamate (MSG) in seasoning blends. MSG provides a source of umami. While I was working for a seasoning company, we discovered that a simple way to increase the consumer liking score of a seasoning blend was to increase the MSG content. Ingredients such as tomatoes, mushrooms, and seaweed all provide 'natural' umami, and the underlying attribute which will drive the use of these ingredients in foods is that they provide a natural source of umami. Umami can be considered biologically relevant, as humans require protein for nourishment. Using MSG or ingredients containing MSG would signal to the body that this food is a source of nourishment.

Another example of a synergistic ingredient is the use of angelica root in gin: 'It 'marries' the other botanicals together into an interwoven blend of flavours' (Regan 2009). Angelica root contains macrocyclic musk compounds which have been identified as pheromones involved in mating (Sommer 2004). Hence, like umami, there is biological relevance as to why macrocyclic musk compounds may be synergistic.

Measuring synergy

Measuring flavour synergy is difficult, in part because there is not a clear scientific definition for it. Flavour synergy can be broadly classified into two categories: elemental/analytical blending and configural/synthetic blending. These categories go by different names depending on which researchers are publishing the work; neuroscientists tend to prefer elemental and configural (Kay et al. 2005), while sensory scientists tend to prefer analytical and synthetic (O'Mahony et al. 1983). Briefly, in elemental/analytical blending components one can enhance or inhibit the effect of another; in configural/synthetic blending new flavours arise from constituent components in an unpredictable manner.

Many tools of the sensory scientist can theoretically be used to evaluate flavour blending. It is known, however, that the manner in which panellists are trained can change sensory perception (Barkat et al. 2012). LaFond and colleagues (2016) developed a technique designed to gain information about how panellists perceive flavour blending while minimising training that could affect perception. For this method panellists were trained to evaluate the overall psychophysical flavour intensity and flavour complexity of sets of teas that were flavoured with objective standards: a concentration series for overall psychophysical intensity and a chemical complexity series for flavour complexity. Trained panellists then rank-rated a set of five teas to evaluate a hypothesis that low doses of civet absolute (a flavour/fragrance compound obtained from the civet cat) can induce flavour blending. These five teas were as follows: a low complexity tea which was unflavoured and lightly sweetened (Tea), a moderate complexity tea consisting of the low complexity tea with two flavour compounds added (Tea+2), a high complexity tea that consisted of the moderate complexity tea with an additional two compounds added (Tea+4), the high complexity tea with a sub-identification threshold dose of civet absolute added (Tea+4+m) and a high complexity tea with a super-identification threshold dose of civet absolute added (Tea+4+M).

For both attributes the panel could clearly differentiate between all teas with differing chemical complexity without civet absolute (Tea, Tea+2, Tea+4). For overall intensity the complex teas with civet absolute were not significantly different from the complex tea ($p > 0.05$). It was found that adding a super-threshold level of civet absolute did not increase the perceived number of flavours of the mixture. The addition of a sub-threshold level of civet absolute reduced the reported complexity of the four-added component blend to that of a two-added component blend.

The addition of the sub-threshold dose of civet absolute to the complex tea altered the pattern of rankings to be more like the moderate complexity tea. This alteration of ranking pattern is unusual, and as the high dose of civet absolute did not have this effect and did not increase overall psychophysical intensity, it is unlikely that this was due to civet absolute overwhelming the other flavour characteristics. It was hypothesised that the observed change in ranking pattern is an example of a complex synergistic effect.

A synergistic effect described as a reduction of complexity that can be observed in minimally trained panellists has broader implications for product design. While this study did not look at the hedonic character of the beverages, it has been shown that there is an optimum level of complexity in food and beverage systems (Giacalone et al. 2014). Consumers are prone to preferring products that are not too complex and not too simple. Having volatile and non-volatile compounds that can modulate an abstract parameter such as complexity can be a powerful tool to increase the balance and integration of a wine. We predict that an increase in balance will increase the hedonic response.

Concluding remarks

Synergy is an elusive property of successful food and beverages. When present, it evokes an emotional response to a given mixture. It is our hypothesis that humans recognise synergy as a result of the presence of biologically active/relevant compounds. Having the ability to create and manipulate the connectedness/synergy of ingredients is of interest to chefs, winemakers and flavourists alike. Research should be continued to identify the compounds that provide such synergy.

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Preventing food fraud

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Abstract

Food fraud is one of the most urgent import food industry issues. The recent focus has been on reducing the human health vulnerability of ingredients. Examples include issues such as Sudan Red carcinogen colorant in sauces, melamine in infant formula, horsemeat in beef, and methanol in spirits. Although those are a public health priority, there is an ever-present threat of finished goods fraud and counterfeit products. For sustained economic growth and brand equity, there must be an overarching focus from the authenticity of raw materials, to detection methods in the marketplace, and there must be a focus on maintaining consumer trust in the markets. The focus on reducing the 'fraud opportunity' must be considered before countermeasures or control systems are implemented. Food fraud prevention must work as a system since we will not test or arrest our way to safety. This presentation will include a review of a country-level food fraud vulnerability assessment as well a holistic analysis of a prevention plan that reduces the overall fraud opportunity. The methods will be presented with a focus on the Australian wine industry.

No paper available, please view this presentation at http://bit.ly/16thSpink.

Traceable, tested and trusted: ensuring the safety, quality and authenticity of Australian wine

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Abstract

Wine is regulated as a food and consumers are increasingly demanding 'authentic' food products from producers they can trust. An authentic bottle of wine is one whose origin is correctly reflected on the label and in other marketing material. Wine provenance comprises various elements. Any indicated vintage, variety and regional origin must be substantiated by the creation and retention of a traceable trail of records throughout the supply chain. Anomalies are rare but, when detected, have resulted in prosecution.

Enforcement of wine origin laws through inspection of records would be redundant if consistently valid analytical tools were available to unequivocally guarantee origin. While a number of such techniques are promising they are often confounded by legitimate wine blending, and rely on the creation of a sufficiently comprehensive database of reference samples of known origin.

A further element of wine provenance is the brand under which the wine is sold. Counterfeit wine, as the term is generally understood, is wine not from the owner of the brand. A 'counterfeit' may, or may not, correctly indicate the vintage, variety and regional origin of the wine. The producer's best protection from counterfeiters is through trademark registration, in multiple jurisdictions, possibly supplemented by technologies such as QR codes or embedded DNA.

Webcast of this presentation available at http://bit.ly/16thSGuy.

Introduction

Consumer trust in Australian wine depends on maintaining confidence in the safety, quality and integrity of our product. This paper will discuss the role of Wine Australia in relation to each of these three elements.

Safety

Wine does not present the same safety hazards as foods such as meat, fish and dairy.

Wine, for example, presents a low risk to health from a microbiological perspective. Of the 15 pathogens considered by the US Food and Drug Administration in 2011 (Food and Drug Administration 2011) only one could grow below pH 4, and this exception, salmonella, is controlled by wine's ethanol content (Moretro and Daeschel 2004). A combination of pH, phenolic composition, ethanol and added sulfites render wine an extremely hostile environment for food-borne pathogenic organisms.

Nevertheless wine is regulated as a food, both in Australia and in most of our export markets, and therefore tends to be subjected to a level of scrutiny that is not always justified based on the risk to consumer health. Vietnam, for example, requires all wine to be tested for the presence of the bacterium, *Escherichia coli* (*E. coli*), despite wine being a hostile environment for these organisms and the pathogenic strains of *E. coli* being unable to grow below pH 4 (Sugita-Konishi et al. 2001). Indonesian authorities also regularly request evidence that wine has been tested to be free from contamination by pathogenic organisms, such as *Staphylococcus aureus*.

Governments that insist on testing wine for the presence of pathogenic organisms are not making best use of their resources. Those resources would be far better directed to the inspection of products that do present a threat to human health and safety.

The use of agricultural chemicals in the vineyard may be a source of one potential threat posed by wine, and maximum limits on residues of these chemicals are applied in most international markets. It is not commonly appreciated, however, that these limits are determined as indicators of good agricultural practice and that a breach of a maximum limit does not generally mean there is a threat to health. Nevertheless, Wine Australia regularly commissions surveys of the residue content of random samples of Australian wine to monitor compliance with the limits imposed by the Australian Food Standards Code. Residues are rarely detected and, when present, are well within legislated limits (for example, Wine Australia 2014).

Many of the 20 materials permitted to be added to Australian wine are naturally occurring components of grapes (Australian Government 2017), exceptions include the sulfite and sorbate preservatives, hence their use is unlikely to pose a health risk. Nevertheless, even this very limited range of additives can prove to be controversial as recent events at the international food standards setting body, Codex Alimentarius, have demonstrated.

Codex Alimentarius develops and publishes a General Standard for Food Additives (Codex Alimentarius 1995) which sets out the conditions under which various additives can be used in foods, categorised by food type. Only five additives are authorised for use in wine; dimethyl dicarbonate, lysozyme, sorbates, sulfites and carbon dioxide (plus caramel for fortified wines).

Some countries, notably India, Vietnam and Sri Lanka have domestic legislation that refers to the Codex Alimentarius standard. There is, therefore, a risk that wine made using an additive not listed by Codex will be refused entry to these markets. Hence there have been recent international efforts to extend the list of approved Codex additives to include those most commonly used in wine producing countries.

These efforts have, unfortunately, so far proved unsuccessful. A working group led jointly by France and Australia, charged with developing the conditions under which new additives would be approved, has been unable to agree on these conditions. On the one hand are those, such as Australia, who want the use of additives to be limited only by guidelines for good manufacturing practice and, on the other, those who insist that additive use should be constrained by defined numerical limits, even when those additives are also naturally occurring components of grapes (Codex Alimentarius 2015).

Nevertheless, there is consensus within the working group that numerical limits can be appropriate when an additive has been assigned an Acceptable Daily Intake (ADI) by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). JECFA assigned an ADI to sulfur dioxide in 1998 (IPCS 2001), and Wine Australia insists that the sulfur dioxide content be declared when any wine is registered for export. With very limited exceptions no wine can be exported from Australia unless it complies with the Australia New Zealand Food Standards Code. For an exception to be made the wine must (obviously) comply with the requirements of the importing country and Wine Australia must be convinced that allowing the exception will not damage the reputation of Australian wine.

Some 50,000 consignments of Australian wine are exported each year to over 120 countries. The only serious safety incident in the past 15 years was attributed to the grossly excessive addition of sulfur dioxide by a German bottling facility to a wine that had been imported from Australia in bulk. The legislated export controls administered by Wine Australia include the power to prevent future sales of wine to particular customers in circumstances such as these (Australian Government 2014).

Quality

For many years Wine Australia, and its predecessors, acted as the arbiter of quality, requiring all wine proposed for export to be approved in advance by a panel of experts. The panel was charged with screening wine for faults, of which few were ever detected – 37 wines were not approved for export from the 14,569 wines submitted in the last full year during which assessments were conducted by Wine Australia Corporation, July 2010-June 2011. In early 2012, Wine Australia changed from reliance on pre-export tastings to an approach based on risk-assessment. At the time the tasting procedure was introduced, in the middle of the 20th century, Australian table wine was rarely exported and there was a risk that a few oxidised or volatile wines could severely damage our reputation. Australia is now an established and respected wine producer, the world's fifth largest exporter and the market leader in some countries. Wine Australia helps ensure Australian wines are 'fit for purpose' by monitoring compliance with the Food Standards Code and by verifying the truthfulness of provenance claims. Degrees or grades of quality are best left to the market to determine.

Integrity

Consumers are increasingly demanding products they regard as 'authentic' from producers they can trust. There has been a recent tendency to apply the term 'authentic' exclusively to a narrow range of wines made by small, preferably family-owned, producers using organically or bio-dynamically grown grapes and a limited number of 'traditional' winemaking techniques. In this paper, however, the word 'authentic' is used in its usual sense of 'genuine'. Hence a wine will be considered authentic if it is what it claims to be.

Wines are differentiated from each other in many ways. Typically these include differentiation based on the identity of the producer, and by indication of the wine's vintage, variety and regional origin.

The identity of the producer is usually communicated via the brand, although this has become blurred with the rise of 'virtual producers' and retailer-owned brands. Whether or not the brand reveals the identity of the producer, the brand remains a key factor in a consumer's purchasing decision. Counterfeit wine, where the product is not entitled to the brand under which it is presented, not only damages the brand owner through lost sales, enforcement costs and tarnished reputation, but consumers are misled as to the wine's origin and may be exposed to potential health risks. Furthermore, governments lose the revenue they otherwise would have accrued through taxes and, especially where health risks are posed, the reputation of Australian wine in general could suffer.

In Australia, trademark rights can be established through usage of a brand; registration is not essential. The situation is the same in other countries having legal systems based on common, rather than civil, law. The US, the UK, New Zealand and Canada are examples of the former, China and most European countries of the latter. Trademark rights established in Australia, however, do not extend beyond our borders, they must be separately established in each country. In China, for example, they can only be established through registration and evidence of prior use of the trademark is not necessary. Hence the first to apply for registration of a particular trademark in China obtains those rights and can then prevent use by others. It is not uncommon for opportunists in China to 'squat' on trademarks they have no intention of using. Huge companies such as Pfizer and Apple have been the victim of Chinese trademark squatters but there is one advantage of the Chinese system in that Australian wine producers can defend their brands by registering trademarks before selling any wine in that country. Companies should also register the Chinese characters corresponding to the English brand name (IP Australia 2006).

Registration of a trademark does not, by itself, protect against blatant copying of a wine label or the use by others of certain brand elements, such as font style, shape and colour, that are characteristic of a particular producer. Enforcement of trademark rights is not always easy and must necessarily be preceded by awareness that counterfeit goods are being traded. This is why a market has emerged in brand protection devices using various technologies such as QR codes, chemical markers added to printing inks, holograms, embedded DNA, and, most recently, the pairing of QR codes with other technologies, allowing consumers to use their smartphones to authenticate products whilst providing producers data on where, and to what extent, their products are being counterfeited.

It is important to distinguish between the fraud associated with counterfeit wine and the fraud that can be committed by legitimate brand owners. Brand owners, like counterfeiters, may be motivated to misrepresent a wine in order to obtain commercial advantage. Although Australia's 107 wine geographical indications and an even longer list of grape variety names are all equal under wine law, some are more equal than others in the eyes of consumers. Grape varieties tend to go in and out of fashion. In the 1980s and 1990s demand for Chardonnay may have exceeded supply but now Prosecco and Pinot Gris enjoy the consumer cachet that has the potential to motivate fraud.

The primary responsibility for protecting a brand lies with the owner of that intellectual property but if wine is falsely represented as to its provenance, whether country of origin, region, grape variety or vintage, then the potential damage may extend beyond one company. Consumers may lose faith in the integrity of <u>all</u> wine of the misrepresented country, region or variety.

Australia, therefore, has robust laws regarding origin claims (Australian Grape and Wine Authority Act 2013, Section 40), which were further strengthened relatively recently. Despite many promising developments there are currently no analytical techniques that can reliably, and in all situations, determine the vintage, varietal and regional origin of a wine with a level of confidence that would satisfy a criminal court. Therefore it remains essential that the history of a wine can be traced through an examination of records made throughout the supply chain. Traceability from the wine retailer to the vineyard via the distributor, broker, bottler and producer provides a guarantee of provenance that, as yet, chemistry cannot.

Until relatively recently, however, only wine <u>producers</u> were required to maintain a traceable trail of records throughout the winemaking process. Others in the supply chain, grapegrowers, brokers, distributors, were not. A traceable trail of records is only as good as its weakest link and the rise of 'buyer's own labels' and 'virtual wineries' ensured there were plenty of potential weak links. In 2010, therefore, the record keeping requirements of Australia's label integrity program were extended to include grapegrowers and all those who supply and receive wine along the supply chain (Australian Grape and Wine Authority Act 2013, Section 39C). For most, the obligation is merely to maintain a traceable trail by recording the classic 'one step forward and one step back', the details of from whom the wine was sourced and to whom the wine is supplied, but wine manufacturers must also keep detailed records of all wine movements, blends and other production processes.

At the same time as the scope of this traceability requirement was widened, two other significant changes were introduced (Australian Grape and Wine Authority Act 2013, Section 39F(2)). Previously there had been no requirement to make a record of a step in the wine production process until three months after the event had occurred. Hence most winemakers would have been under no obligation to make any records about a particular vintage until the end of that vintage! Notwithstanding the threat posed to label integrity, it is difficult to see how any business could successfully operate in an environment where records were only made three months after the event had occurred. This is especially true in the case of a winery during harvest, where the situation is dynamic and constantly changing. The location, and composition, of a particular wine may change multiple times each day. Attempting to construct, from memory, a history of all winery operations at the end of vintage would have been an impossible task. Furthermore, for anyone attempting to verify a label claim through an examination of a trail of records, the possible gap of three months during which no records were mandatory would have proved extremely frustrating. In practice, of course, winemakers generally made records as soon as practicable following the occurrence of the relevant event but it was only in 2010 that the legislation was amended to specify a maximum of three days between the transaction and its record.

Another anomaly was resolved with the raft of legislative amendments introduced that year (Australian Grape and Wine Authority Act 2013, Section 39J). Previously the penalty applying to false statements about a wine's provenance was substantially higher than that which applied to failure to make, or making incorrect, records during the wine's production. Custodial sentences could be imposed on those proved to have made false representations about the origin of a wine but a fine (albeit substantial) was the worst that could be expected for breaches of record keeping obligations. An investigation into allegations of false provenance claims could therefore be thwarted by the culprit's destruction of any incriminating records. In the absence of analytical techniques proving a wine's origin, winemaking records are the only recourse for the investigator, hence a recalcitrant wine producer may have found it prudent to incur the imposition of a fine rather than risk time in gaol.

The penalty for failing to keep adequate records, or making false records, is now aligned with that associated with false label claims so no incentive remains to destroy any incriminating records when being scrutinised by Wine Australia's investigators.

In 2010 Rivers Wines Pty Ltd, and one of the company's directors, were convicted on multiple counts of intentionally making false records relating to the fraudulent representation of Sultana grape juice as Chardonnay (Magistrates Court of South Australia (criminal) File no. AMC-06–13804). Substantial fines were imposed but similar offences today could attract custodial sentences of up to two years.

The culture of compliance prevalent across the Australian wine community is evidenced by the fact no prosecutions other than the Rivers matter have been initiated by Wine Australia, or its predecessors, in the past 15 years. Nevertheless label integrity audits, up to 300 of which are conducted each year, occasionally detect relatively minor breaches resulting in the imposition of administrative, rather than criminal, penalties. In such cases, Wine Australia can suspend an export licence until record keeping systems are improved. In 2015, however, one licence was cancelled, rather than merely suspended when the varietal origin of a substantial quantity of wine was found to have been misrepresented.

There are various analytical techniques that, if demonstrated to be valid, could complement the work of Wine Australia's label integrity auditors. In the cases cited above, for example, where the varietal origin of the wine is in question, analysis of the protein content could be a useful tool, particularly for white wine. If, however, the wine has been treated with bentonite, as is often the case, most of the original protein will no longer be present, thus rendering the technique of limited use in the case of finished wine.

Various methodologies have been developed to extract, and amplify, DNA from wine in an attempt to verify varietal origin. None are used extensively at this stage.

Similarly, techniques such as trace metal analysis, stable isotope ratios and Nuclear Magnetic Resonance can be used for determining geographical origin.

The validity of all such tools, however, relies on the existence of a sufficiently comprehensive database of samples of known origin, with which to compare the sample under test. Without such a database there is a risk of legitimate samples being categorised as fraudulent.

The varietal authenticity of an Australian Chardonnay, for example, was disputed by German authorities in 2002 on the basis of the wine's shikimic acid content when the reference samples consisted primarily of wines from the Chablis region of France. A robust database would need to account for a range of viticultural, environmental, fermentation and post-fermentation factors that can influence the shikimic acid content of wine. A complete set of criteria for establishing whether a database is sufficiently robust to avoid falsely condemning legitimate wine as fraudulent has yet to be developed.

In the meantime, therefore, the authenticity of provenance claims can only be demonstrated through the maintenance of a trail of recorded information that can be tracked and traced through the wine supply chain by an appropriate authority. In the case of Australian wine, the information that must be recorded is specified in legislation, Wine Australia is the relevant auditing body and, when necessary, action is taken to ensure trust in the integrity of Australian wine is not undermined by the actions of a few.

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Measuring up authentication: analytical tools to test wine provenance

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Abstract

Although innovative packaging technology is emerging as a reliable way of proving wine authenticity by supporting document-based traceability systems, there are still cases where it is necessary to use chemical analysis to verify the provenance of wine. These may include situations where 'refilling' of authentically labelled bottles is suspected or when a wine has originated from a bulk shipment. A wide range of methods to analyse for geographical origin have been proposed over the past 30 years including stable isotopes of 'bio-elements' (e.g. carbon, oxygen or nitrogen) or 'geo- elements' (e.g. strontium or lead), trace elements and organic profiles measured by near-infrared or mass spectrometry. In many cases, convincing arguments have been made as to the success of these methods, but on closer inspection they have failed to place themselves within a winemaking framework or to take a sufficiently holistic view of the challenge of wine authentication.

A recent AWRI pilot study, undertaken in conjunction with Wine Australia, has measured strontium isotope ratios and some carefully chosen trace elements in a set of Australian and international wines, in the first steps towards developing an analytical tool to assess wine provenance. Results have shown that this approach not only allows for wines produced in Australia to be differentiated from those produced in other countries but also differentiates between wines from some of the major Australian grapegrowing zones. Analysis of data from Australian wines suggests a substantive link to the underlying geology. Although compelling in its current form, additional parameters are set to be included in the next stage of this applied research project.

Webcast of this presentation available at http://bit.ly/16thDay.

Introduction

According to the Organisation Internationale de la Vigne et du Vin (OIV), the volume of bulk wine produced in 2014 was 377,300 ML (OIV 2015); that is nearly 40% of the world's production of wine that is not protected by tamper-evident bottle seals, holographically authenticated labels or similar devices. It is therefore important to have a set of tools to enable the provenance of a wine not protected by these devices to be determined by analysing the wine itself. The volume of bulk Australian wine produced in the same period was a mere 402.8 ML (Wine Australia 2014) which equates to more than half of Australia's annual production. With Australia's strong label integrity program and customs control, wine fraud within Australia's bulk wine is unlikely but what is of concern is that there is such a large volume of bulk wine across the world that could end up labelled fraudulently, potentially even as Australian. And for bottled wine, only a very few brands will be able to afford to physically tag each bottle. This means that intrinsic analyses to determine wine provenance are important for everyone in the Australian wine community.

There are many facets to authenticity and wine fraud and for analytical scientists the challenges are many—as are the proposed solutions. This paper will give an overview of what has happened around the world, largely in Europe, and what is driving the approach Australia is now working towards.

Learning from European research on wine authentication

One of the earliest areas of research in Europe concerned chaptalisation, the addition of sugar before fermentation to increase alcohol. After nearly a hundred years of research in Bordeaux, largely using variants of dry extract, Professor Gérard J. Martin from Nantes University in France finally solved the problem using stable isotopes measured using ^{2}H-NMR, a laboratory version of the MRI scanner (Martin et al. 1986; Martin and Martin 1988). Although there is obviously little need for such measures in Australia, this demonstrates the first use of complex analytical equipment to solve a wine authenticity problem. Often paired with chaptalisation, extension by dilution can now be detected using oxygen-18 techniques (Calderone and Guillou 2008). One of the old tricks of the past to get more colour into Burgundy was to add North African wine, but just looking at the anthocyanin profile would have easily shown this (Pisano et al. 2015). However, determining the varietal blend is still one of the hardest challenges, until DNA can be reliably isolated from commercial wine. Working out how the fizz got into sparkling wine, whether bottle-fermented, from the Charmat process, or carbonated, is relatively simple using stable carbon isotope analysis (Martinelli et al. 2003). This paper is about determining the provenance of a wine however, it is an expensive undertaking and rarely straightforward. Ultimately, if there is a way of determining if a wine is Australian or not, the Australian wine community can avoid catastrophes such as melanin in infant formula (Anon. 2008; Knechtges 2011) or diethylene glycol in wine (van der Linden-Cremers and Sangster 1985). All of the techniques discussed above are used in Europe along with the necessary databases and could potentially be used in trade disputes. To date no data from these techniques on Australian wines is available.

From current knowledge of the hundreds of aroma and phenolic molecules found in wine, using organic compounds would seem a good starting point to work out a wine's place of origin. Anthocyanins (Pisano et al. 2015), amino acids (Saurina 2010) and polyphenolic compounds (Makris et al. 2006) have all been used as targets for analysis. But the winemaking process itself is likely to modify the concentrations of these compounds, for example through exposure to oxygen (Day et al. 2015). The 'metabolomics approach' has been so important lately in understanding complex systems in wine biochemistry. Essentially numerous compounds are determined without specifically knowing their identity and by using multi-dimensional statistics a great deal of discriminating information is available. Typically, this work is realised using complex analytical equipment such as ^{1}H-NMR, time-of-flight mass spectrometry or even mid-infrared spectroscopy have been used (Riovanto et al. 2011).

Wine composition is not static – it changes over time during maturation, ageing and storage. Indeed, many consumers expect that it will change and improve with age. In addition, if wine is exposed to heat during transport or storage, then there is a chance the complex organic matrix will change. So, unlike human fingerprints which never change, these fingerprinting techniques are not a reliable basis on which to build a robust authenticity system.

Investigating trace elements and stable isotope ratios

The OIV defines terroir, in resolution OIV/VITI 333/2010, as follows:

> Vitivinicultural "terroir" is a concept which refers to an area in which collective knowledge of the interactions between the identifiable physical and biological environment and applied vitivinicultural practices develops, providing distinctive characteristics for the products originating from this area.
>
> "Terroir" includes specific soil, topography, climate, landscape characteristics and biodiversity features.

Taking inspiration from this, other bases can be drawn upon to define the chemical authenticity of a wine. The soil represents both lithography (bedrock) and lower and surface horizons; the topography reflects vineyard altitude and distance from the sea; climate is represented by long-term characteristics in temperature, rainfall and sunshine hours; landscape characteristics might be slope, orientation, peripheral vegetation and proximity of bodies of water; and biodiversity clearly indicates myriad indigenous biota. These parameters, which we see as ultimately defining what a wine tastes like, can also be used to find analytical proxies. To avoid one of the pitfalls of data which has a lot of correlation between parameters, it is essential that the different data channels are completely independent of each other. Multiple sets of data that are unrelated should therefore be the foundation of the most robust model for authentication purposes.

Of these defining terms, soil composition could very reasonably be considered to play the key role in defining terroir and a good basis for authenticity testing. Trace elements (i.e. those present in wine around the tens of mg/L level or less) get transported from the soil to the grapes through the roots. This is certainly a good place to start and researchers have been looking here for over 40 years (Versari et al. 2014).

Analysis of some key papers published over the past 20 years looking at wine regions from over 30 countries reinforces that there are just a handful of elements that constantly prove useful in discriminating different wine regions (i.e. they are significant for geographical discrimination in 40 to 55% of the papers considered). These elements are: lithium, manganese, rubidium and strontium, with barium, calcium, chromium, copper, magnesium and zinc appearing as significant in more than 25% of papers. However, there are issues with many of the elements chosen merely by statistical analysis but without considering the oenological framework. Several reviews of winemaking processes highlight this problem (Castiñeira Gómez et al. 2004), which calls into question the usefulness of trace elements on their own for determining wine provenance and suggests that other, additional parameters should be explored. Natural abundance isotope ratios are the one type of analytical parameter which is able to act as a proxy to allow assessment of the distance of a vineyard from the sea, or how high up a mountain it is, or how little rain falls there.

From a simple viewpoint, it is the naturally occurring differences in atomic weight of the stable isotopes of hydrogen and oxygen that are sufficient to make, say water molecules, heavier and therefore more difficult to evaporate or easier to condense. This means that in the water cycle, as sea water evaporates into clouds and then rains inland, there will be a natural isotope distribution in the surface water available for agriculture. On a world scale these differences are seen in surface waters across the planet, forming a basis for their use in authentication studies. There have been several reviews recently that tie all this information together (Camin et al. 2015; Raco et al. 2015).

One possible downside to this approach is that because isotope ratios of water depend on the annual weather conditions, there will be year-to-year variation. An example from Germany (Aurand et al. 2015) demonstrates that the extensive databases to which unknown samples are compared need to be supplemented every year to account for annual variations. It would therefore be useful to have other additional variables which do not vary year on year. One of the many ratios which has attracted the attention of authenticity scientists over the past 10–15 years is the ratio of strontium 87 to strontium 86 ($^{87}Sr/^{86}Sr$). This is demonstrated by the variation of $^{87}Sr/^{86}Sr$ in natural mineral waters overlaid on a map of the varied geology of Europe (Voerkelius et al. 2010).

A pilot study of Australian wines

To date no comprehensive, multi-region study of the $^{87}Sr/^{86}Sr$ ratio has been made of any agricultural produce in Australia, although some values in milk have been published (Crittenden et al. 2007). With the financial and logistical help of Wine Australia, AWRI researchers determined the $^{87}Sr/^{86}Sr$ ratio on 194 finished wine samples from eight major wine-producing regions of Australia and 37 non-Australian wines that represent countries with important bulk trade markets. Samples were made available through statutory sample requests from the Export Approval scheme. Measurements were made using thermal ionisation mass spectrometry (TIMS) according to Balcaen et al. (2010). To minimise variables in the experimental design, only wines made from Cabernet Sauvignon or Chardonnay grapes were included (Pinot Noir replaced Cabernet Sauvignon in Tasmania). The mean ratio value for Australia was 0.71131 (standard deviation 0.0022) which is higher than the mean of the other countries analysed (0.70897; standard deviation 0.0024) and although analysis of the variance demonstrated statistically significant differences there is sufficient overlap of the Gaussian distribution to warrant additional parameters being required to successfully differentiate Australian wines from those of other countries. These values agree with some recently published data for wine from McLaren Vale (Kristensen et al. 2016). Of the 25 trace elements also measured by ICP-MS, only nine (arsenic, beryllium, chromium, lithium, nickel, lead, rubidium, vanadium, zinc) in addition to $^{87}Sr/^{86}Sr$, were shown to have significant differences between Australia and other countries, with lithium, rubidium, vanadium and zinc being the most important. The scores plot of the Partial Least Squares Discriminant Analysis is shown in Figure 1 and when used in a quadratic discriminant analysis yielded a classification rate of 94.7%.

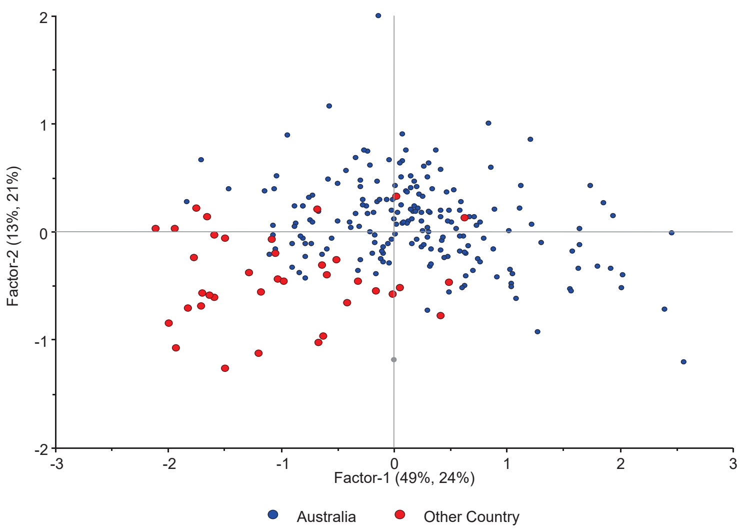

Figure 1. PLS-DA scores plot using $^{87}Sr/^{86}Sr$ and As, Be, Cr, Li, Ni, Pb, Rb, V, Zn for differentiating Australian wines from those produced in other countries

Within the different regions of Australia, the scores plot in Figure 2 confirms that wines group together according to their region, and some regions are quite separate from others.

The important parameters for this analysis were among those that have appeared frequently in the literature as important. Despite the visual overlapping, the results of the discriminant analysis are generally promising, with an overall classification rate of 87.6% and several regions (Tasmania, Hunter Valley, Margaret River) achieving greater than 95% classification.

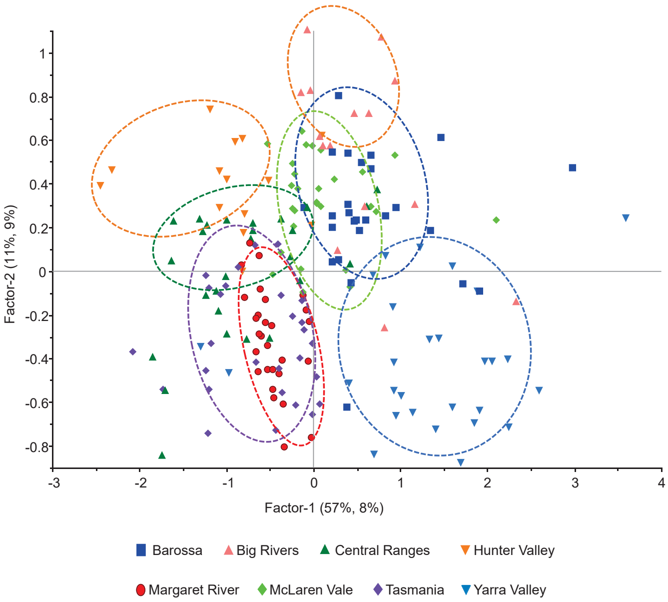

Figure 2. PLS-DA scores plot using $^{87}Sr/^{86}Sr$ and As,B, Ba, Co, Li, Mn, Ni, Rb, Sr, V, Zn for differentiating wines from major regions within Australia

Conclusion

To sum up, the Australian wine community is now going down a concerted path of defining its own wine provenance authentication toolkit by using the knowledge gained by European researchers. This work has shown that trace metals and their isotope ratios are very powerful parameters but other unrelated data is required. In the next steps of this research, the usefulness of other isotope ratios of boron, lithium and lead will be explored to add further independent data.

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Smart packaging technologies and solutions to preserve authenticity: an overview

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Abstract

Smart packaging can take on several meanings with respect to authenticity. We can define 'smart' in several ways. Prior to modern times, when you heard someone talk about 'smart,' it probably meant something along the lines of being clever, intelligent or knowledgeable, all complimentary terms. Negatively, smart can take on other meanings that describe someone being witty, saucy or even rude. 'Don't get smart with me,' is probably something we heard from our parents a few times during our 'know-it-all' years. Today in the age of microprocessors, radio frequency identification (RFID), proximity chips, near field communication (NFC) chips, the word smart has taken on a meaning that probably is first to mind compared to years gone by. It is used to describe forms of artificial intelligence (AI). We are manufacturing products and packaging to give them an 'element' of intelligence. True AI advocates would quickly point out that this is not really artificial intelligence, because true AI requires complete mechanisms of thought. This would be considered, 'somewhat intelligent.' Regardless of the 'level' of intelligence, the use of these technologies has introduced a level of sophistication beyond anything we had in the past. In the world of counterfeit deterrence, one must look at 'smart technologies,' but also be smart. Today, there is not a lot of 'smart' packaging in the market, though it is evolving. Use of human intelligence to make decisions about practical and economical ways to preserve and protect your IP is crucial. The use of modern technology can enhance the level of sophistication. Every program/product must be evaluated on its own merits. There will be different solutions that are 'best fit' depending on the given circumstances of the product, the method(s) of manufacturing and the distribution channels. There is no single solution when it comes to protecting the authenticity of a product. The following paper will try to take an honest and practical look at the current state-of-the-art technologies in the marketplace, their strengths, drawbacks and general costs.

Webcast of this presentation available at http://bit.ly/16thMerchant.

Introduction

The International Chamber of Commerce (ICC) estimated that the value of counterfeit goods globally would exceed $1.7 trillion by 2015 (Hargraves 2012). That is approximately 2% of the total world's economic output and larger than many countries' gross national product (GNP). This is having serious impact on companies' profits, jobs, goodwill and governments' ability to collect taxes. Between October of 2005 and September of 2006, Homeland Security in the USA made 14,000 seizures of counterfeit products worth a total of $155 million. In New York City alone, the trade was worth $80 billion and cost an estimated $1 billion in lost sales tax revenue (The International Herald Tribune 2007).

Existing markets vary for several reasons, but two of the most important are culture and financial social status. We have seen the emergence of a new middle class in Asia, making this an ideal time to implement a smart packaging strategy. In China for example, consumers interact with products in a very different way than the Western world. The use of WeChat (a free instant messaging service in China) is very widespread and a consumer in China is much more likely to scan a QR code than one in the Western world. There is also a cultural aspect to this. It is well known that more counterfeiting comes out of China than any other country. In the Western world, people are brought up to believe and trust in products. In China and most parts of Asia, the mentality is the opposite. Supply chains are infiltrated with counterfeit goods and the level of 'smart' in the packaging for the most part does not exist. Let's take a look more specifically at the wine industry.

Wine has become increasingly popular in China. It is a real growth industry and recently a 70-million-euro winery was constructed in the Ningxia province. The building of Chateau Changyu Moser along with other significant investments by LVMH (owners of Lafite Rothschild) to produce wine in China is a clear indication of the potential for the wine market in China. But in a fashion so common in recent times, these market growth opportunities also come with challenges of how to protect the investments. China is a very large country and in recent times has become a very wealthy country. It is not much of a surprise to think about the wealthy Chinese and how they want to enjoy the best things life has to offer and even to a degree brag about their wealth. The ability to 'connect' with the Western world is thought of as having obtained higher social status. Western wine drinking has become a key part of this. But with that comes another challenge, the Chinese are very 'fickle' in their choices of wine. They will switch from one brand to another showing no real brand loyalty. Online shopping allows them to source and purchase budget wines. Internet purchases are increasingly common and contribute to the ease of 'attracting' unscrupulous businesses that are able to offer very attractive deals such as low prices, fast deliveries and other advantages that seem too good to pass up. Often they are no more than cases of fraud and sales of counterfeit wines.

Doing business in China – like all cross-border commercial activities, carries certain risks that Australian companies might not be aware of. These include:

- commercial fraud
- breaches of contract
- intellectual property infringement and theft
- bullying, intimidation and threats to physical safety
- restrictions on movement
- criminal charges for engaging in activities that may not constitute crimes under Australian law.

Australian companies should spend time investigating the market, obtain professional advice and conduct thorough due diligence before establishing business relationships (Australian Trade and Investment Commission 2016).

In 2012, China imported approximately 68 million gallons of wine worth over $1 billion dollars! This placed China as the fifth largest importer of wine in the world, ahead of the United Kingdom and many other large wine drinking nations (The Wine Cellar Insider 2013).

Wine exports to China have increased more than tenfold since 2006 as rapidly increasing wealth transforms lives and tastes in the world's fastest growing major economy. More than half of the 2012 total – 139.5 million litres – came from France. The iconic Chateau Lafite has become the 'poster child' for wine forgery. A bottle of Lafite from 1982, considered one of the greatest vintages of the 20th century, can cost upwards of US $10,000. That has led to a thriving industry in Lafite knock-offs in China. Aficionados say there are more cases of 1982 Lafite in China than were actually produced by the chateau that year. Christophe Salin, president of Domaines Barons de Rothschild, which owns Chateau Lafite Rothschild, says fake Lafite however isn't the major problem:

> 'I have never seen a bottle of fake '82 Lafite,' says Salin, who has been travelling to China for 20 years. 'The problem we have is the creative attitude of some Chinese. They sometimes use our name in funny ways,' he told Reuters in a telephone call from Paris.

Nobody knows how much of the market is cornered by fakes and copycats, says Jim Boyce, who follows China's wine industry on his blog, grapewallofchina.com (Boyce 2016). Several wines on the market are branded with names close to Chateau Lafite, including 'Chatelet Lafite'. Chatelet is the name of one of the busiest subway stations in Paris. Lafite 'is such a generic brand in China that it has widespread appeal as a name and as a status symbol,' says Boyce. The mystique extends beyond the wine -- in Beijing there is a 'La Fite British Exotic Bar' and the 'Beijing Lafitte Chateau Hotel.' The first step for anyone counterfeiting wine is to find or manufacture a bottle that is close to the original. 'People will also use real bottles with something else inside, or make labels that are spelled differently,' says Cheng Qianrui, wine editor for the Chinese lifestyle website Daily Vitamin. 'If you know wines, you can tell, but not a lot of Chinese do.'

The copyright problems, however, tend to focus on the better-known marques. Importer Torres Wines includes Chateau Mouton Rothschild, another top-ranked Bordeaux, in its portfolio. Sales Director Sun Yu says phoney wine brands such as 'Mouton & Sons' or 'Edouard Mouton' pop up in the Chinese market. 'It happens in secondary or third-tier cities where they don't have much wine knowledge,' Sun says.

Elite winemakers are trying to fight back, sometimes by smashing bottles after tastings, to prevent their being refilled for resale. Anti-counterfeiting measures by major international spirits brands, which also fall victim to fakes in China, include bottle buyback programs, tamper-proof caps and covert tagging of bottles. But such measures are less common with wine brands, according to an executive at an international beverage company in China. Domaines Barons de Rothschild has been putting tamper-proof tags on bottles of Chateau Lafite and its second label, Les Carruades de Lafite, since the 2009 vintage. But the producer has been protecting its elite bottles since 1996, company president Salin says, with four other identification techniques that he will not reveal. 'If you show me a bottle of Lafite, I can instantly tell you when it was bottled, a lot of things,' he says. 'To counterfeit it is not easy.' (Jones 2013).

At least half the Chateau Lafite sold in China is fake and, like other high-end Bordeaux counterfeits, probably made on boats moored in international waters off the mainland coast, a senior Chinese government official has said. Over the last few years, China has become the biggest market for Bordeaux wines, accounting for almost of 20% of the area's wine exports by volume in 2013. Although Li said he had no idea how many boats were used as faking stations, he described their existence as one of the most shocking aspects of the counterfeiting sector. Their modus operandi is to use low-end wine to make high-end fakes for exorbitant profit, he said. (Kevany 2014).

In July of 2013 an investigation led to the arrest and confiscation of over $32 million dollars of fake wine by the Yantai police. In dollar value, this could be the largest bust of its kind in China. Some of the wines being counterfeited were Chateau Lafite Rothschild, Chateau Latour, Chateau Mouton Rothschild, Chateau Beychevelle, Chateau Pichon Baron, and other famous brands. A small French brand, Rafi was also being counterfeited by the same team of suspects as well. This investigation and arrest follows a July, 2013 meeting in Beijing with representatives from the EU where an agreement was produced to increase efforts to bring down the rampant amount of wine counterfeiters operating in China.

In the raid, police found the supplies needed to make counterfeit wines, rolls of labels, bottles, corks, etc. At least 10 suspects were arrested. More than 40,000 bottles of fake wine were seized in the raid. The suspects were reportedly buying inexpensive wine and placing it into bottles of the famous and expensive, counterfeit brands they were selling. The suspects are thought to have been selling the wines in various popular Chinese cities such as Shanghai, Beijing, Tianjin, Shanghai, Guangzhou, Qinhuangdao, Dongguan, Shenzhen, Suzhou and Nanchang.

This was not the first time a ring of this size was discovered. Previously in 2012, an operation discovered counterfeit wine totalling $1.6 million including 350 cases of fake Chateau Lafite Rothschild and 60 cases of fake Chateau Margaux along with others. The exposure to the opportunities that come with a growing market make wine a natural target (The Wine Cellar Insider 2013).

Authentication technology in the market today

Technology continues to evolve. 'Outside influencing' technologies are also having a major impact on the market. An example of this is modern cell phone or as it is often referred to, the 'smart phone.' The smart phone has taken on so many forms of functionality and is having a major influence in many areas, including authenticity. We all have experienced a major change in our lives from advancements in cellular technology and no doubt, this will continue for years to come. There are several companies developing technology that uses the smart phone to perform an aspect of authentication for brand protection. YPB is in the process of developing and commercialising a nanotechnology that will become a benchmark of future smart phone authentication. The technologies today that would form a part of a smart solution would fall into one or more of the following categories:

- Conventional design with or without security graphics
- Manufacturing technologies
- Cloud/server-based technologies
- Pattern recognition technologies
- Serialisation technologies
- Electronic chip technologies.

Conventional design with or without security graphics

For purposes of clarity, conventional design is defined here as the design elements that one would normally see in packaging. These are the least expensive of the design elements in most cases and include graphics that are created to attract the consumer. They are carefully selected and placed into a design to market the brand, to portray good aesthetics, attract the senses and give harmony to the product and instant recognition to the brand. Brands will often have guidelines on how the elements are to be used and, for example, what PMS (Pantone Matching System) inks are to be used. These may be referred to as the 'corporate colours' so that any package stays consistent wherever it might be printed. An example of a design guideline is shown in Figure 1.

These type elements would normally be considered conventional design elements, not security elements, however even with conventional graphics there are techniques that can be used to add security to conventional design elements. This will be discussed in the section covering forensics of design. Security elements can be incorporated

Figure 1. Example of a design guideline for conventional design elements

Figure 2. Packaging from a pharmaceutical product from China that uses some fine guilloche patterns in a mix of colours to provide an authentication element

Figure 3. Packaging from a pharmaceutical product with elements of security design mixed with a manufacturing technology, a 'windowed thread.' This is a common feature found in many banknote papers.

into some designs. Adopting a design with forms of security graphics and/or materials generally increases costs by 10% over conventional materials and graphical designs (Figures 2 and 3).

Manufacturing technologies

Figures 4, 5 and 6 show examples of manufacturing technologies on a bottle of Moutai, a popular spirit. The front of the Moutai bottle features intaglio printing and a security label on the neck of the bottle. The security label uses a technology based on retro-reflective images. A handheld viewer is supplied by the manufacturer to authenticate the label. The intaglio printing process is considered to be the most secure printing process available and the choice of every central bank in the world for production of currency. In the case of intaglio printing, the design uses security elements from an engraving, not conventional process colours of cyan, magenta, yellow and black (CYMK) half-tone dots. The area of the picture is referred to as a vignette. It is very similar to a portrait engraving such as seen on most banknotes.

Inside the Chinese character, there are 'cross-hatched' lines. This is another technique that is often deployed in large areas that would otherwise be 'solid' if printed conventionally. The cross-hatching is a technique used when printing intaglio to keep the ink in the plate during the wiping process. Without these cross-hatched lines, there can be printing problems with voids created in the finished print due to the ink being wiped out of the image area prior to transferring onto the paper.

Figure 4. Images from the packaging of the product Moutai, a very popular white spirit alcoholic drink in China

Figure 5. Back label of Moutai bottle, showing a 'latent image' security feature

Figure 6. Enlargement of back label of Moutai bottle, showing more detail of the security elements present

Another distinguishing characteristic of this process is the 'relief' or tactility that can be felt in the label. Intaglio printing is a process whereby the paper is forced up into the printing plate and the ink is literally 'pulled out of the printing plate' leaving a relief image on the paper. This can easily be felt by running fingers across the surface because the surface is raised, not smooth.

Looking at the back label on the Moutai bottle (Figure 5), further use of intaglio printing can be seen, as well as a design element called a latent image. The 'MT' shown is only viewable when the label is tilted and held between the viewers' eyes and a light source. The 'raised surface' and the use of lineal elements running at oblique angles to one another allow some areas to reflect off the surface to the eye, while other areas reflect off the surface, but can't reach the eye due to the ink acting as a type of 'wall' or barrier to the incident light.

These design elements are security elements. Further details can be seen in the enlarged image in Figure 6: microtext, guilloches, line modulation and the formation of the latent image, as described above.

Figure 7 shows two more examples of intaglio printing on the labels of two French wines. In each case, the use of a fine vignette forms an integral part of the design element. The use of specialised printing technologies or holographic technologies adds cost to the labels or packaging. Volumes play a key part in pricing, but in general these specialty manufacturing processes add 10–15% to the cost.

Holography is another manufacturing technology that offers some authentication value in packaging, although it is also used to attract consumers' attention. It can be argued that holography is more valuable as a marketing technique than an anti-counterfeit deterrent.

Figure 7. Further examples of intaglio printing on French wine bottle labels

Figure 8. Examples of holography used in packaging

Figure 8 shows some examples of holography in packaging including the use of Fresnel lens technology, a rarer form of holography that has far more limited sources for manufacturing than conventional forms of holography.

Figure 9 shows a combination of manufacturing security technology and conventional design elements as was produced for Kodak in China. In the foil area, micro embossing has been used to create optical movement in the foil. The word 'Kodak' will change from a positive to a negative image as the box is tilted back and forth.

Foils such as that shown in the Kodak box make ideal points for adding covert tracer technology. The foil can be manufactured using technology that allows for the authentication of rare earth inclusions that are not visible with ultraviolet or infrared lights. The tracer materials can be authenticated with handheld readers. An additional product feature is the capability to integrate a brand logo and/or brand name in the foil that can only be authenticated in a forensic environment.

Cloud/server-based technologies

The technologies used in platforms that are based in the cloud or on servers are most often combined with either serialisation or pattern recognition. In cases of serialisation, a randomly generated number or a sequential number is produced and added to the product in some material form. It could be a label, ink jet printed directly onto the product/package or laser etched onto the product. The 'authentic numbers' are a known entity and stored in the cloud or on a server. The authentication process involves reading the number, normally with a smart phone and then sending it to the cloud/server for authentication. This is a very general and simplified explanation. Depending on the specific technology, the codes can be encrypted or require a custom reader or app (smart phone) in order to be scanned. These types of technologies are generally associated with a royalty cost and vary from company to company.

Figure 10 shows an example of a cloud/server-based serialisation matching technology. The QR code is scanned and sent to the cloud/

Figure 9. Combination of manufacturing security technology and conventional design elements on a Kodak package

Figure 10. Example of a cloud/server-based serialisation matching technology on a soft drink package

server and the product is authenticated by a previously known set of numbers. If the consumer does not have a QR code scanner application, an alternative is to enter the 12-digit string of numerals at the website of the technology provider.

Figure 11 shows further examples of this type of technology. The 2D barcode serves as a 'pointer' and sends the person to a link on either the cloud or a server where the number is retrieved and allows the user to authenticate it. These technologies may have custom apps associated with them or they may work with any standard 2D scanner.

The cloud/server-based technologies must also use an element of 'smart' in designing the authentication methodology. There have been cases of fake websites that take an authentication attempt to a site that will authenticate the counterfeit product.

Pattern recognition technologies

Another form of cloud/server technology is pattern recognition. Such technology can be cloud/server-based or self-contained for authentication directly from information on the product itself. For example, if a label contains a graphical element that is scanned, a read-out could be generated that matches a serial number in some other place on the label. There is no need to go to the cloud/server in this case. More advanced methods of cloud/server authentication involve scanning on a production line, capturing images, reducing file sizes and sending them to the cloud/server for future authentication using pattern 'matching' or recognition.

Figure 12 shows an example of this type of technology. The label on the neck of the bottle contains a pattern of random 'bubbles' that has been uploaded to a server or the cloud. The 2D barcode is scanned by the consumer and it brings up the image of the bubbles. The consumer is then required to look at the label on the bottle and compare it with the picture on the screen of his phone or computer to manually make the authentication.

Figure 13 shows another form of pattern recognition technology, but this one is a bit more sophisticated and automated. The graphics going around the outside of the label actually form a custom font. This technology requires production line scanning. The image is captured as a fractal image meaning the font, 2D code and every other graphical element is being captured in a 3D frame grab. The images are captured along with the fibre pattern that is in the paper or substrate underneath the image. At the point of authentication, an app is used on a smart phone to capture and send the image to the cloud for an automatic pattern match with the image captured at the point of manufacture.

Figure 11. Further examples of serialisation matching technology

Figure 12. Example of pattern recognition technology on a label on the neck of a wine bottle

Figure 13. A more sophisticated example of pattern recognition technology

Serialisation technologies

Serialisation technologies are monitoring tools in the form of numbers or a code system that is widely used to indicate and identify the product. An example of serialisation is the lot number and serial number frequently found in pharmaceutical products. They are mostly found on the packaging, making them very useful for supply chain security. The tracking and traceability of the product and packaging can be done from the manufacturing process until the products are in the end user's hand.

In efforts to minimise the risk of counterfeiting and adulterated drugs, many national regulators have developed serialisation methods for supply chain security. A complete serialisation program represents the history of a given product, much like a chain of custody would provide a complete accountability for evidence. Serialisation allows a manufacturer to track products from manufacturing through to the final product dispensing.

Pharmaceutical manufacturers have invested in ways to uniquely serialise each unit and to register relationships such as parent/child units into larger packages, cases, cartons and even up to pallet level. Serialisation information is very dependent on different national laws and standards. There are many programs already being implemented in Europe and the United States as the pharmaceutical industry is now coming under legal deadlines to control the complete supply chain.

There are two types of serialisation, random and sequential code (Figure 14).

- Random code uses randomised numbers. One of its applications is protected identification (limited access code) because it is unpredictable.
- Sequential code is an ordered running number. It is used for unprotected identification.

Serialised numbers and the other data can be encoded into a barcode. There are many systems and barcode technologies (symbologies) presently available that will facilitate track and trace systems including the benefit of brand identity. Serialisation can be the intermediary between the brand owner and their customers.

Figure 14. Two types of serialisation – random and sequential codes. Credit: Verify Brand

Types of barcodes

- 1D barcode (linear code)
  This is a first generation symbology that is made up of lines and spaces of various widths that create specific patterns. These codes are defined by standards that are in the public domain (Figure 15).
- 2D code (matrix)
  There are two-dimensional symbologies that represent information and contain greater amounts of data than one-dimensional symbologies per unit area (Figure 16).

Barcode technologies are constantly evolving. They are being developed to have more capacity and contain more data. In terms of anti-counterfeiting solutions, they are normally used in combination with other authentication solutions as a part of a track and trace system for supply chain security providing a much stronger solution.

Electronic chip technologies

One of the increasingly interesting areas in chip technology is near frequency communication (NFC) chips. These proximity chips evolved from radio frequency identification (RFID) chips. The technology is really quite simple. The NFC chip operates as a part of a wireless link. Once it is activated by another chip, small amounts of data can be shared between the two devices. They do not have to be in contact with each other, but they do have to be within a few centimetres of each other. In this manner, technology is enabled in close proximity and one can communicate without the need to have an internet connection. It is very easy and fast to use. No pairing code is necessary to link up and because it uses chips that run on very low amounts of power it is much more power-efficient than other wireless communication technologies. NFC identifies people by their enabled cards and devices as well as their bank accounts and other personal data. The cost associated with high volumes of perhaps a million or more chips such as NFC will add cost in the range of US $0.07 - 0.09 per label or package. While this may be viewed as expensive, the added applications and benefits that can be derived are numerous.

Virtually every mobile operating system maker has their own apps that offer unique NFC functionality. Android users have the widest variety to choose from, with examples including Google Wallet, which accesses funds for contactless payments and Samsung Pay, which operates similarly.

Apple's iPhone 6 and iPhone 6 Plus received NFC functionality, though with limited use thus far, only for Apple Pay. This is similar to Google Wallet, in that it is an app which gives users the ability to pay for goods and services at participating retailers. For people who prefer Microsoft's Windows Phone, they will be able to use Microsoft Payments.

Passive NFC 'tags' are being built into posters and informational kiosks to transmit additional information, similar to the way scanning a QR code can launch a web address, offering a discount coupon, or a map to download to a smart phone. A clever use of NFC is combining it with packaging to engage the consumer and gather valuable market information about the customer base. YPB's platform including 'nTouch', with consumer engagement and many other functionalities such as track and trace is leading the way in this market segment.

Since NFC occurs in the free and open air, one can easily be concerned about the security aspect of data. It is easy to get caught up in the idea that your data can be stolen or 'skimmed' by anyone who tries to intercept it. Technically it can happen, but it is not difficult to prevent and with a bit of effort, one can eliminate the chances of being skimmed. First, you must consider that the NFC chips in your cards or phones can't be skimmed unless they are within mere centimetres of a would-be thief. There are those that are out there and may try to use a skimming app to capture your personal information, including your address and account details. But this is getting more and more difficult, as apps like Apple Pay and Google Wallet are implementing some clever safeguards to protect users.

According to information from Apple, Apple Pay stores payment information on only the device, encrypting the card information only for use by the merchant and payment network for verification. The information is not in the cloud, nor does it reside in the iOS source code. It is possible to erase financial transaction information manually using the 'Find my iPhone' feature.

Google uses SSL (secure socket layer) technology to protect financial information on Google Wallet. They recommend a very practical approach in making sure your details stay safe: the use of a passcode on the phone. Additionally, the NFC antennae in Android phones is only activated when the screen is both on and unlocked. People using a credit or debit card with NFC capabilities should protect themselves by getting an anti-skimming sleeve to go over the card. These deflect radio frequencies from attracting the card's NFC chip.

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Figure 15. Examples of 1D barcodes

QRcode (general used)

Datamatrix (industry)

Figure 16. Examples of 2D barcodes

Using forensics as a key element in smart packaging

Part of any 'smart packaging' effort should include features that are designed intentionally to be authenticated in a forensic environment. It is not difficult to incorporate elements of forensics that are totally covert to the consumer and do not pose any problem for the marketing teams. These features can become key in legal proceedings or final determination on whether a product has been counterfeited. Designing with forensic elements serves as a back-up should internet connections be unavailable. One 'creates' the ability to authenticate product when necessary using handheld instruments in the field or laboratory instruments.

An example of ways in which forensics might be added to product packaging without any impact on the design of the brand image is shown in Figure 17, which shows a current version of Diageo's Johnny Walker Blue Label.

This package features a tracer technology that can be used in almost any material. In this case, the gold ink and the gold capsule on the bottle has had a tracer added to it. The tracer is detectable by a handheld reader (Figure 18).

A further 'level' of sophistication can also be added. A 'second level' covert feature is detectable with a handheld reader, but it does not detect the presence of an image. This is referred to as a 'third level' feature. The Johnny Walker logo is manufactured covertly in the foil. This feature can be detected by a laboratory device using a mouse connected to a videoscope and/or a spectrophotometer using wide field infrared illumination.

The design feature shown in Figure 19 is a very effective forensic feature. It is based on the use of IR matched pairs or IR matched inks. Visibly, there is no difference in the appearance of the label, but under IR examination, the area of the 'JW' drops out providing clear forensic evidence of the genuine product.

Figure 20 shows a 2D barcode with serialisation which is used to make an authentication via the cloud or a server. This could also serve as a starting point for a track and trace platform which provides many benefits for the brand owner such as marketing, geo-location, and consumer engagement. For the consumer, it is authentication and a link to the brand website where other benefits may be derived.

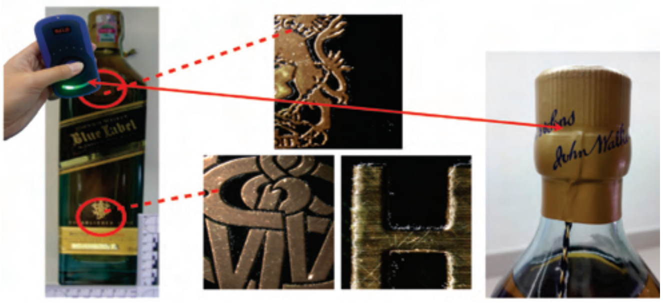

Figure 17. Example of tracer technology used in the packaging of Johnny Walker Blue Label Whisky

Figure 18. Use of a handheld reader to detect a tracer material present in the packaging and a third level security feature of an image in the foil that is only detectable in a laboratory

Designing and implementing true smart packaging

Smart packaging today is much more than just being 'wise' about choices. Today, with the use of the smart phone, there are many additional ways to protect and monitor your products. Track and trace, temperature and exposure to climate changes, authentication of various physical security features, chip (NFC) authentication and consumer engagement. The ability to gather information from your customer base brings an added dimension to smart packaging.

If brand owners wish to preserve authenticity and protect themselves from would-be counterfeiters, the use of several of the techniques discussed in this paper are highly advised. With the conventional means of protecting product, which forms one of the three pillars of 'smart packaging' (protect), the brand owner establishes the means to authenticate. In many countries, the USA included, if a brand owner has not taken the initial step to add some 'protection' technology, the ability to defend a brand in a court of law can be seriously diminished. Another aspect of the conventional means of protecting your product is the ability for 'back-up.' What is meant by this term is the use of security technology in the materials so that authentication can be made in the event a computer chip is damaged or the cloud/server cannot be accessed. This can happen and if a brand is putting all their 'eggs in one basket' so to speak, one could easily find product that cannot be authenticated simply because a chip cannot be read or the internet is down. This is the reason we will never see an ID card or passport issued with just a chip. It might be secure enough in an ideal environment, and contain encryption but electronics can be damaged and can fail. There needs to be a secondary or back-up method for authentication.

Figure 19. 'JW' image only visible under infrared (IR) illumination

Figure 20. A 2D barcode on the neck of the bottle

Moving to the second pillar of 'smart packaging,' (detect) the brand owner is provided with the tools to authenticate the products. The detection of tracers, security features and manufacturing technologies are all key in building a sound deterrence against counterfeiters. With the advancements made in the cellular telephone and continued evolutions of the smart phone, this will become a key part of the second pillar in the future. Forms of it are already happening in the industry.

The third pillar of a sound 'smart packaging' strategy is the consumer engagement (connect). There is added value and benefit to engaging the customer. The ability to understand customers, their needs and satisfaction levels is going to help a brand's business to grow. It is not just the prevention or detection of counterfeit product alone, but the 'connect' becomes a key part of a brand manager or senior management's ability to make sound business decisions about where to invest and what products to bring to the consumers. In addition, if the occasion arises where there are complaints, it gives firsthand information and feedback on quality issues so they may be addressed quickly. The consumer ends up with a very positive experience and the brand image grows as they share their satisfaction on the many social networks in use today.

Choosing a company with a diverse technology portfolio including a range of services and technology covering all the key elements of brand security and consumer engagement is key to preserving authenticity and protecting the brand.

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Grape berry counting based on automated 3D bunch reconstruction from a single image

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Abstract

Developing accurate yield estimation methods for vineyards has become an emerging research topic in Australia. Estimating the yield prior to harvesting aims at reducing waste and increasing profit margins for growers and wineries. The traditional approach of yield estimation involves manually counting the number of berries in sampled bunches, which proves to be a tedious and time-consuming task. More recent methods attempt to count berries via stereo imagery, which requires high quality cameras assisted by lighting rigs in order to capture the finer details of a bunch. However, the overall set-up for this method proves to be bulky and thus impractical for field applications. This paper presents a novel lightweight method for berry counting that involves constructing a 3D model of a bunch based on a single image. The proposed method produces an accuracy of 87.6% on Shiraz and Cabernet Sauvignon crossing multiple cultivars. Furthermore, the proposed method was extended to achieve an accuracy of 84.5% in counting berries on Chardonnay (green). An Android app developed based on this reconstruction method allows customers in the field to get real-time results by simply capturing a photo of a bunch with a backing board.

Webcast of this presentation available at http://bit.ly/16thSLiu.

Introduction

Yield estimation in viticulture is notorious for producing poor estimates due to a range of sampling factors and dependency on subjective interpretation of the state of vine maturity. This poor estimation costs hundreds of millions of dollars each year in contract adjustments, harvest logistic management, oak barrel purchases and tank space allocation amongst others. The structure of vineyards means aerial imagery is only able to contribute a small amount to the yield estimation, and other on-ground estimation methods are time-consuming. Recent work by Nuske et al. (2012) in the US has shown the potential for image processing to speed up this analysis as well as generate unbiased estimates which are orders of magnitude smaller than manual estimates, leading to substantial cost savings.

As to traditional yield estimation in vineyards, berry number is a critical parameter for early forecasting production since the number of berries remains stable after fruit setting (Martin et al. 2003). Also the ratio between berry number per bunch and bunch size is one of many factors governing the quality of the fruit at harvest. At current vineyards, berry counting is accomplished by hand, which is work intensive and time-consuming. Liu et al. (2013), Diago et al. (2014) and Ivorra et al. (2015) demonstrated the advantages of image processing on yield components analysis for the sake of saving time and energy for grape production forecasting. Grossetete et al. (2012) and Diago et al. (2014) applied image processing techniques for berry counting one side of a bunch, achieving average R^2 value of 0.92 and 0.82 between actual berries and detected berries per bunch. However, the image processing algorithm proposed in Grossetete et al. (2012) cannot be utilised after veraison since the reflection on berry skin is affected by pruine (which causes matte surface on berries on both green and purple grapes). As presented by Diago et al. (2014), a data set with 70 bunches from 7 varieties was tested, with an R^2 value varying from 0.62 to 0.95 based on 10 bunches for each variety (0.817 for 7 cultivars on average). Leaving the image techniques described by the author aside, 10 bunches is not representative for validating an image processing procedure in one cultivar. Especially for Cabernet Sauvignon as well as Shiraz which are known for their non-uniform bunch shape, Diago et al. (2014) obtained the lowest R^2 value with 0.62 based on a single image of Cabernet Sauvignon from a total of 7 cultivars tested.

Other than determination of berry number by processing a single image from one side of a bunch, other work has shown the advantages of performing 3D reconstruction of grape bunches for the purpose of estimating the number of berries in a bunch by stereo images (Ivorra et al. 2015; Herrero-Huerta et al. 2015). Their accuracy improved to an R^2 value of 0.78 compared to more traditional 2D estimation techniques by Liu et al. (2013) which have been a standard for the image processing community (Chamelat et al. 2006; Reis et al. 2012). Their 3D reconstruction relies on substantial manual input (semi-automatic) for each bunch, which is tedious even given an impressive user interface and thus cannot be applied on a large scale for reliable yield estimation. As to the scope of these experiments, data sets in Ivorra et al. (2015) and Herrero-Huerta et al. (2015) are small, 10 bunches from one cultivar (10 cultivars) and 20 bunches from 14 vines in one block, respectively. Also the R^2 achieved in both papers are 0.71 and 0.78, which is not satisfactory for practical implementation in current vineyards. In addition, a specialised stereo camera arrangement was required, along with controlled lighting conditions, limiting the applicability to *ex vivo* analysis. Stereo cameras also have a minimum range which restricts the level of detail which may be achieved by moving closer; therefore in-field application within the confines of a sprawling canopy is impractical.

In order to increase the precision of these image processing methods, lower cost and simpler solutions are needed that can be applied by growers on the ground. Thus, the objective of this paper is to produce a representative 3D reconstruction of grape bunches from a single image for the purpose of accurate berry counting. The use of only a single image is a key feature, which simplifies the data capture process and keeps the cost manageable, to the point where cameras such as those contained in current smart phones can be used. This feature is particularly attractive to growers in difficult economic circumstances.

Methodology

Two major image processing components form the basis of the berry counting method. Firstly, a 3D reconstruction of the bunch is produced to give an initial estimate of the number of berries. A sparsity factor is then calculated from the colour of the berries and used to generate a final estimate of the number of berries. This paper is based on three assumptions:

1. The actual number of berries in a bunch is equal to the number of berries that fit in a volumetric shell derived from a single image of a bunch.

2. All sizes of invisible berries follow the normal distribution of sizes of visible berries on the same bunch/sub-bunch.
3. The sparsity factor has an effect on estimating the number of berries per bunch.

3D reconstruction and initial estimate of berry number

Given an image of a single bunch of grapes, the outline of the bunch is extracted from R-channel by Otsu (1975). The image is rotated until its major axis is approximately vertical. Each point on the outline is considered a candidate berry location to which a circle is fitted using a Hough transform, as demonstrated in Figure 1. These circle locations and diameters are used to seed the 3D model by placing spheres of corresponding diameter in a single plane normal to the direction of view. For addressing overlapping of berries at the edge of a bunch, 'neighbour searching' within specific distance is applied for finding two berries that have extreme metrics within this distance. Then the berry with largest metric is moved forward in z-direction (normal to the paper plane) pixel by pixel while the berry with smallest metric is moved backward until there is no overlapping. Beginning at the top detected berry, the 3D model is populated using the following process until the bottom of the bunch is reached:

1. Find the first and last pixel of a horizontal section through the image and subtract the diameter of one berry.
2. Revolve this section about a vertical axis through its centre, forming a virtual circle.
3. Randomly pick a sphere diameter within the observed range of berry diameters.
4. Moving around the circumference of the virtual circle, attempt to place a new sphere at regular (one degree) intervals.
5. At each interval, place a sphere at that location on the circumference only if no intersection with any existing sphere is detected.
6. Move down a defined step size (in this paper, step size is two pixels) and repeat. Once the model is fully populated, the number of berries is tallied and denoted as Initial Berry Number (IBN).

As to step 3) above, Hough transformation is applied on an image of a bunch to detect all visible berries and a normal distribution model is built based on all radius of detected berries. Then in aforementioned step 3), a radius is randomly generated by the built normal distribution. Assumptions 1) and 2) are embedded here. Figure 2 illustrates examples of single images and the corresponding shaded 3D reconstructions.

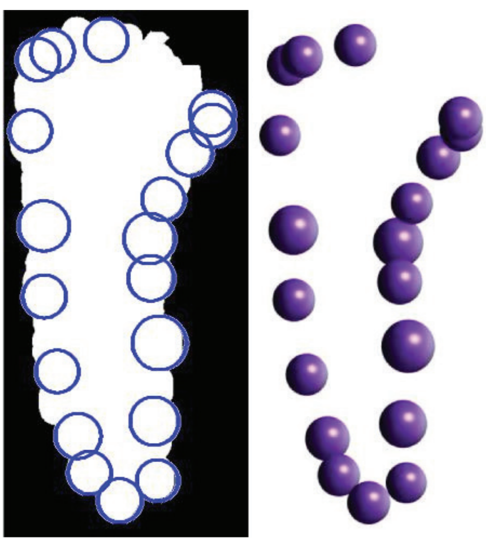

Figure 1. Berries as seeds on the edge of a bunch

Sparsity factor

Assumption 1) refers to a convex hull for a healthy and compact grape bunch. However, there are not always compact bunches so that IBN is not accurate enough for a bunch with a looser pattern by applying previously mentioned image processing techniques. Hence in this paper, a sparsity factor (SF) is proposed for defining the compactness of a bunch. This indicator will be used for final estimation of berry number. Assumption 3) is embedded here. In order to get SF for each bunch, each image was processed according to the following sequence of operations:

1. Automatically crop image to the outline of the bunch as detected above.
2. Automatically threshold the red channel to obtain a binary image using Otsu's method.
3. Automatically threshold the saturation (from HSV) channel to obtain a binary image using Otsu's method.
4. Calculate the area of each of these two threshold images, giving AR and AS, as shown in Figure 3.
5. Calculate the sparsity factor according to: $SF = (AR - AS)/AR$

In addition to aiding the estimation process, the sparsity factor, along with the bunch volume can be identified as important indicators of various berry-related diseases.

Final estimate of berry number

The sparsity factor is then used to improve the estimation of the number of berries through the following formula:

$$BN = SF \times IBN \quad (1)$$

where BN is the final estimate of the number of berries.

Development of the mobile application

Next, a mobile application was developed using the proposed 3D berry reconstruction theory. The application allows any customer in the field to get real-time berry estimations by simply capturing a photo of a bunch in front of a backing board. The app consists of a

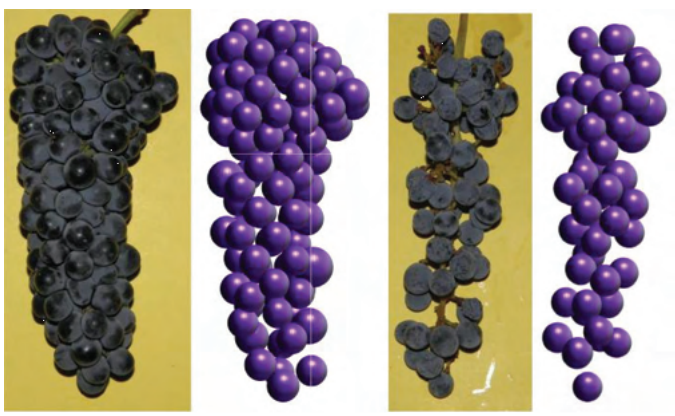

Figure 2. 3D reconstruction by a single image

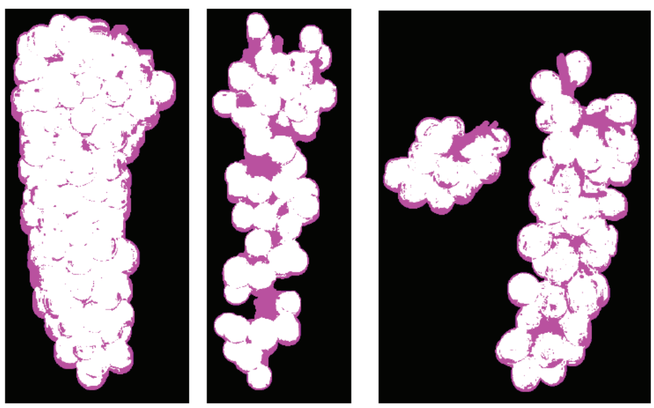

Figure 3. Sparsity factor calculation

simple interface where the user can simply load an image captured and estimate the corresponding berry number. In addition, the app provides the facility of saving the processed images for later analysis. The final outputs of the app are the berry number and the sparsity factor.

Android was chosen as the target platform to run the app. The existing MATLAB Code was converted to Java and C, languages which are compatible with Android. Libraries and a tool such as OpenCV and MATLAB Coder were used in the process. Figure 4 depicts the different stages of the berry estimation process in the Android berry counting app. The completed app was tested on an LG G3 smartphone, which was able to execute the berry estimation task in 9–14 seconds, depending on the bunch size.

Results

Data were collected by viticulturists at Treasury Wine Estates, Camatta Hills, California in September and October 2013. Photographs were taken of a total of 112 individual bunches randomly comprised of two red wine-grape varieties, Shiraz and Cabernet Sauvignon.

Images were captured at a resolution of 3968 × 2976 pixels using a consumer-grade compact camera (Olympus SP600UZ) on automatic mode with the flash turned on. These images were then processed using MATLAB according to the method outlined earlier. Firstly, a 3D reconstruction of each bunch was generated from a single image of that bunch, providing an initial estimate of the number of berries. Secondly, the sparsity factor for each bunch was calculated and applied to the initial estimate to obtain a final estimate of the number of berries.

Each bunch was then de-constructed, with manual counts of the number of berries on each bunch being recorded. In addition, the diameters of a small number of berries on each bunch were measured. The number of berries was compared with the final estimate from the proposed method, and the following metrics calculated:

Average absolute error: taking the absolute values of the differences between the actual and estimated number of berries divided by the actual number of berries and then averaging these differences over all bunches.

Accuracy: one minus average absolute error.

Average error: taking the values of the differences between the actual and estimated number of berries divided by the actual number of berries and then averaging these differences over all bunches.

R^2 value: based on a linear correlation between the actual and estimated number of berries.

Figure 5 represents the relationship between the actual berry number and the initially estimated berry number without the sparsity factor. Results are presented for both purple (Shiraz) and green (Chardonnay) bunches. An absolute average error of 23.1% was observed for Shiraz and 30.49% for Chardonnay. The corresponding R^2 values for the two grape types were 0.63 and 0.67 respectively.

Figure 6 shows the relationship between the actual and finally estimated berry number for both Shiraz and Chardonnay with the sparsity factor. The sparsity factor ranged from 0.32 to 0.89. On the data set of 112 images for purple bunches described above, an average absolute value error of 12.4% (i.e. accuracy of 87.6%) was achieved. Following the application of the sparsity factor, a final R^2 value of 0.85 was achieved for all purple bunches. Similarly, as shown in Figure 6, the berry estimation process with the sparsity factor for green bunches returned an accuracy level of 84.5% with an R^2 value of 0.78 for a sample data set of 45 bunches. The processing time was approximately 0.5 seconds per image, prior to any optimisation. The proposed method fits berries to the outer profile of the bunch, which matches in-field observations to the structure of real grape bunches and produces good models. It is notable that larger bunches induced larger errors in the method, most likely due to a larger number of interior berries.

The proposed method was then applied to different data sets from Orange, NSW, Clare Valley, SA and the Treasury Wine Estates database to evaluate the robustness of the 3D reconstruction and sparsity factor method. The results are presented in Figure 7. The linear fit for each data set lies very close to each other suggesting that

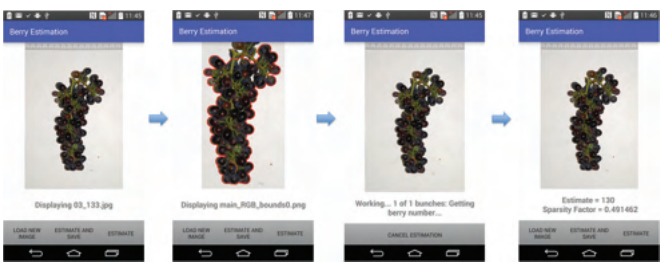

Figure 4. Different stages of the berry estimation process in the Android berry counting app

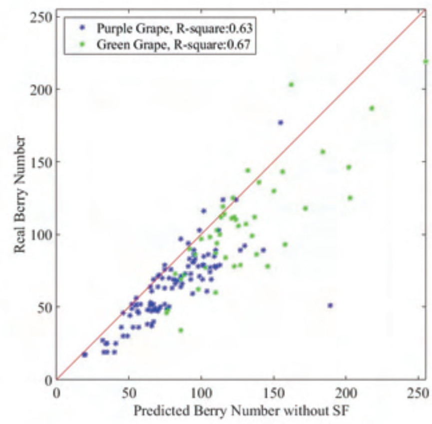

Figure 5. Initial berry number estimations for purple (Shiraz) and green (Chardonnay) bunches without the sparsity factor. The two data sets returned average absolute errors of 23.1% and 30.49% respectively.

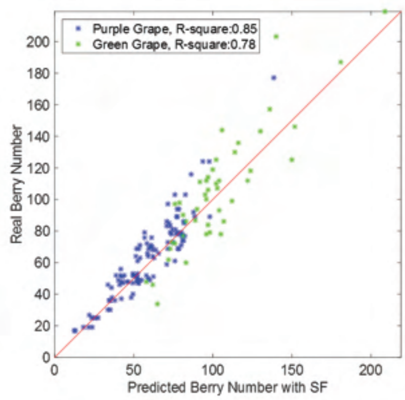

Figure 6. Final berry number estimations for purple (Shiraz) and green (Chardonnay) bunches with the sparsity factor. The two data sets returned average absolute errors of 12.4% and 15.53% respectively.

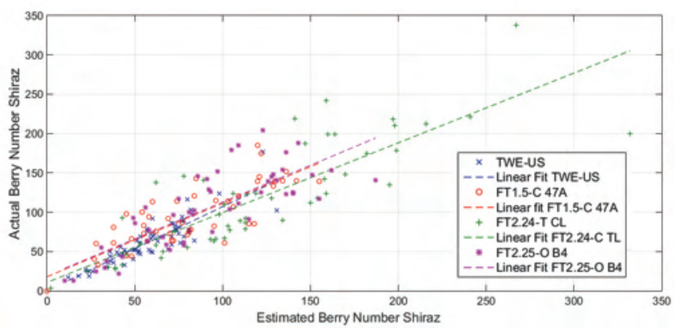

Figure 7. Linear fitting between real berry number and estimated berry number per bunch, across different maturity stages and cultivars.

the proposed method is a generalised approach for estimating the berry number in an accurate manner. Thus, the proposed 3D reconstruction methodology can be applied to estimate the berry number in different vineyards without requiring any major modifications.

Next, the accuracy of the 3D reconstruction method was tested on the green bunches at different maturity levels. The results are shown in Figure 8. The absolute weighted average error from lag stage to mature stage for the green bunches is within 20%. Although the proposed method returns a weighted average error of 18.2% at the lag stage, the error values gradually decrease to 10–12% as the bunches reach more mature stages. The ability of the proposed method to accurately estimate the berry number in the early stages of maturity allows the user to predict the overall berry yield well before the harvesting date.

A comparison of the proposed method with three berry counting methods is demonstrated in Table 1. In terms of processing type, the proposed method in Diago et al. (2014) requires calibrating the relationship between visible and invisible berries in a testing data set, while approaches presented in Ivorra et al. (2015) and Herrero-Huerta et al. (2015) need human interaction with software. Therefore, these three methods are not totally automatic while the proposed approach can immediately estimate berry number based on one image of a bunch. It is also important to note that the proposed method works accurately with purple (Shiraz and Cabernet Sauvignon) as well as green (Chardonnay) cultivars.

Conclusions

This paper has presented an improved method for estimating the 3D structure of grape bunches from a single image. Experiments on two varieties of red grapes showed an average absolute accuracy of 87.3% relative to the actual number of berries on a bunch. The method achieved an R^2 value of 0.85 using a linear relationship between the estimated and actual number of berries. The same method was then applied to green bunches, which returned an R^2 value of 0.78 with an accuracy level of 84.5%. These results were obtained with nothing more than a standard compact camera.

The proposed 3D model based on a single image also works on a winged bunch (Figure 9). However, it cannot achieve a good estimation of berry numbers on a bunch with overlapping shoulders. Comparison of the results with analysis of the same bunches as photographed *in vivo* is expected to demonstrate the viability of the method for reliable counting of berry numbers and in turn estimating block yield. Furthermore, more features are being developed for the current berry counting mobile application.

The processing time may also be improved by using a larger distance between horizontal sections. Some varieties of grapes elongate noticeably following veraison, and this method could be extended to fitting ellipses and reconstruction using corresponding ellipsoids. Furthermore, the 3D structure may be used for large scale analysis of the bunch structure, as it allows rapid estimation of many bunch parameters which are tedious to calculate via existing manual methods.

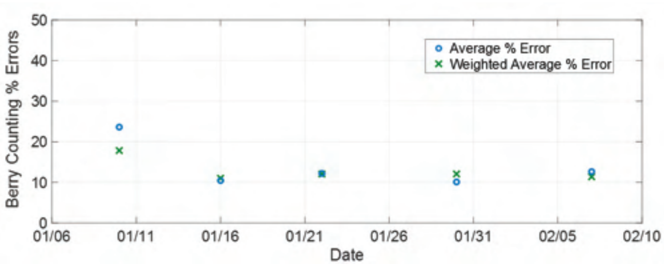

Figure 8. Average absolute error for Chardonnay over time with multiple vineyard visits

Table 1. Comparison of proposed method with other methods

Method	Cultivar	Data Set Size	Type	R^2
Diago et al. 2014	Cabernet Sauvignon (CS)	10 bunches	Semi-auto	0.62
Ivorra et al. 2015	10 cultivars	100 bunches	Semi-auto	0.71
Herrero-Huerta et al. 2015	Tempranillo	20 bunches	Semi-auto	0.78
Proposed method	CS and Shiraz	112 bunches	Automatic	0.85
	Chardonnay	45 bunches	Automatic	0.78

Figure 9. 3D model of a winged bunch

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Effect of machine harvesting with and without optical berry sorting on Pinot Noir grape and wine composition

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Abstract

Despite the high efficiency and economic advantages of machine harvesters, there is some resistance to their application for premium wines based on a belief that wines made from mechanically harvested grapes are inferior to those made from hand-harvested fruit. It has been proposed, however, that coupling mechanical harvesting with optical berry sorting technology may lead to wine that is as good as wine made from hand-harvested grapes. The objective of this study was to determine the possible synergistic effects, if any, of using machine harvesting in conjunction with optical berry sorting on Pinot Noir grape and wine composition. Pinot Noir grapes from the Russian River Valley AVA in California were harvested by hand, by a standard bow-rod mechanical harvester, and by a mechanical harvester with a Selectiv' Process on-board. For each harvest method, half of the grapes were unsorted and half were optically sorted at the winery. The grapes, wines at bottling, and wines after three months of bottle ageing were analysed by UV-Vis spectroscopy, RP-HPLC, and HS-SPME-GC-MS for colour expression and phenolic and aroma profiling. In some cases, such as anthocyanins in the grape samples and flavan-3-ols and tannins values in the wines, the different harvest treatments did result in significantly different values. In general, differences in wine composition that arose from harvest type were diminished or eliminated with the use of optical berry sorting. Descriptive sensory analysis conducted on wines three months after bottling resulted in only two significant differences among the 18 aroma, taste, and mouth-feel attributes evaluated. It was concluded that all treatments led to wines of similar character.

Webcast of this presentation available at http://bit.ly/16thOberholster.

Introduction

Mechanising the grape harvesting process for wine production has become increasingly important due to the ever-increasing cost and shortage of qualified labour and the desire to economise vineyard operations. Concerns associated with mechanical harvesting include: physical damage to the fruit resulting from the rapid shaking required to separate berries from rachis; the inclusion of undesirable second crop, overripe or mouldy clusters, and material other than grapes (MOG); the increased risk of oxidation, enzymatic activity, and the development of microbial populations in the broken and therefore vulnerable fruit during transport from vineyard to winery; and lastly the loss of juice in the vineyard. Only a few studies have investigated the impact of mechanical harvesting on grape and wine composition. Two studies that evaluated wines (Chardonnay, Petite Sirah, French Colombard and Chenin Blanc) made with machine- and hand-harvested grapes found that subjects had no preference between wines made from grapes harvested by the two methods (Noble et al. 1975; Clary et al. 1990).

Ideally, only fruit within desired parameters will be harvested while excluding MOG. Some of the new mechanical harvesters include an on-board picking head that eliminates pieces of rachis, leaves and shoots. However, these mechanisms are not capable of excluding mouldy or overripe berries. Typically, sorting is done at the winery to eliminate undesirable fruit. Hand sorting is slow and requires extensive resources to inspect individual berries. Optical sorters, however, are well suited for rapidly sorting destemmed grapes and their use has become more common in wineries. Sorting is based on a variety of parameters including berry size, colour and shape, while also eliminating foreign material. There is, however, very little research that investigates their impact on the chemical and sensory properties of wine. One study found that wines made from optically sorted Chardonnay grapes had higher total phenols, pH, and residual sugar than the unsorted control (Falconer et al. 2006) and sensory analysis showed that the wines were very similar in character with the wine made from optically sorted fruit having only more 'tropical fruit' aroma and sweetness (Falconer et al. 2006). In a much earlier study using older optical berry sorting technology, Carroll et al. (1978) sorted Muscadine grapes into four groups using absorbance parameters. Chemical analyses of the processed grapes showed that Brix level and pH increased with successive sorting (ripeness) levels, while titratable acidity decreased; pH and tannin levels increased and titratable acidity decreased in the wines made with successive sorting levels. Sensory analysis found that the wines made from the first and fourth sorting groups were inferior to those made from the two middle groups, which were deemed to have optimal ripeness. Although this study employed outdated technology, it shows that optical berry sorting can successfully segregate grapes by ripeness and can have chemical and sensory impacts on resulting must and wine.

There are, to our knowledge, no studies that investigate the impact of both mechanical harvesting and optical berry sorting on grape and wine composition. Although the limited amount of research available indicates that mechanical harvesting has a relatively small impact on wine, the general perception by the grape and wine industry is that mechanical harvesting negatively impacts quality. Additionally, the use of optical berry sorters is promoted to remove the potential impact of harvest method on grape composition. The aim of this study was twofold: one was to determine the impact of both mechanical harvesting and optical berry sorting on grape and wine composition; and the second was to determine whether grapes obtained by a combination of 'new age' mechanical harvesters with optical berry sorting are comparable to hand-picked grapes.

Grape harvest and processing

The grapes in this study were sourced from a commercial *Vitis vinifera* L. cv. Pinot Noir vineyard (clone Dijon 667 grafted on 1103 Paulsen rootstock) located in the Russian River Valley AVA, California during the 2014 harvest. The grapes were in good condition with no rot or mould visible. The harvest treatments were as follows: one metric tonne (1000 kg) of grapes was picked by hand (HH); one tonne was mechanically harvested with a Pellenc over row tractor 8590 with a Selectiv' Process On-Board picking head (Pellenc America, Santa

Rosa, CA, USA) (henceforth referred to as 'Selectiv', PS); and one tonne was mechanically harvested with the same Pellenc harvester with the on-board picking head disengaged and thus operated like a standard bow-rod machine harvester (henceforth referred to as 'machine', MS). The Selectiv' Process On-Board picking head does on-board sorting of the harvested grapes which mostly removes MOG with all grapes accepted. For each harvest treatment, half of the grapes received no sorting at the winery (NS) and half were sorted with a 2011 Delta Vistalys R1 optical sorter (Bucher-Vaslin, Chalonnes sur Loire, France) (VS) set to a stringency level of four out of five, resulting in six total treatments. The rejection rate was 9 ± 1 % of the fruit based on a weight per weight basis. Wines were made from each treatment in triplicate using 200 L stainless steel fermentation vessels as described previously (Lerno et al. 2015) with minor alterations. The diammonium phosphate (DAP) needed to achieve total yeast assimilable nitrogen levels of 300 mg/L was added in two additions (before inoculation and following one third sugar depletion). The musts were inoculated 36 hours after arrival at the UC Davis Research and Teaching Winery with *Saccharomyces cerevisiae* strain Lalvin® D254 (Lallemand, Montreal, Canada) according to the manufacturer's rehydration procedure and fermentation temperatures were controlled at 22 ± 1°C. Pumpovers with one tank volume of wine were performed three times per day (one aerative) until the wines reached five degrees Brix, then twice per day (one aerative) until the wines were dry. All wine fermentations fermented similarly and completed MLF within the same week. The chemical composition of the grapes from each treatment prior to winemaking was determined as well as of the wines at time of bottling and after three months of bottle ageing to coincide with sensory analysis. This included basic chemical parameters (EtOH % (v/v), pH, titratable acidity (TA)) as well as phenolic profiling by Adams-Harbertson assay (Harbertson et al. 2003) and RP-HPLC using a method based on Peng et al. (2002). Phenolic profiling of grape samples was done on extracts prepared from sequential extractions using 50% EtOH and 70% acetone solution as described in Lerno et al. (2015). The aroma compositions of both the grapes and wine treatments were determined by HS-SPME-GC-MS using a method adapted from Hjelmeland et al. (2013). The grape and wine samples were prepared for volatile analysis by an adaptation of a procedure previously described by Canuti et al. (2009).

Chemical composition of grapes from different harvest methods with and without optical sorting

The Brix, pH, and titratable acidity of the grape musts were relatively uniform among treatments with some minor differences with only Brix of the optically sorted hand and machine treatments significantly lower than the other treatments (Table 1). The removal of overripe and raisin-like berries from the product stream by the optical sorter, both of which have high sugar content, could be responsible for the lower Brix in these treatments. Although there were also significant differences in the pH levels of the musts, the difference in values did not exceed 0.1 pH units. No impact on TA due to sorting was observed potentially due to the fact that the grapes showed mostly uniformed ripeness with no visible unripe berries. The differences in must chemistry were small from a practical perspective and would likely have had minimal or no impact on the wine's sensory or future reaction chemistry.

There were small but significant differences among wine treatments at the time of bottling. For the most part, differences in ethanol content were driven by sugar content differences in the grapes following treatment. Larger sugar differences due to soak up than reflected in the Brix measurements of the grapes at harvest could explain discrepancies in grape sugar and final ethanol content in the wines. This was taken into account during sensory evaluation of the wines. In general, the machine-harvested treatments resulted in wines with lower TA's which could be the result of more potassium leaching from the skins which combined with tartaric acid to precipitate as potassium bitartrate. pH values were similar to those seen for the respective grapes.

There were no significant differences in total phenol and tannin concentrations in grape samples among treatments according to the Adams-Harbertson assay (data not shown). This was not unexpected as one would not expect different harvesting and sorting methods to significantly change the chemical composition of grapes that were harvested from the same vineyard given that only grapes with no MOG were used to prepare the grape extracts. Additionally, these extracts were prepared using strong organic solvents to determine total amount of available phenolics, not extractable phenolics which may have been more influenced by berry intactness. This is part of the reason why differences in phenolic content were found in the finished wines but not in the grape samples themselves. The grapes from the unsorted hand-picked treatment did, however, have a significantly lower anthocyanin concentration compared to the other treatments (Figure 1) according to both Adams-Harbertson and RP-HPLC analyses. This could potentially be due to lower extractability from the skins due to a higher percentage of raisin-like berries in this

Table 1. Brix, pH, and titratable acidity of must and wines at bottling for all treatments. Treatments sharing a common letter do not differ significantly at p≤0.05 (n=3).

	Treatment*	°Brix	pH	TA (g/L tartaric acid)
Grape samples	HHNS	24.6 ± 0.1 a	3.7 ± 0.0 a	5.3 ± 0.0 a
	HHVS	24.3 ± 0.2 b	3.7 ± 0.0 a	5.3 ± 0.0 a
	PSNS	24.5 ± 0.0 a	3.8 ± 0.0 b	5.1 ± 0.1 a
	PSVS	24.6 ± 0.0 a	3.8 ± 0.0 b	5.1 ± 0.1 a
	MHNS	24.5 ± 0.1 a	3.8 ± 0.0 b	5.1 ± 0.1 a
	MHVS	24.3 ± 0.1 b	3.7 ± 0.0 a	5.2 ± 0.1 a
	Treatment*	**%EtOH (v/V)**	**pH**	**TA (g/L tartaric acid)**
Wine at bottling	HHNS	13.9 ± 0.1 a	3.7 ± 0.0 ab	4.9 ± 0.1 ab
	HHVS	13.2 ± 0.3 b	3.7 ± 0.0 b	5.0 ± 0.1 a
	PSNS	14.4 ± 0.0 c	3.8 ± 0.0 a	4.6 ± 0.0 c
	PSVS	14.0 ± 0.2 a	3.8 ± 0.0 a	4.8 ± 0.1 bd
	MHNS	14.4 ± 0.0 c	3.8 ± 0.0 c	4.5 ± 0.1 c
	MHVS	14.4 ± 0.0 c	3.7 ± 0.0 b	4.6 ± 0.0 cd

*HHNS = hand-harvested, no sort; HHVS = hand-harvested, Vistalys sort: PSNS = Pellenc Selectiv', no sort; PSVS = Pellenc Selectiv', Vistalys sort; MHNS = mechanical harvest, no sort; MHVS = mechanical harvest, Vistalys sort

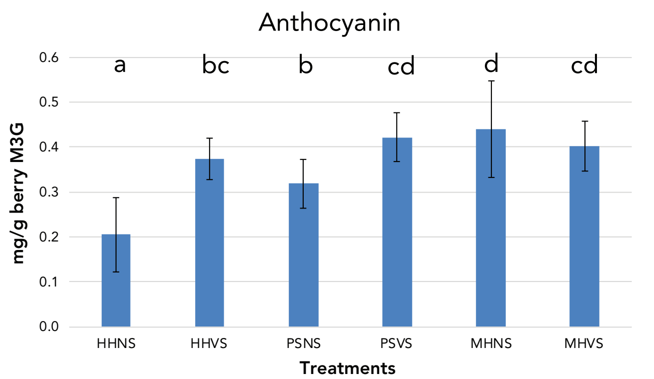

Figure 1. Anthocyanin concentration in grape samples as determined by the Adams-Harbertson assay (Harbertson et al. 2003). Treatments sharing a common letter do not differ significantly at p≤0.05 (n=3). Refer to Table 1 for the explanation of treatment codes.

treatment. When looking only at the unsorted treatments, the two mechanically harvested lots had greater anthocyanin levels than the hand-picked treatment by an average factor of two. This large difference may be the result of a greater skin to flesh ratio in the machine-harvested grapes caused by damaged berries losing juice and pulp. Since anthocyanins are derived solely from the skin in Pinot Noir grapes (Boulton 2001; Ribéreau-Gayon et al. 2006), a greater skin to flesh ratio from damaged berries would lead to artificially elevated anthocyanin concentrations after back-calculating to mg/g berry units. These differences, however, were not reflected in the total phenolics and tannin measurements which could indicate solubility limitations similar to those found by Gawel et al. (2001) when the impact of saignee (juice run-off) was investigated using different fruit sources.

Optical sorting led to a significant ($p \leq 0.05$) increase in anthocyanin concentration for the hand-harvested grapes. This is possibly due to the fact that the optical sorter removed raisin-like and sun-damaged berries that provide poor anthocyanin extraction, thus increasing the concentration in the optically sorted sample. Optical sorting had a smaller and non-significant impact on anthocyanin concentrations in the mechanical treatments due to slightly higher variability in anthocyanin content within replicates. Significant differences in anthocyanin concentrations between the unsorted hand and both unsorted mechanical treatments were removed in the optical sorted treatments.

Twenty-two of the 44 volatile compounds determined by HS-SPME-GC-MS in the grape samples were significantly different among the treatments. They were ethyl acetate, ethyl hexanoate, nerol oxide, benzyl alcohol, isoamyl acetate, cis-3-hexen-1-ol, trans-2-hexen-1-ol, trans-3-hexen-1-ol, cis-2-hexen-1-ol, β-citronellol, ethyl vanillate, ethyl 2-methylbutyrate, geraniol, unidentified sesquiterpenes I and II, γ-nonalactone, nerol, ethyl octanoate, β-linalool, β-myrcene, damascenone and α-terpinene. The PCA score plot shows that treatments with the same harvest method are grouped near one another (Figure 2), which indicates that harvest method had a greater influence on volatile profile in the grapes than did sorting. The grapes that were picked by hand had notable greater concentrations of the terpenes and alcohols located in the right quadrants of the loadings plot. The separation of treatments is also driven by β-linalool, β-myrcene, β-damascenone and α-terpinene as shown by the PCA loadings plot. These compounds, which are characterised by floral, spice, and perfume aromas, had greater concentrations in the machine-harvested treatments. This may be due to glycosidic hydrolysis of their non-volatile precursors in the mechanically harvested treatments. Aromatic compounds in grapes are, to a great extent, glycosidically bound, serving as an important reserve of aroma in wine (Williams 1993). The grape-derived glycosidase enzymes, located in the juices and pulp of the berry, are capable of liberating aroma compounds (Aryan et al. 1987). Berry damage incurred during mechanical harvesting, which disrupts the compartmentalised flesh fraction of grapes, would have released glycosidase enzymes which may have led to the greater concentrations of aroma compounds in machine-harvested treatments. Another possibility is the induction of synthesis as a wounding response to berry damage (Niinemets et al. 2013; Rodríquez et al. 2013). However, further study is needed to determine why these particular terpenes were present in higher concentrations in the non-hand-harvested treatments.

Chemical composition of different wine treatments

The total phenol content of the wines three months after bottling indicate that optical sorting led to a decrease in total phenolics except for the machine treatment (MH) (Figure 3). A previous study, however, found a general increase in total phenolic levels in optically sorted wine compared to its unsorted counterpart (Falconer et al. 2006). Our Adams-Harbertson data is supported by RP-HPLC data that shows that optical sorting also led to decreasing levels of gallic acid, (+)-catechin, (-)-epicatechin, and tannin in wines at bottling and after three months of bottle ageing (data not shown). It has been shown before that wines made with the addition of MOG, which can contain high levels of phenolic compounds, have greater total phenolic content than the control (Huang et al. 1988). It is possible that the general decrease of phenolic compounds observed in the optically sorted treatments is due to the removal of MOG by the sorter. Treatment PSNS had the greatest concentration of phenolics which may be due to the fact that during this harvest method the grapes experienced an additional physical process due to the Selectiv' Process on-board which could have led to greater berry damage (Figure 3). Damage that occurred during harvest would lead to greater extraction during subsequent transport of the fruit to the winery and during fermentation, since all treatments underwent whole berry fermentation. The extraction of tannin during fermentations when different percentages of crushed fruit were used have been studied by Cerpa-Calderón and Kennedy (2008) using Merlot grapes. They found that the final wine tannin amount increased with the percentage of crushed fruit used with a maximum reached at 75% crushed fruit. Thus, the assumption can be made that a higher percentage of crushed or damaged fruit in

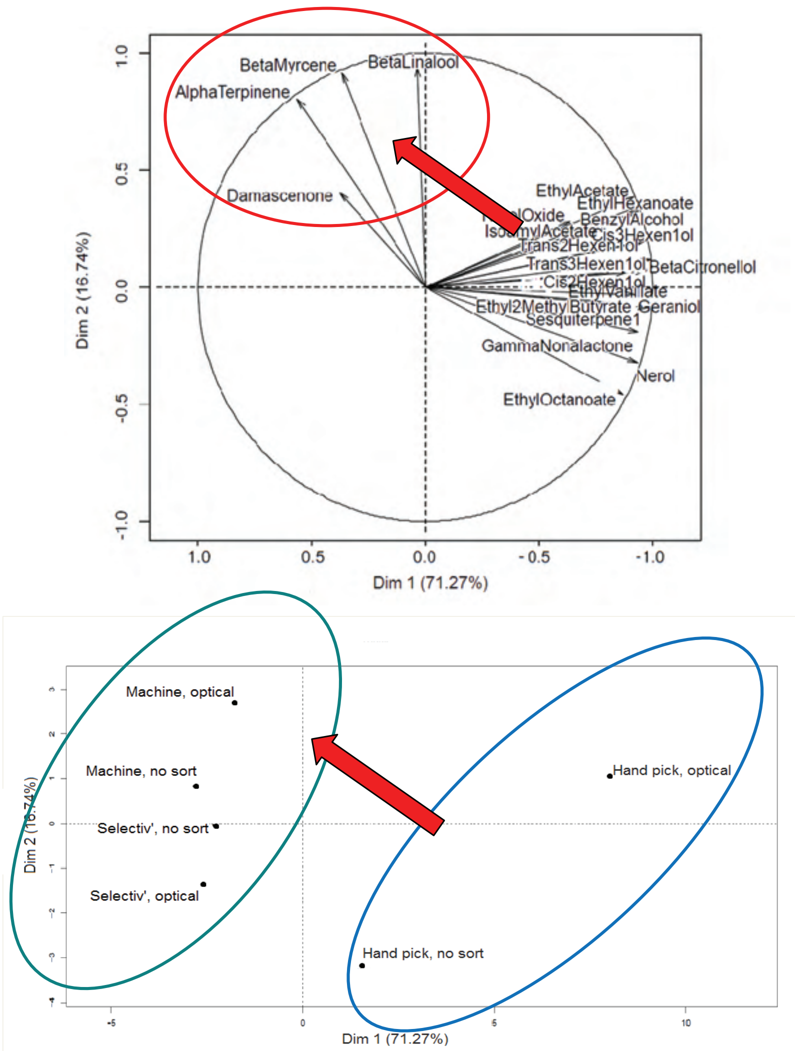

Figure 2. PCA loadings (top) and score (bottom) plots of the volatile compounds that differed significantly ($p \leq 0.05$) among grape samples as determined by HS-SPME-GCMS analysis

a ferment will result in more extraction of phenolics during fermentation. In our study, the mechanically harvested fruit did show visible damage upon arrival at the winery, and the half-tonne bins in which the grapes were transported contained a large amount of juice that had leached from ruptured berries. The PSVS treatment had phenolic concentrations more consistent with the other treatments, suggesting that the sorter may have effectively removed damaged berries from the process stream, thereby limiting extraction.

Figure 4 shows the total anthocyanin levels in the different wine treatments at bottling and after three months of ageing. A consistent decrease in anthocyanin concentration is observed with bottle ageing, which is expected as anthocyanins react with wine components to form mainly polymeric pigments (Fulcrand et al. 2006), although precipitation and/or oxidation reactions could also contribute to these decreases. All treatments had increased polymeric pigment levels after three months of ageing (data not shown). As seen for other phenolic compounds, the PSNS treatment generally had the highest concentration of anthocyanins. The higher phenol concentrations in the PSNS treatment can be the result of greater extraction from less intact berries during fermentation, as discussed earlier (Cerpa-Calderón and Kennedy 2008). If all treatments had been crushed rather than undergoing whole berry fermentation, these differences may have been eliminated or decreased. Sorting did not lead to large differences in anthocyanin concentrations in the hand- and machine-harvested treatments.

Fifty-one wine aroma compounds were determined of which 45 and 40 were significantly different among treatments at zero and three months of bottle ageing respectively. This indicates that the volatile composition of the different wine treatments became more similar with ageing. Commercial red wines are often aged for considerably longer than three months before they are bottled and released. Thus, if the trend of decreasing aromatic differences with time in this study were to continue, the volatile profiles of the wines will be even more similar across treatments with further ageing. The wine treatments were similarly separated as seen for the grapes (Figure 2) based on volatile composition (data not shown). However, no specific aroma compounds were responsible for the separation among wine treatments.

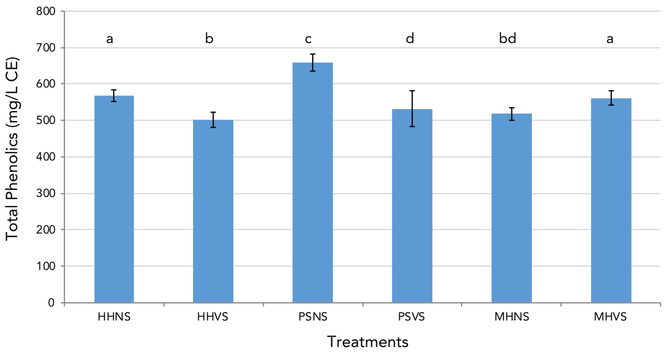

Figure 3. Total phenol concentration in wines after three months of bottle ageing as determined by the Adams-Harbertson assay (Harbertson et al. 2003). Treatments sharing common letters do not differ significantly at p<0.05 (n=9). Refer to Table 1 for explanation of treatment codes.

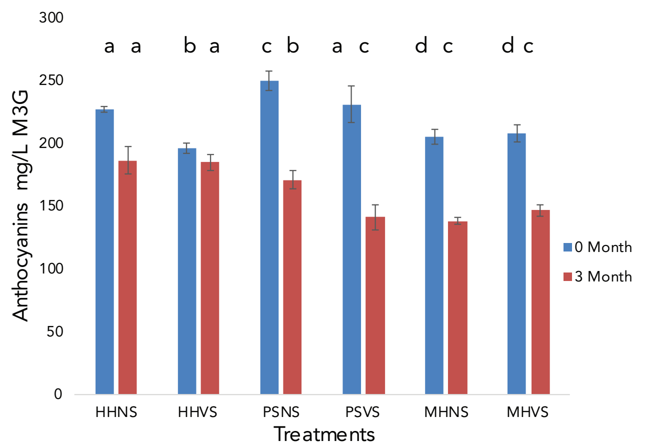

Figure 4. Anthocyanin concentration in wines at bottling and after three months of bottle ageing as determined by the Adams-Harbertson assay (Harbertson et al. 2003). Treatments at the same time point sharing common letters do not differ significantly at p<0.05 (n=9). Refer to Table 1 for explanation of treatment codes.

Sensory analysis of the wine treatments

The Pinot Noir wines were analysed sensorially approximately three months after bottling using descriptive analysis (DA) in the J. Lohr Wine Sensory Room, University of California, Davis, CA. Thirteen panelists evaluated two fermentation replicates of each treatment which were randomly selected for the descriptive analysis, totaling 12 wines. Training for the panel consisted of five one-hour sessions over two weeks. The study was approved by the Institutional Review Board of the University (IRB ID 571920–1). The research wines were presented blindly to the panelists who generated a comprehensive list of attributes which were reduced through group discussion and consensus to 12 aroma attributes, five taste and mouth-feel descriptors, and one visual assessment of hue saturation intensity (Figure 5). Wine attributes were rated on an unstructured line scale anchored by the words 'low' and 'high'. Wines were evaluated in triplicate in a random block design and presented in black tasting glasses to eliminate biases introduced by possible colour differences. Panelists evaluated the hue saturation intensity of the wines in a Macbeth light box.

Statistical analysis revealed that only 'tropical fruit' and hue saturation were significantly different among treatments out of the 18 attributes used to analyse the wines (Figure 5). The HHNS and PSNS treatments had significantly higher 'tropical fruit' aroma than the other treatments. Interestingly, this is in disagreement with a previous study on optical berry sorting that found greater 'tropical fruit' character in wines made from sorted fruit (Falconer et al. 2006) although in that case Chardonnay grapes were investigated. Additionally, even though 'tropical fruit' was significantly different among treatments, it was not a prevalent characteristic in general, with the highest rating at only 2.47 on a 10-point scale. This is not surprising as 'tropical fruit' is a relatively uncommon descriptor for Pinot Noir and most other red wines.

Optical sorting led to a significant decrease in hue saturation for each harvest treatment. This makes sense in the context of this study since the grapes were not crushed before fermentation. The optically sorted treatments would have had more intact fruit due to the removal of damaged berries, thus limiting extraction of pheno-

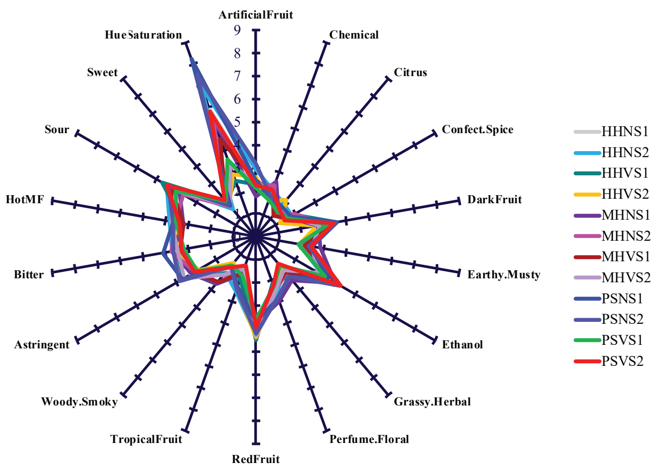

Figure 5. Spider plot of the attribute means from descriptive analysis. *Attributes that were significantly different among the wines (p≤0.05). Refer to Table 1 for explanation of the treatment codes.

lics during fermentation and leading to wines of lighter colour. As only two attributes differed significantly, of which one was a visual assessment, it is safe to conclude that the wines made from different harvest and sorting treatments were quite similar in taste and flavour profile. Thus, in the context of this study, mechanically harvested fruit did not produce inferior wines, as they were mostly indistinguishable from the wines made from hand-picked grapes. Similarly, wines made from grapes that were optically sorted were seen as very similar by the sensory panel to wines made from non-sorted fruit. Aroma compounds that may have contributed to the 'tropical fruit' aroma are β-citronellol and ethyl acetate (both of which can be perceived as 'fruity' at low concentrations), geraniol (which is found in many essential oils and is used in pineapple and grapefruit flavourings) and nerol (a monoterpene with a fresh scent that is found in lemongrass and hops) (Fahlbusch et al. 2003).

Conclusion

One of the main objectives of the study was to investigate the potential synergistic effects, if any, of using mechanical harvesting in conjunction with optical berry sorting on grape and wine composition. In some instances, such as anthocyanins in the grape samples and flavan-3-ols and tannins, the different harvest treatments did result in significant differences. The use of optical berry sorting either reduced or in many cases eliminated these differences. Thus, in this study, optical sorting was successful in diminishing the differences that arose from mechanical harvesting. Differences in anthocyanin and total phenol content which persisted in the final wines were likely exaggerated by the whole-berry fermentations employed by this study. By allowing differences in berry condition (presumably caused by treatment) to persist throughout fermentation, this technique allowed for differences stemming from treatment method to be seen more clearly. However, since it is common practice to crush fruit during red wine production, the differences in phenolic extraction seen among the treatments in this study may have been eliminated if more typical processing occurred. In general wines were not distinguishable using aroma, taste, and mouth-feel attributes, although significant chemical differences were found among treatments. As only two of the 18 wine sensory attributes were significantly different among treatments, the wines were seen as very similar in overall character. Pinot Noir was chosen as a potentially more sensitive variety to investigate the possible impact of mechanical harvesting due to its lower phenol content in comparison to varieties such as Cabernet Sauvignon and Merlot. This study, although only conducted for one season, is in agreement with the few studies completed on other varieties, as well as with many anecdotal studies. Mechanical harvesting is not recommended in years with high rot or mould. As mechanical harvesting and optical sorting become more commonplace in wine production, future studies of a similar nature using other grape varieties are merited.

Acknowledgements

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Objective measures of grape quality

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Abstract

Grape composition is critical to achieving a desired wine style, yet in Australia grape quality assessment has remained largely subjective. Recognising that objective measures of grape quality may contribute to achieving transparency and maximum value, research at the AWRI aimed to assess whether existing grape grading allocations could be predicted using targeted and non-targeted chemical measurements. Grape samples from 56 Cabernet Sauvignon, 62 Shiraz and 64 Chardonnay grape vineyards were obtained, from up to 9 quality grades. Chemical analyses included basic compositional factors such as berry weight, acid/pH, °Brix, moisture and nitrogen. Possible negative quality markers included laccase activity and chloride. In addition, the UV-visible spectrum and tannin concentration (red varieties) were measured. Aroma compounds quantified included the 'grassy', 'green' C_6 compounds, free β-damascenone, and the broad flavour measure phenolics-free glycosyl-glucose. Full spectral fingerprints in the mid-infrared (MIR) and near-infrared (NIR) regions were also acquired. Using multivariate statistical modelling, many of the targeted and non-targeted measures provided a strong prediction of quality grade, in some cases up to 100% accuracy. Differences were found between grape varieties in terms of the chemical measures which could best be used to predict grade, as well as a small degree of vintage variation. The work shows that there is potential for objective chemical measures to be defined, requiring refinements by variety and calibrated across multiple vintages.

Webcast of this presentation available at http://bit.ly/16thBindon.

Introduction

A recent survey of current practices to assess grape quality and value in the Australian wine industry indicated that the primary measures used include yield, sugar, pH and titratable acidity (TA), together with field assessments (Longbottom et al. 2013). This revealed that the approach taken has not changed significantly from that identified by a previous survey taken in the early 2000s (DeGaris et al. 2001). The survey of Longbottom et al. (2013) raised concerns about the degree of subjectivity in field assessments, and indicated support for standardisation of grape assessment methods, with a call for greater reliability and accuracy in their application in the Australian wine industry.

In the time between the two Australian surveys, comprehensive guidelines were published outlining best practices for wine-grape assessment in Australia (Allan 2003; Krstic et al. 2003). These guidelines notably included the measurement of grape colour (red varieties) by NIR or UV-visible spectrophotometry. In the guide to negotiating the sale of grapes provided by the Wine Grape Council of South Australia (WGCSA 2016), colour is also identified as one of the measures to assess minimum grape quality standards. Some use of colour as a pricing tool for grapes has been seen within the Australian wine industry, but a number of issues have arisen. These may account for the observation that while the 2001 survey of grape assessment practices had suggested that the use of colour as a quality measure for red grapes was expected to become more prevalent, the 2013 survey indicated that this was not the case. Some pitfalls or problems in the use of colour as a quality metric for red grapes in Australia have been:

- a failure to accommodate for the large seasonal variation in grape colour, requiring price point adjustment on a vintage by vintage basis, leading to a loss of confidence in colour as an objective quality metric
- a perceived lack of standardisation and reliability in the measurement of colour across different analysis platforms
- concerns regarding a lack of transparency and regulation in the administration of colour measures to set price
- changes in vineyard management practices to improve colour resulting in a trend in colour increase across the board, leading to 'shifting goal posts' for producers
- increasing recognition that colour is not necessarily indicative of other important quality attributes such as aroma or flavour.

Since the guidelines for wine-grape assessment were published in 2003 (Allan 2003; Krstic et al. 2003) significant progress has been made toward understanding the relationship between grape and wine composition, and in turn wine sensory properties (with implications for wine style). Smith (2013) reviewed research on the key classes of grape-derived compounds which affect the sensory properties of wine. The review did not incorporate those aspects which are consistently measured and well understood to influence wine composition such as acidity and sugar. Important compounds were categorised into a number of subgroups based on their form in the grape and conversion during the grape processing and winemaking process:

- compounds that are directly extracted into wine and undergo minimal conversion, such as rotundone ('black pepper'), methoxypyrazine ('capsicum') and β-ionone ('violets')
- compounds produced from precursors in the grape by the action of yeast, such as glycosidically bound aroma compounds and C_6-alcohols ('grassy', 'green')
- compounds produced from precursors in the grape without the action of yeast, such as volatile esters ('fruity') and higher alcohols including 2-phenylethyl alcohol ('rose') and isoamyl alcohol ('whisky')
- compounds influenced by both post-harvest processing and yeast; examples are the varietal thiols ('passionfruit', 'grapefruit', 'box hedge'), tannin (astringency) and anthocyanin (colour)
- environmentally derived compounds such as 1,8-cineole ('eucalyptol') and smoke taint.

The techniques used to measure these compounds in grapes and wine are complex, labour-intensive, time-consuming and expensive. Considering this, incorporating some or all of them into a suite of objective measures to be combined with traditional analysis is unrealistic. However, it is interesting to note that some of these more advanced analytical measures have been adopted by international producers such as E. & J. Gallo Winery to support decision-making for grape streaming and harvest date (Cleary et al. 2013). Through years of consistent analysis and the development of kinetic models tracking changes in important compounds during ripening, and also observing variation by vineyard and season, a number of important chemical measures were identified by E. & J. Gallo scientists (Cleary et al. 2013). Among these were pH, malic acid, yeast-assimilable nitrogen (YAN), colour, tannin, C_6 compounds, glycosyl-glucose (GG), β-damascenone and methoxypyrazine. Both the ripening-related evolution of these compounds and their relative proportions were found to be important in defining value and/or style. Certain of these compounds, for example tannin, colour, sugar/acid ratio and glycosidically bound volatiles have already been shown to be of relevance to red wine quality in other contexts (Abbott et al. 1993; Mercurio et al. 2010; Ristic et al. 2010).

Following on from these observations, and through discussion with Australian producers (Accolade Wines and FABAL) a project was designed to apply new and traditional analytical techniques in an Australian setting. The initial aim of the project was to determine from a range of measurable chemical compounds in grapes which of these, independently or in combination, were able to differentiate between grape grades. The primary research objective was to determine whether existing grading allocations could be predicted using previously identified, and some new, chemical measurements. This approach made the assumption that the existing current grading system was accurate and sought to establish an objective basis for it. Accolade Wines uses one standardised approach for grading of all fruit and pays growers independent of the wine grade outcome. For the study, grapes of three varieties [Cabernet Sauvignon (CAS), Shiraz (SHZ) and Chardonnay (CHA)] from a range of quality grades were sourced by representative sampling of vineyards. A range of chemical analyses were performed for the key measures known to affect wine style and sensory properties, shown in Table 1. These included some traditional measures commonly used within the industry such as total soluble solids, pH, TA, colour (anthocyanin) and total phenolics. Less frequently used measures, such as malic acid, and YAN were also included as well as new measurements: amino acid profile, methoxypyrazine, C_6 compounds, phenol-free GG assay, tannin, β-damascenone, laccase and complete spectral fingerprints in the UV-Vis, MIR and NIR regions. Univariate or multivariate statistical techniques of partial least squares (PLS) regression, principal component analysis (PCA) and discriminant analysis (DA) were applied to assess the extent to which these measures could be used to predict the grape grade. This paper reports results from one season of the study, 2014.

Materials and methods

Grape samples (~ 2 kg) for CAS, SHZ and CHA varieties were obtained across grades 1–9 (lower number = higher value/grade). Samples were sourced from a number of geographical areas across Australia: Swan Valley (Swan), Western Australia (WA), Riverland, McLaren Vale (MCV), Langhorne Creek (LHC), Clare Valley (Clare), Padthaway (PTW), Coonawarra (COO), Wrattonbully (WRA) and Tasmania (TAS) (CHA only). Basic chemical measures performed were berry weight, TA, pH, malic acid, °Brix, nitrogen as alpha amino nitrogen (AAN), ammonia (NH_3) and total YAN (Dukes and Butzke 1998). The whole amino acid profile was also assessed (Boughton et al. 2011). Potential negative quality markers included were laccase (Li et al. 2008) and chloride (Wheal and Palmer 2010). Methoxypyrazine was analysed in CAS (Bindon et al. 2013) in a previous season (2013) and found to be very low or absent in the samples, and was excluded from the 2014 analysis. Other targeted compositional measures (dependent on variety) included 'grassy', 'green' C_6 compounds (Capone et al. 2012), volatile thiol precursors (Capone et al. 2010) and β-damascenone (Perestrelo et al. 2011). The broad measure of glycosidically bound flavour precursors was assessed as phenolics-free GG (Zoecklein et al. 2000). Total phenolics, (280 nm), colour (520 nm), the whole UV-visible spectrum, and tannin (Mercurio et al. 2007) were assessed in the red varieties, and a subset of UV-visible wavelengths were analysed in CAS juice. Non-targeted MIR and NIR spectral fingerprinting were performed on homogenates and juices (MIR only). Data were analysed with PLS regression, linear DA (LDA), quadratic DA (QDA) and PLS-DA approaches. For multivariate analyses, the UnscramblerX 10.3 software package (Camo, Norway) was used.

Results and discussion

The results demonstrated that both partial least squares regression (PLS) modelling and quadratic discriminant analysis (QDA) modelling could be used to develop predictive models for fruit grade. The relative strength of prediction varied depending on the type of analytical data (e.g. spectral or targeted chemical analysis) and the type of modelling (PLS versus QDA) used. To summarise the results, using targeted chemical analysis for the three varieties studied, values of R^2 (validation, R^2_{val}) achieved with PLS modelling were from 0.66 (66% of the variation in grade explained by the linear relationship with the selected objective chemical measures) to 0.78. Somewhat better models were achieved using 'non-targeted' measurements (i.e. not measuring specific compounds, but rather overall 'fingerprints'), in particular the mid-infrared (MIR) spectrum of either grape homogenate or juice, with R^2_{val} of between 0.78 and 0.86 achieved, indicating that grade could be more accurately predicted using this approach. The use of PLS modelling for the prediction of grade was limited by the underlying assumption that grades are linearly separated, that is, by consistent, numerically defined increments between grades. This is in fact not the case, and grade could rather be said to be categorically defined. To overcome this, categorical models (which consider grades as categories that are not necessarily consistently linearly separated) could potentially be applied using PLS-discriminant analysis (PLS-DA), but for the particular data sets defined in this study could not be used successfully for grade prediction.

The use of QDA overcame the limitations of PLS-DA, and was found to be the most effective modelling technique to predict allocation grade, with prediction accuracies between 70 and 100%. Using QDA, certain analytical data sets better predicted grade by variety and season. CAS grade was well predicted from non-targeted grape

Table 1. Grape compositional measures used in this study; *indicates compounds routinely analysed for red grape varieties by E. & J. Gallo Winery, California

Berry basics	Phenolics	Aroma compounds	Other measures
berry weight (g) *pH titratable acidity (g/L) total soluble solids (°Brix) moisture (%) *malic acid (g/L) alpha amino nitrogen (mg/L) amino acid profile ammonia (mg/L) *yeast assimilable nitrogen (mg/L)	complete scan (SHZ, CAS only) total phenolics A280 (AU) *colour A520 (AU) (SHZ, CAS only) A420 (AU) flavonols A370 (AU) *tannin (mg/L) (SHZ, CAS only)	*C-6 compounds (µg/L) *phenolic-free glycosyl-glucose (µmol/kg) *β-damascenone (µg/L) volatile thiol precursors (CHA only) *methoxypyrazine (CAS 2013 only)	laccase activity (units/mL) chloride (mg/kg) near-infrared scan, juice near-infrared scan, homogenate mid-infrared scan, juice mid-infrared scan, homogenate

homogenate spectra in the infra-red range, with 100% accuracy achieved using MIR and 87% for NIR. This was a significant outcome, as it showed that spectral data could be used for the development of highly predictive models for fruit grade classification. This is important, since some of the more complex targeted analyses (i.e. of individual compounds) are not viable for many producers due to cost and time requirements, and spectral methods are relatively low cost and rapid by comparison.

Similarly, SHZ allocation grade was predicted most effectively by QDA using homogenate NIR spectra and the UV-visible spectrum, at 94% and 95% accuracy respectively. Using non-targeted juice MIR data, a 93% accuracy of prediction was the maximum achieved for CHA with QDA, which was similar to the accuracy achieved using targeted chemical data, at 95%. Interestingly, for CHA, no loss of prediction accuracy occurred when a limited suite of simple analytes were used: °Brix, pH, TA, malic acid, nitrogen (YAN, ammonia, alpha-amino nitrogen), malic acid, chloride, berry weight and the UV-visible spectrum between 280 and 370 nm (phenolics). Many of these techniques are readily accessible to the wine industry and this was a significant result that showed grade could be easily predicted for CHA using a combination of these simple measurements. The results showed that QDA modelling provided a promising tool for grade prediction, in particular using non-targeted MIR and NIR spectra but also with simple chemical measures (CHA), and could be highly valuable for the wine industry given the simplicity and accessibility of these techniques.

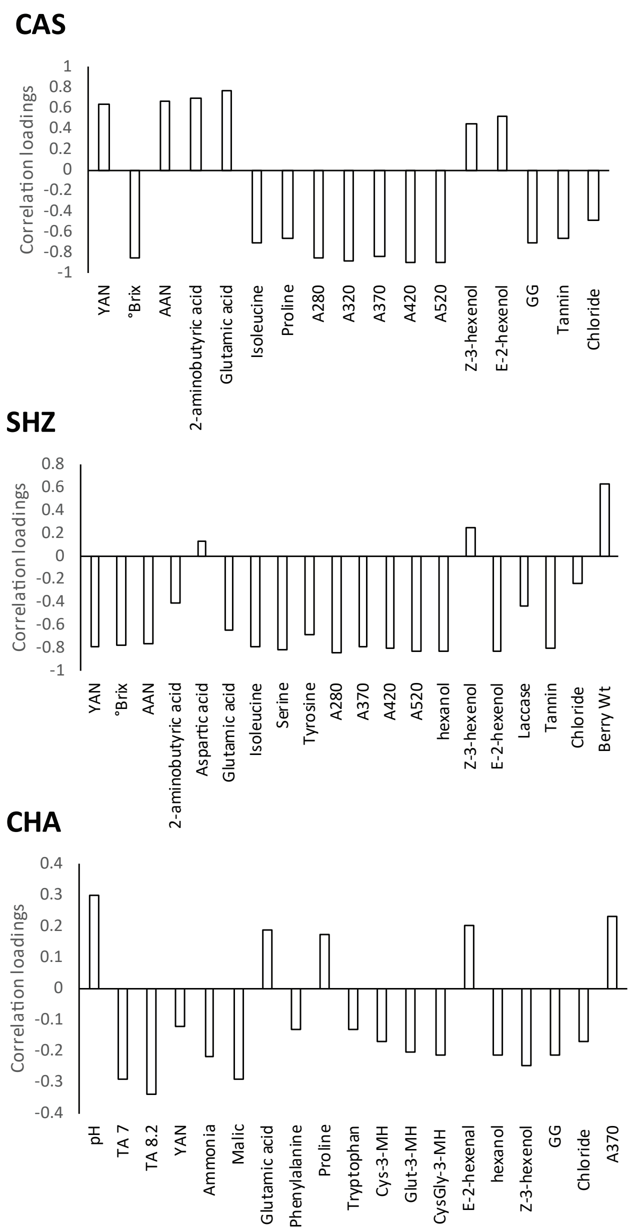

Figure 1. Correlation loadings from PLS regression models developed for the prediction of commercial allocation grade from targeted chemical analysis data for CAS, SHZ and CHA. Negative correlation loadings indicate higher concentrations of a given compound occur in higher value grades, and positive correlation loadings indicate higher concentrations are associated with low value grades.

A limitation of the QDA modelling approach is that it cannot provide an indication of which individual grape compositional variables are important for predicting grade allocation. Although not as highly predictive as QDA, PLS modelling was useful in defining the important compounds which differentiated fruit grades from one other. The correlation loadings for these models are shown in Figure 1. Since lower-value grade allocations were assigned higher numbers, a positive correlation loading for a given analyte indicate that higher concentrations occur in lower value grades. Conversely, a negative correlation loading indicates that higher concentrations of a compound are present in higher value grades. Some traditional measures of quality were found to be important for grade prediction, for example TA, pH, °Brix and berry weight which, depending on grape variety, were either positively or negatively associated with grade. The UV-visible spectra of grape extracts, in particular the measurements 280 nm (total phenolics), 370 nm (flavonols), 420 nm (yellow) and 520 nm (red varieties, colour), were shown to be important. For the red varieties CAS and SHZ, grape tannin was also important, and higher levels of both tannin and UV-visible measures were associated with higher value fruit grades. This is in agreement with previous studies which indicated a relationship between fruit phenolics and quality (Mercurio et al. 2010; Ristic et al. 2010). In CHA, higher levels of the 370 nm (flavonols) measure, were associated with poorer quality fruit. This may reflect decisions made through visual vineyard assessments of the grapevine canopy (greater fruit exposure leads to increased flavonols) and is the first time a phenolics measure has been demonstrated as being of importance in defining quality in white grapes.

The relevance of grape juice nitrogen measures (as YAN, alpha-amino nitrogen, ammonia and amino acid profile) to quality were assessed for the first time in this study, and were significant for CAS, SHZ and CHA. These measures were found to be both positively and negatively associated with quality, driven by changes in specific amino acids which varied seasonally. A significant finding was that the amino acid glutamic acid was strongly related to fruit allocation grade across multiple varieties. Glutamic acid was negatively associated with quality (i.e. higher amounts in lower value quality grades) in CAS and CHA, but positive in SHZ (i.e. higher amounts in higher value quality grades). Grape-derived aroma compounds, either in the free volatile or precursor form were also explored as potential objective quality markers. For CAS, phenolics-free GG (which indirectly represents aroma potential) was found to be an important predictor of higher value grape allocations. For all the grape varieties studied, the C_6 volatiles which contribute to 'grassy', 'green' aromas (E-2-hexenal, Z-3-hexanol and hexanol) were significant, being either positively or negatively associated with allocation grade. For CHA in particular, the C_6 volatiles Z-3-hexanol and hexanol together with precursors to the volatile thiols 3-S-cysteinylhexan-1-ol (Cys-3-MH) and 3-S-glutathionylhexan-1-ol (Glut-3-MH), 3-S-cysteine-glycine-3MH (Cys-Gly-3MH) were elevated in higher value grapes.

Conclusions

Together these findings support some of the current measures used to define grape quality but have also highlighted the importance of compounds which have not previously been demonstrated as being of importance to the objective measurement of grape quality in the Australian context. Both targeted (individual compounds) and non-targeted (spectral fingerprint) analytical approaches were shown to be useful for grade prediction, as well as the identification of specific chemical markers important for quality. Potential impacts of these results for the wine sector include the ability for grapegrowers to more efficiently produce grapes to defined specifications, and for winemakers to select fruit with greater confidence that it will be appropriate for a targeted wine style. In addition, it is significant to grapegrowers because it relates to confidence and transparency in the realisation of maximum economic value for their grapes. For both growers and winemakers, objective chemical measures can provide specifications that allow the most value to be achieved from grapes. Developing an understanding of the synergistic relationships between objective measures and well-established subjective systems has the potential to significantly increase value by ensuring that fruit is used in the most efficient production stream. It also may lead to significant savings in the costs of monitoring crops through more effective application of resources.

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Compositional variation amongst Australian sparkling white wines

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Abstract

Australian sparkling white wines range from fruit-driven styles, typically derived from either carbonation or the Charmat method, to more complex sparkling wines, exhibiting 'toasty', 'yeasty', 'bready' notes, attributable to bottle fermentation and/or lees ageing undertaken during production using the transfer method or Méthode Traditionelle. This study aimed to investigate composition (basic chemistry, amino acids, proteins, polysaccharides and volatile compounds) and foaming properties of 50 Australian sparkling white wines produced via the four key production methods. Méthode Traditionelle wines generally had higher titratable acidity and alcohol content, but lower residual sugar and total phenolics, compared to other sparkling wines. Méthode Traditionelle wines also had significantly higher protein content, which likely explains the higher foaming capacity observed for these wines upon pouring. Carbonated wines were highest in total free amino acids, which may be a consequence of a proportion of these wines being produced from higher yielding, non-traditional wine-grape varieties grown in warmer climates. Furthermore, carbonated wines only undergo primary fermentation, so it is likely higher levels of amino acids in these wines reflect the lower microbial activity associated with this production method compared to others which involve secondary fermentation. Méthode Traditionelle and transfer wines contained a higher percentage of yeast-derived mannoproteins, while there was a shift towards lower molecular weight rhamnogalacturonans for Charmat and carbonated wines. Chemometric analysis of volatile profiles indicated that the Méthode Traditionelle and transfer wines exhibited similar volatile compositions, as did Charmat and carbonated wines. There were higher concentrations of ethyl decanoate and fatty acids (octanoic and decanoic acids) in carbonated wines, and acetates (isoamyl, isobutyl and hexyl), associated with 'banana' and 'confectionery' notes, were the most abundant volatile compounds in Charmat wines. The floral compound, phenethyl acetate, was strongly associated with both Charmat and carbonated wines. In contrast, the volatile composition of Méthode Traditionelle and transfer wines was characterised by higher concentrations of compounds such as furfural, diethyl succinate, ethyl hydrogen succinate and 3-methyl-1-pentanol, which are associated with more aged aromas and flavours of 'caramel', 'earthy', 'wood', 'cooked apple' and 'whisky'. These research findings enable industry to better understand the diverse compositional differences in different styles of Australian sparkling white wines.

Webcast of this presentation available at http://bit.ly/16thCulbert.

Introduction

In Australia, sparkling white wines are produced by four key production methods: carbonation, Charmat, transfer and Méthode Traditionelle (analogous with the Méthode Champenoise used for Champagne production). All methods initially involve the production and blending of base wines using similar processes to those used for table wine production including crushing, pressing and alcoholic fermentation by yeast (Iland and Gago 1997). Transformation of the base wine into a sparkling wine is ultimately determined by the introduction of dissolved carbon dioxide in the wine which is responsible for the 'sparkle'. In the simplest production method, carbonation, carbon dioxide is directly infused into the base wine whereas the other methods involve the generation of carbon dioxide from secondary fermentation, either in a pressurised tank (for Charmat) or enclosed bottle (for transfer and Méthode Traditionelle) (Iland and Gago 1997). Bottle fermentation often involves a period of lees ageing, which results in the formation of more complex 'toasty', 'yeasty' and 'bready' notes compared to the fruit-driven styles derived from either carbonation or the Charmat method.

The process of lees ageing has received considerable attention, particularly in the production of Spanish Cava and French Champagne, since this process results in changes in organoleptic properties of the wine due to the release of chemical constituents during yeast autolysis (Alexandre and Guilloux-Benatier 2006). Yeast autolysis can alter mouth-feel and foaming properties by influencing amino acid, protein and polysaccharide content. Isoamyl and hexyl acetates, ethyl decanoate and 2-phenylethyl acetate, which exhibit 'fruity' and 'floral' sensory characteristics, are markers of young sparkling wines (Francioli et al. 2003). As sparkling wines age on yeast lees, however, the concentration of these volatile compounds decrease along with fatty acids (Riu-Aumatell et al. 2006; Hidalgo et al. 2004), while compounds such as 1,2-dihydro-1,1,6-trimethyl-naphthalene (TDN), vitispirane and diethyl succinate significantly increase in concentration (Riu-Aumatell et al. 2006; Francioli et al. 2003; Gallardo-Chacón et al. 2010; Hidalgo et al. 2004; Torrens et al. 2010). For sparkling wines produced via the traditional method, the concentrations of free amino acids can fluctuate during ageing and yeast autolysis (Martínez-Rodríguez et al. 2002), are not released at the same time in all wines (Moreno-Arribas et al. 1998) and are positively correlated to foam height (Moreno-Arribas et al. 2000). Other studies have shown that protein concentration and composition influence sparkling wine foam stability (Manteau et al. 2003; Brissonnet and Maujean 1991; Pueyo et al. 1995) and contribute to wine body and quality (Luguera et al. 1998). Similarly, polysaccharides, and in particular, the yeast-derived mannoproteins have been linked to improved foaming properties (Pueyo et al. 1995; Martínez-Lapuente et al. 2013; Coelho et al. 2011; Abdallah et al. 2010) and wine stability by reducing protein haze (Dupin et al. 2000) and potassium bitartrate crystallisation (Waterhouse et al. 2016).

This study investigated the compositional differences (basic chemistry, amino acids, proteins, polysaccharides and aroma and flavour volatiles) and foaming properties of fifty Australian sparkling white wines produced via the four key production methods.

Methods

Fifty Australian commercial sparkling white wines produced via the four key production methods, i.e. Méthode Traditionelle (MT, n=20), transfer (Tr, n=10), Charmat (Ch, n=10) and carbonation (Ca, n=10), were sourced from wineries and retail outlets. Wines were prepared and analysed for basic wine parameters (pH, TA, alcohol, residual sugar and total phenolics) as described previously (Culbert et al. 2015; Iland et al. 2004).

Amino acids were derivatised to their highly fluorescent 6-amino-quinolyl-N-hydroxysuccinimidyl carbamate (AQC) derivatives using the AccQ-Fluor Reagent Kit (Waters, #WAT052880) and analysed by high performance liquid chromatography (HPLC) with fluorescence detection as described previously (Boss et al. 2015). Amino acids were quantified based on comparison against the responses of calibration solutions containing known concentrations of amino acids.

The concentration of chitinases and thaumatin-like proteins (the major haze-forming proteins) were determined in the wines using a published HPLC method (Van Sluyter et al. 2009) with some modifications. Proteins were quantified using an external standard curve of thaumatin, with results being presented in mg/L of thaumatin equivalents.

Polysaccharides, including mannoproteins, arabinogalactans (AGP's) and rhamnogalacturonans (RG's) were isolated by precipitation with ethanol, purified by dialysis and analysed by size-exclusion HPLC using methods previously described (Bindon et al. 2013). Polysaccharides were quantified by comparing the peak areas with those of a standard curve of dextran and results were presented as mg/L 50 kDa dextran equivalents.

Foaming behaviour was investigated in triplicate by pouring wine (approx. 10 mL) into the base of a 50 mL measuring cylinder. Videos were recorded (typically between 45 and 90 s for each wine) and used to determine foam volume (mL) generated per mL of wine poured, rate of foam collapse (mL/s) and foam collar volume remaining after collapse (mL).

Aroma and flavour volatiles were determined by gas chromatography-mass spectrometry (GCMS), with samples prepared via solid phase extraction (SPE) using LiChrolute-EN cartridges (Merck). GCMS data (3D) was exported into excel and subjected to multivariate curve resolution and alternating least squares (MCR-ALS) analysis using methods described previously (Schmidtke et al. 2013). Positive compound identification was achieved using the NIST 05 Mass Spectral Library database as well as comparing retention times and mass spectra to those observed when analysing known volatile standards.

Data were analysed using a combination of descriptive and multivariate techniques, including Analysis of Variance (ANOVA) with post-hoc Tukey's test at $P<0.05$, correlation analysis and principal component analysis (PCA) using XLSTAT (version 2015.1, Addinsoft).

Results and discussion

Basic chemical composition of sparkling white wines

The ranges and means for the pH, TA, residual sugar, alcohol and total phenolics for the sparkling wines by production method are presented in Table 1. While chemical variability does exist between wines produced by the same production method, there were also significant differences between production groups for most parameters. Méthode Traditionelle wines were, on average, significantly higher in TA (8.0 g/L) compared to transfer (6.9 g/L) and Charmat (6.8 g/L) wines. These differences may reflect the quality of the grapes used for sparkling wine production. Premium quality sparkling wines, typically used in the production of Méthode Traditionelle wines, are classically produced from grapes sourced from cooler regions, such as Tasmania; such grapes, at flavour ripeness, naturally possess high acid levels and low sugar levels (below 11°Baumé). Méthode Traditionelle and transfer wines were, generally, higher in alcohol but the lowest in residual sugar and phenolics compared to those wines produced by the other methods. Higher alcohol content directly relates to production method. The Méthode Traditionelle and transfer methods involve a secondary (in bottle) fermentation to generate wines of higher pressure (5–7 atmospheres, compared to 3–5 for Charmat and carbonation). In order to generate higher dissolved carbon dioxide levels, higher sugar levels are added during tirage which subsequently convert to alcohol during yeast fermentation, therefore resulting in higher alcohol wines. Carbonated wines were highest in total phenolics, containing more than double that observed for Méthode Traditionelle wines. This is likely due to fractions arising from heavier pressings used for production of carbonated wines. In addition, phenolic levels are minimised for Méthode Traditionelle wines since premium grapes typically undergo whole bunch pressing to reduce skin maceration, therefore producing higher quality musts. In Champagne production, strict regulations only allow approximately 100 L of must from 160 kg of fruit (25.5 hectolitres per 4,000 kg (Comité Champagne)), thereby minimising poor quality musts and ensuring the quality of product.

Table 1. Basic chemical composition (ranges with means in parentheses) of Australian sparkling white wines, by method of production. Values are mean scores for triplicate samples of each wine within each production method. Means within rows followed by different letters are significantly different (p = 0.05, one way ANOVA, Tukey's post-hoc).

	Méthode Traditionelle (n=20)	transfer (n=10)	Charmat (n=10)	carbonated (n=10)
pH	2.9-3.4 (3.2)	3.1-3.5 (3.2)	3.2-3.5 (3.3)	3.1-3.4 (3.3)
TA (g/L)	6.4-9.6 (8.0 a)	5.8-7.6 (6.9 b)	6.1-7.4 (6.8 b)	6.4-9.2 (7.6 ab)
Residual sugar (g/L)	0.5-13.1 (8.8 b)	3.9-15.8 (12.0 ab)	8.5-19.0 (14.0 a)	7.9-13.5 (12.4 ab)
Alcohol (% abv)	11.2-13 (12.3 a)	11.0-13.1 (12.0 ab)	11.0-12.2 (11.6 bc)	10.3-12.5 (11.1 c)
Total phenolics (au)	0.3-4.9 (2.2 b)	0.9-4.3 (2.4 b)	0.5-4.5 (2.9 b)	2.5-5.8 (4.7 a)

Amino acids

Amino acids in wine originate from grapes and yeast, either in their free form or as a product of grape protein degradation or yeast autolysis (Lehtonen 1996; Waterhouse et al. 2016). They play an important role in alcoholic fermentation as they provide an energy source for yeast metabolism. In this study, carbonated wines contained the highest concentrations of total amino acids, averaging 1274 mg/L, while Méthode Traditionelle, transfer and Charmat wines contained similar concentrations of 949, 931 and 976 mg/L, respectively (Table 2). The variation between production methods was highlighted in the mean concentration of proline, which was significantly higher ($p<0.05$) in the carbonated wines compared to wines produced by the other methods (Table 3). Proline was the most abundant amino acid in the wines contributing approximately 40% of the total content, with arginine, lysine and alanine collectively contributing a further approximately 35%. Numerous studies have reported proline to be the most abundant amino acid in wines (Casoli and Colagrande 1982; Huang and Ough 1991; Lehtonen 1996; Waterhouse et al. 2016) and this may be a consequence of proline not being consumed as a nitrogen source for yeast growth during fermentation as well as the accumulation of proline in berries due to vine stress. Amino acid composition in grapes can also be influenced by vineyard management/ water availability, nitrogen application and grape variety. For example, studies by Huang and Ough (1991) found that arginine, rather than proline, was the most prominent amino acid for many of the grape varieties they investigated. The higher total free amino acids observed for the carbonated wines may be a consequence of many factors. Firstly, carbonated wines only undergo primary fermentation in comparison to the other methods of production which involve a secondary fermentation. Therefore, since amino acids are consumed during yeast fermentation, wines which undergo two fermentations (Méthode Traditionelle, transfer and Charmat production methods) are more likely to have lower amino acid concentrations. Secondary, the higher proline (and overall total amino acids) in the carbonated wines may be more indicative of where the grapes are grown for the base wines rather than other winemaking factors. For instance, carbonated wines are more commonly produced from higher yielding vines grown in warmer climates and under water stress higher accumulation of proline may occur (Bertamini et al. 2006). In this study, half the carbonated wines were produced using non-traditional sparkling wine-grape varieties (i.e. varieties other than Chardonnay, Pinot Noir and Pinot Meunier) and consequently this may impact on amino acid concentration and composition.

Proteins

Two major haze forming proteins in wine, chitinases and thaumatin-like proteins, were quantified in the sparkling wines using HPLC. A previous study (Brissonnet and Maujean 1993) showed that these proteins, of size 20–30 kDa, comprise the majority of proteins in Champagne base wines. In this study, total protein content was significantly ($p<0.05$) higher in the Méthode Traditionelle wines, averaging 67.3 mg/L thaumatin equivalents, which was approximately double that contained in the transfer, Charmat and carbonated wines (averages of 28.8, 34.9 and 34.6 mg/L thaumatin equivalents, respectively; Table 2). The protein content of the sparkling wines solely comprised thaumatin-like proteins, except for one wine (MT03). Similarly, other studies on sparkling wine have observed no chitinases in the finished wine (Manteau et al. 2003). Chitinases and thaumatin-like proteins are grape-derived, rather than being yeast derived, so the higher concentrations observed in Méthode Traditionelle wines may be indicative of less protein fining of base wines. Stabilisation treatments, such as fining, have been shown to significantly reduce the protein content during sparkling wine production (Luguera et al. 1998; Vanrell et al. 2007). Since protein content can greatly influence foaming properties and, in turn, wine quality, it is likely that Méthode Traditionelle wines undergo less fining of their bases wines to ensure desirable organoleptic characteristics typical of this style of wine.

Polysaccharides

The average total polysaccharide content for the sparkling wines in each production group was similar, ranging between 622 and 736 mg/L 50 kDa dextran equivalents (Table 2). However, when considering the contribution of each of the individual polysaccharide classes, mannoproteins (200 kDa), arabinogalactans (100 kDa) and rhamnogalacturonans (10–50 kDa), clear differences were observed between production methods (Figure 1). The Méthode Traditionelle and transfer wines contained a higher percentage of the yeast-derived mannoproteins while for the Charmat and carbonated wines the profile shifted towards lower molecular weight polysaccharides (i.e. 10 kDa RG's) (Figure 1). This relates to the ageing of the bottle fermented wines on yeast lees where yeast autolysis results in the release of mannoproteins from the degradation of yeast cell walls (Alexandre and Guilloux-Benatier 2006). Studies on sparkling wine production via the traditional method (for Spanish Cava and French Champagne) have shown that mannoproteins increase during bottle ageing (Charpentier 2000; Martínez-Lapuente et al. 2013). However,

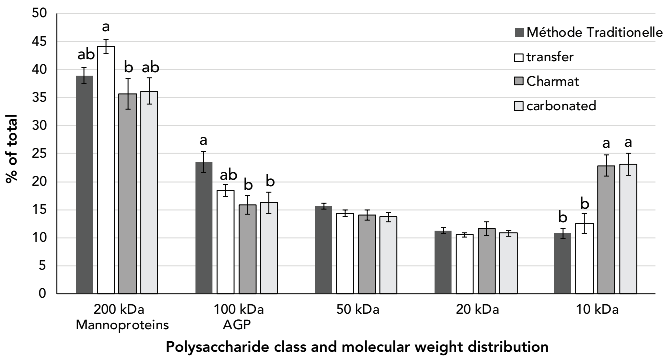

Figure 1. Polysaccharide content of Australian sparkling wines based on percentage contribution of each polysaccharide class for each production method. Different letters within individual classes indicate significant differences (p = 0.05, one way ANOVA, Tukey's post-hoc). Note: AGP = arabinogalactans; molecular weight ranges 10-50 kDa relate to RGI&II, where RG = rhamnogalacturonans

Table 2. Total proteins, free amino acids, polysaccharides and foaming properties (ranges with means in parentheses) of Australian sparkling white wines, by method of production. Values are mean scores for two wine replicates for each wine within each production method. Means within rows followed by different letters are significantly different (p = 0.05, one way ANOVA, Tukey's post-hoc).

	Méthode Traditionelle (n=20)	transfer (n=10)	Charmat (n=10)	carbonated (n=10)
Total proteins (mg/L Thaumatin equiv.)	7-161 (67.3 a)	8-77 (28.8 b)	9-70 (34.9 ab)	9-88 (34.6 ab)
Total free amino acids (mg/L)	450-1452 (949 b)	602-1168 (931 b)	665-1254 (976 ab)	471-1924 (1274 a)
Total polysaccharides (mg/L 50 kDa dextran equiv.)	239-1285 (723)	393-792 (622)	341-801 (660)	443-975 (736)
Foam collar volume (mL)	1.5-8.0 (3.1 a)	0.0-4.0 (1.9 ab)	0.0-3.0 (1.2 b)	0.0-6.0 (1.6 ab)
Foam collapse (mL/s)	0.33-1.26 (0.86)	0.94-1.55 (0.94)	0.68-1.75 (1.11)	0.23-1.45 (0.89)
Foam volume (mL) per mL of wine	1.2-3.7 (2.4 a)	0.2-3.0 (1.7 b)	1.3-2.8 (2.0 ab)	0.9-2.6 (2.0 ab)

Table 3. Concentrations (mg/L) of the most abundant amino acids (mean and ranges) for Australian sparkling white wines, by production method. Values are mean scores for two wine replicates for each wine within each production method. Means within columns followed by different letters are significantly different (p = 0.05, one way ANOVA, Tukey's post-hoc).

		Proline	Arginine	Alanine	Lysine	Glutamic acid	Aspartic acid
Méthode Traditionelle (n=20)	Range	259-621	20-277	18-172	33-134	12-67	14-63
	Mean	405.6 b	131.2	85.9	75.7	42	39.1
Transfer (n=10)	Range	227-521	32-237	43-152	39-106	23-70	28-52
	Mean	389.9 b	114	92.9	69.1	47.2	39.2
Charmat (n=10)	Range	285-569	41-470	48-136	20-114	28-66	15-37
	Mean	381.4 b	198.8	97.2	64.8	46.8	28
Carbonated (n=10)	Range	272-884	19-647	43-189	15-167	19-100	15-66
	Mean	556.7 a	232.6	115.2	77.3	55.8	37.9

other winemaking practices such as filtration and stabilisation can reduce polysaccharide content (López-Barajas et al. 2001). This may explain some of the variation in wine polysaccharides observed amongst wines produced by the same production method.

Foaming properties

The foaming behaviour of the 50 Australian sparkling white wines was observed by manually pouring approximately 10 mL of wine into a 50 mL glass measuring cylinder. Experiments were repeated until three adequate replicates for each wine had been obtained. Wine was poured directly onto the base of the measuring cylinder to ensure maximum foaming and consistency of results (i.e. less foaming occurs if the wine flows down the sides of the measuring cylinder). The foaming behaviour was recorded so that foam volume (mL) per mL of wine poured (Figure 2a), rate of foam collapse and foam collar volume remaining after foam collapse (Figure 2b) could be captured. The averages for each of these foaming parameters based on production method are listed in Table 2. Méthode Traditionelle wines had, on average, the highest level of foam per millilitre of wine poured, the largest remaining foam collar volume after collapse, and the lowest rate of foam collapse, though not all these observations were statistically different to the wines produced by the other methods of production. The higher foaming capacity for Méthode Traditionelle wines is likely a consequence of their higher protein content. However, the likely higher dissolved carbon dioxide concentrations and pressures inside the bottles containing Méthode Traditionelle wines may also contribute to foaming behaviour.

Volatile profiles

Sparkling wines were analysed by GCMS in full scan mode and multivariate curve resolution of the obtained data provided areas under the curve for each of the peaks in the chromatograms for each of the wines, in triplicate (separate bottles analysed). Chemometric analysis of the entire chromatographic spectrum for all wines indicated that Méthode Traditionelle and transfer wines exhibited comparable volatile compositions, as did Charmat and carbonated wines (data not shown). Statistical analysis of the individual peak areas identified those compounds which were significantly driving the differences among production methods. Those compounds showing the greatest variation and abundance between the sparkling wines included: the esters ethyl propanoate, ethyl decanoate, ethyl isobutyrate, ethyl lactate, ethyl hydrogen succinate and diethyl succinate; the alcohols 3-methyl-1-pentanol, benzyl alcohol and 2,6-dimethyl-7-octene-2,6-diol; the acids of decanoic and octanoic; the acetates of isoamyl, isobutyl, hexyl and phenethyl and the aldehyde, furfural. PCA was performed on the average compositional data (i.e. basic chemistry, total amino acids, proteins, individual polysaccharide classes and volatile compounds) and foaming characteristics for each production method (Figure 3). The first and second principal components explained 72% and 28% of variation, respectively, and highlighted the chemical differences in wines produced by each production method (based on average data for each method of production). The volatile compounds ethyl decanoate, octanoic acid, decanoic acid and to a lesser extent 2,6-dimethyl-7-octene-2,6-diol were associated with the carbonated wines, while the acetates (isoamyl, isobutyl and hexyl), which are associated with 'fruity' (e.g. banana and apple) and 'confectionery' notes (Etievant 1991; Smyth 2005; Capone et al. 2013), were strongly related with Charmat wines. The floral compound phenethyl acetate was strongly associated with both Charmat and carbonated wines. Méthode Traditionelle and transfer wines were more closely related in chemical composition, with volatile compounds such as furfural, diethyl succinate, ethyl hydrogen succinate and 3-methyl-1-pentanol notable. These compounds are more associated with aged wine, exhibiting aroma and flavours such as 'caramel', 'earthy', 'wood', 'cooked apple' and 'whiskey' (Wright 2012; Capone et al. 2013; Wang et al. 2016). Ethyl lactate and ethyl propanoate were also related to Méthode Traditionelle and transfer wines but to a lesser extent. These results are in line with other studies which indicate isoamyl and hexyl acetates, ethyl decanoate and 2-phenylethyl acetate are markers of young sparkling wines (Francioli et al. 2003), with these compounds significantly decreasing over time (Riu-Aumatell et al. 2006) along with a reduction in fatty acids content (Hidalgo et al. 2004). Furthermore, secondary fermentation and ageing in contact with yeast lees has been shown to increase esters such as diethyl succinate (Francioli et al. 2003; Hidalgo et al. 2004; Riu-Aumatell et al. 2006) and furans (Torrens et al. 2010).

Conclusions

The chemical composition and foaming properties of Australian sparkling white wines varied according to their method of production. Méthode Traditionelle and transfer wines generally had similar

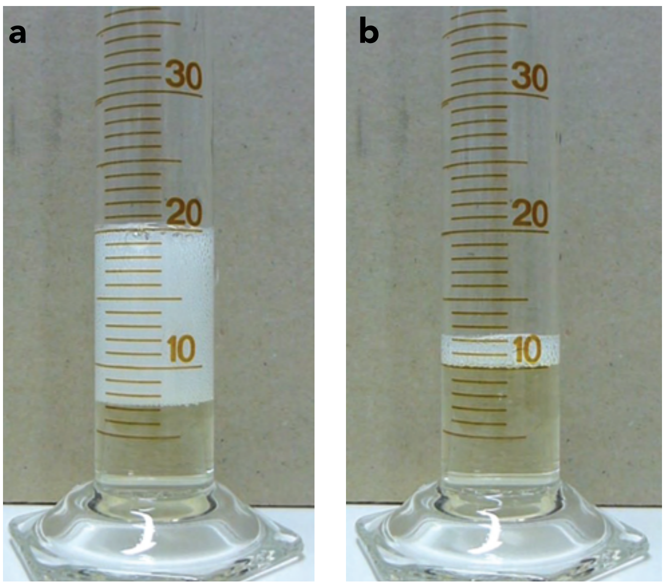

Figure 2. Images from foaming experiments showing (a) maximum foam volume reached (used to determine foam volume per mL of wine poured); and (b) foam collar volume remaining after foam collapse.

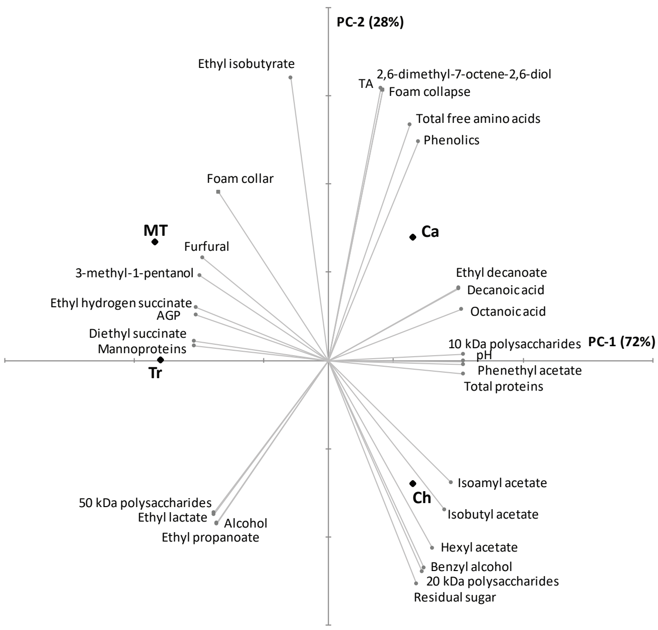

Figure 3. PCA biplot of the average chemical composition (basic chemistry, protein, polysaccharides, amino acids and volatile compounds) and foaming properties for each production group. Ca = carbonated wines, Ch = Charmat wines, Tr = transfer wines, MT = Méthode Traditionelle wines

chemical properties, as did Charmat and carbonated wines. Protein content was higher for Méthode Traditionelle wines suggesting that it influenced foaming behaviour. Future work will focus on measuring foaming parameters using a robotic pourer and image analyser (Condé et al. 2017). In addition, the chemical data obtained in this study can be examined against wine quality ratings and sensory data to identify key markers of quality. Variations observed between wines produced from the same method of production also offer an avenue for future studies. These research findings enable industry to better understand the diverse compositional differences between Australian sparkling white wines.

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Why is the genetic diversity of *Brettanomyces bruxellensis* important for winemakers and is it related to sulfur dioxide tolerance?

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Abstract

The environmental conditions of wine are unfavourable for growth of many microorganisms; however, Brettanomyces bruxellensis *is highly adapted to the winemaking process, which implies resistance to sulfur dioxide, high ethanol tolerance, growth on limited nitrogen sources and tolerance of low pH. This yeast's metabolism results in an alteration of the wine's flavour profile (unpleasant 'leathery' and/or 'mousy' characters), thus leading to economic losses.* B. bruxellensis *is also associated with other industrial fermentations such as beer, cider, kombucha (fermented tea), kefir and bioethanol. In these products, the desirability/undesirability of this yeast is unclear and still debated.*

The industrial importance of B. bruxellensis *has led to the study of its genome and population structure. Previous studies revealed a high genotypic diversity at intra-species level and that phenotypic characteristics are strain-dependent. Furthermore, a comparison of genome assemblies revealed the coexistence of diploid and triploid populations and high dissemination of a triploid population in wine fermentations in Australia. We have conducted a genotyping study of a large population of* B. bruxellensis *isolates from five continents and different substrates using microsatellite markers. The results suggest that* B. bruxellensis *species is structured according to ploidy level and substrate. The potential contribution of the triploid state to the adaptation to industrial fermentations and to the dissemination of* B. bruxellensis *is discussed. This works focuses on the ability of different strains of* B. bruxellensis *to survive in the presence of sulfur dioxide.*

Webcast of this presentation available at http://bit.ly/16thAvramova.

Introduction

Brettanomyces bruxellensis (*B. bruxellensis*) is a spoilage yeast associated with major wine aroma defects which are present in about 25% of red wines (Gerbaux et al. 2000; Conterno et al. 2006). The 'Brett' character often leads to consumers' rejection and therefore economic loss. Even if numerous prevention and elimination methods are available for winemakers, the problem often persists from one year to another. Thus, controlling *B. bruxellensis* contamination risk is often a priority when vinification and wine ageing management decisions are made. The importance of the *B. bruxellensis* issue is underscored by the fact that this species is detected worldwide and in substrates other than wine.

Sulfur dioxide (SO_2) addition is the most common technique to prevent *B. bruxellensis* development during the winemaking process. However, high SO_2 levels are not always associated with premium wines styles and many wine consumers are seeking wines with lower SO_2 levels. Furthermore, it was recently shown that some *B. bruxellensis* strains are tolerant to SO_2. The use of SO_2 as a prevention method against *B. bruxellensis* contamination might not prove a long-term strategy.

Here, we present our results concerning the intraspecific diversity of *B. bruxellensis* – on both genotypic and phenotypic level – and we focus particularly on *B. bruxellensis*' sensitivity to SO_2.

Brettanomyces bruxellensis and its genetic diversity

Numerous tools allow the detection and quantification of the species *B. bruxellensis*, although few were developed for genotypic analysis at intra-species level. Therefore, little evidence is available on the species' ecology and genetic diversity. Even though the population level can be quantified in a wine or must sample in a reliable way, it was, until now, relatively challenging to assess the nature of the strains present in the sample and their contamination and spoilage activity.

A collection of 1280 *B. bruxellensis* isolates from 29 countries was assembled and considered in this study (Figure 1).

Scientific teams from all over the world have focused their work on the genome sequencing of different strains of *B. bruxellensis*. Thanks to this recent knowledge, and particularly the sequences provided by the work of Curtin et al. (2012b), our team devel-

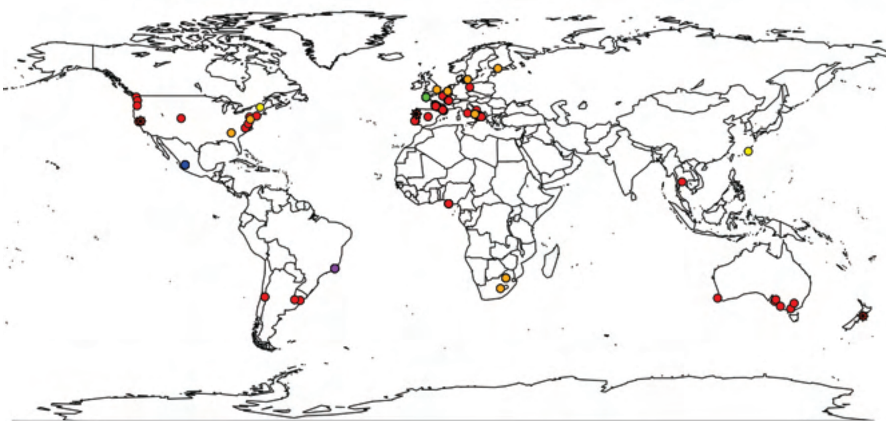

Figure 1. Geographic origin of *B. bruxellensis* strains used in this study. The substrate of origin is indicated by the colour as follows: red – wine, grape and wine equipment; orange – beer; blue – tequila; green – kombucha; violet – bioethanol; yellow – others (cider and other fermented beverages).

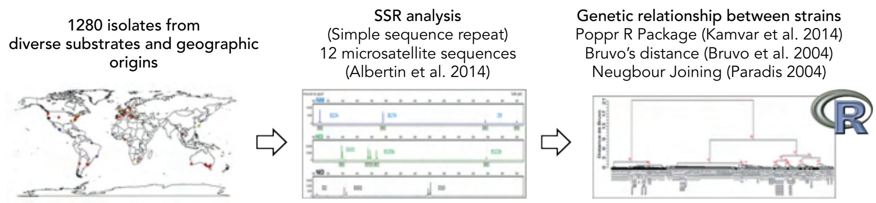

Figure 2. Schematic of experimental approach

oped 12 genetic markers based on microsatellite sequence repeats (Albertin et al. 2014) (a scheme representing the main steps of the method is provided in Figure 2). The strains used in the study were isolated from wine, sourced from other laboratories or were already present in Centre Ressources Biologiques (CRB) oenologie strain collection. DNA extraction was performed from a single fresh colony by treatment with 30 µl of 20 mM NaOH and 99°C heat for 10 minutes. Microsatellite analysis was done by amplifying Simple Sequence Repeat (or SSR) regions as described by Albertin et al. (2014). Amplicon sizes were measured by ABI3730 DNA analyser and GeneMarker® software. Raw data was treated on R software using Poppr Package (Kamvar et al. 2014).

This method allows the establishment of the genetic links between strains, revealing significant genetic diversity within the species. In agreement with the first genome studies of the species (Curtin et al. 2012b; Borneman et al. 2014), we confirm the existence of triploid strains possessing every gene in three copies instead of the common two. These triploid strains possess an additional genome whose origin remains unknown at present. Remarkably, a high proportion of genotyped strains are triploid. The hypothesis is that the triploid state could confer specific traits to *B. bruxellensis* which would be advantageous for the adaptation to wine-type environment.

The 1280 *B. bruxellensis* isolates were genotyped by microsatellite analysis highlighting 617 genetic profiles clustered in three main genetic groups (A, B, and C). Group A consists of strains that are triploid and isolated from wine, group B consists of a second type of triploid strains isolated from beer and wine, and group C consists of mostly diploid strains isolated from wine and other substrates (kombucha, tequila, bioethanol, etc.). This significant genetic diversity may help to explain the considerable phenotypic variation of the strains shown in previous studies, particularly growth capacity, ethyl phenol production and/or SO_2 tolerance. Interestingly, different genetic groups have been shown to co-exist in the same winery or wine sample and from one sample to another taken from the same source.

Brettanomyces bruxellensis and use of sulfur dioxide

The active form of SO_2 (i.e. molecular SO_2) is the most common method to fight against *B. bruxellensis.* The efficiency of SO_2 depends on the dose applied, the pH, ethanol content and temperature of the medium. It is generally considered that 0.5 mg/L molecular SO_2 is sufficient to inhibit *B. bruxellensis* growth and that 0.7–0.8 mg/L of molecular SO_2 is a lethal dose (Chatonnet 2012), although the levels change with wine pH: 30 mg/L of free SO_2 at pH 3.6 equates to 60 mg/L at pH 3.9.

The existence of strains that are tolerant to SO_2 was recently highlighted. In Australia, a strategy to control *B. bruxellensis* based on the use of SO_2 was applied over the last ten years or more. *B. bruxellensis* isolates were collected from different wines treated with SO_2 and the studied population clustered in eight different genetic profiles as estimated by Amplified Fragment Length Polymorphism (Curtin et al. 2007). A group comprising the majority among these strains (85% of the isolates) was shown to be highly tolerant to SO_2 and cells could grow at 0.6 mg/L of molecular SO_2 (Curtin et al. 2012a). These findings suggest that using SO_2 to manage *B. bruxellensis* may apply a selective pressure to the population and ultimately lead to the emergence of highly resistant strains. Thus, it is important to understand any links between the genetic linkage of the strains and their resistance to SO_2.

To spread these observations to other winemaking regions, we performed at laboratory scale a phenotypic test to evaluate the SO_2 tolerance of 33 strains representative of the genetic diversity of the species (strains varied in their geographical region, substrate of isolation and genetic group A, B or C as previously defined by microsatellite analysis). Strain growth was characterised in a synthetic laboratory medium in triplicate and under anaerobic conditions.

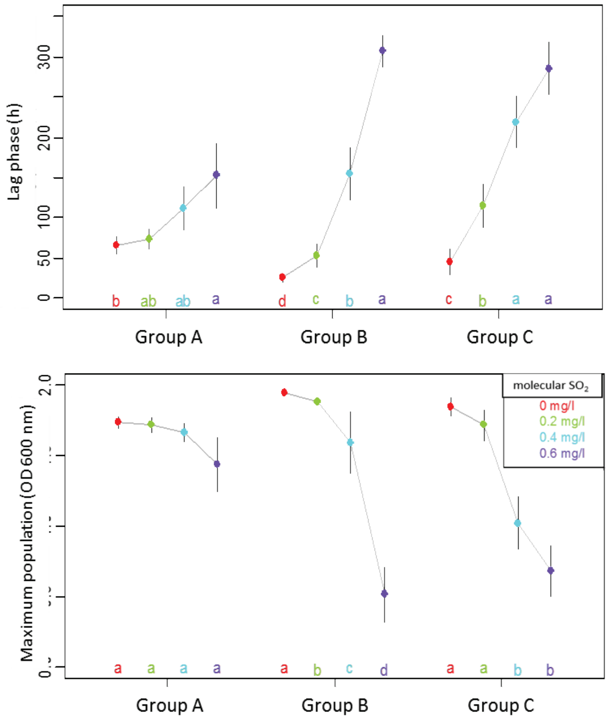

Figure 3. Growth parameters of different *B. bruxellensis* strains belonging to three major genetic groups (A – 8 strains, B – 8 strains and C – 17 strains) in media with increasing molecular SO_2 concentrations. Kruskal-Wallis test was performed for every parameter and every group of strains, different letters (a, b, c, d, ab) indicate significantly different mean values at 5% threshold.

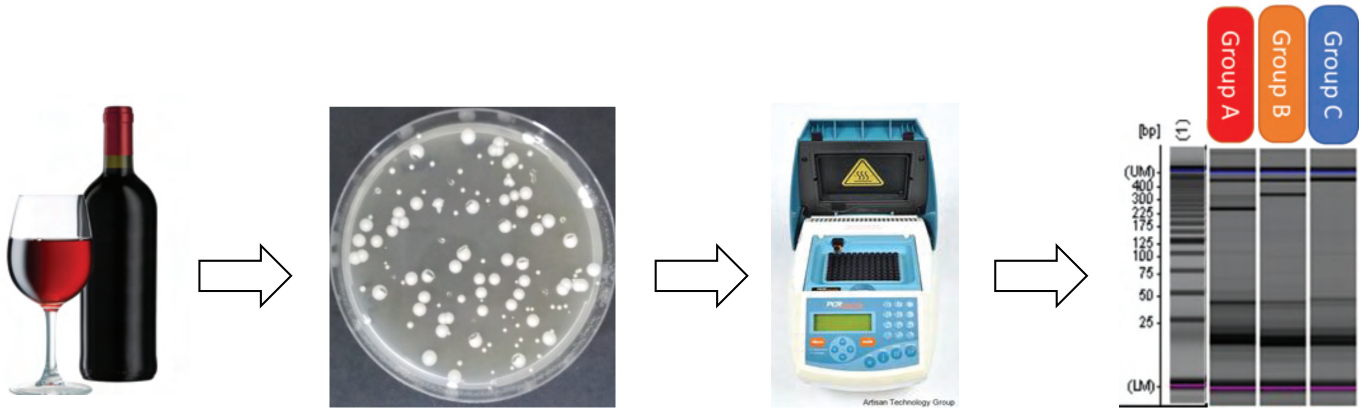

Figure 4. Result from the PCR to determine if a *B. bruxellensis* isolate belongs to the group A, B, or C. The 470 bp band is specific to the *B. bruxellensis* species (Ibeas at al. 1996), a 281 band is specific to strains from group A, a 356 band is specific to strains from group B and there is no band specific to group C.

It was observed that growth in the presence of increasing concentrations of SO_2 is significantly different for each genetic group (Figure 3). For the strains from group A, the lag phase is slightly but significantly longer with an increasing SO_2 concentration. However, once the growth has started, the SO_2 concentration does not have any significant effect on the other growth parameters (i.e. growth rate, maximum population attained and time taken to attain the maximum population; only maximum population is shown) and this observation was valid even for the concentration of 0.6 mg/L of molecular SO_2. Thus, these strains are considered tolerant to SO_2: apart from the longer lag phase, they have a 'normal' growth from 0.2 to 0.6 mg/L molecular SO_2. On the other hand, the growth of the strains belonging to strains B and C is strongly affected by the concentration of molecular SO_2 and this is valid for doses higher than 0.4 mg/L (and even 0.2 for several strains). These strains are considered sensitive to SO_2.

Consequently, the adjustment of the molecular SO_2 even at 0.6 mg/l could be insufficient when SO_2 tolerant strains are present.

A tool for diagnosing *Brettanomyces bruxellensis*' SO_2 sensitivity

In our laboratory, we have developed and filed a patent for a simple molecular test which highlights the genetic group of a given *B. bruxellensis* isolate (A, B or C) and therefore predicts its SO_2 sensitivity. This test is based on simple Polymerase Chain Reaction (PCR) analysis on colonies isolated on selective solid medium. The analysis relies on the size of the amplification fragment produced at the end of the PCR, which varies with the genetic group of the strain. An example of a result after the test is performed on three different colonies is shown in Figure 4.

Thus, a single analysis permits (i) to confirm that the isolate belongs to *B. bruxellensis* species and (ii) to predict its sensitivity to SO_2. Of the 1280 isolates studied, 435 belong to group A, 206 to group B and 639 to group C. The group A isolates come from various wine regions in France (Bordeaux, Bourgogne, Jura, Languedoc, Côtes du Rhône) but also from Italy and Australia. No link was established between the genetic group and the geographical origin of the strains.

As a next step, we aim to develop a quantitative PCR tool that would eliminate the cultivation step of the analysis and allow faster quantification.

Conclusion

The study of *B. bruxellensis*' genetic diversity revealed an unexpected genomic complexity. Various *B. bruxellensis* groups exist which differ in terms of sulfite tolerance. In the collection of isolates studied by microsatellite analysis, 34% of the strains are potentially very tolerant to SO_2, illustrating the fast adaptation capacity of the species. The phenomenon is widespread – the sulfite-tolerant isolates were detected not only in different French winemaking regions but also in other countries such as Australia.

In the actual context of chemical input reduction in the wine industry (particularly the use of SO_2), it is now possible to assess the SO_2 sensitivity of *B. bruxellensis* contaminating a wine sample. This may help the winemaker to select a strategy to prevent and control spoilage, and avoid the use of SO_2 when it is not likely to be effective – winemakers should use SO_2 addition only on wines that are not affected by tolerant strains.

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Using cross-linked polymers to sequester metals and extend shelf life of wine

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Abstract

It is generally recognised that one of the most significant limiting factors affecting the shelf life of a wine is the impact of oxidation. This indirectly reduces the level of antioxidants present, such as sulfur dioxide, and renders the wine susceptible to microbial spoilage. Attempts to minimise the impact of oxidation typically focus on reducing the availability of oxygen during bottling and through selection of appropriate closures with low oxygen transmission properties. However, recent studies have shown that for oxidation to occur in wine, trace metal ions such as copper and iron must be present to act as a catalyst for the oxidation process. Little research has been carried out to evaluate strategies that can make wine more immune to the impacts of oxygen by binding or scavenging these metals.

Studies carried out at the AWRI have shown that cross-linked polymers (CLPs) can be used to sequester metal ions such as copper and iron and reduce the speed with which oxidation reactions subsequently occur, thereby extending wine shelf life. White and red wines treated with two different types of CLPs were shown to be less susceptible to the impact of oxygen on sulfur dioxide levels and browning when subjected to an accelerated oxidation treatment.

Webcast of this presentation available at http://bit.ly/16thScrimgeour.

Introduction

Oxidation during maturation and bottle ageing can have a significant impact on the development and resulting shelf life of a wine. Oxygen can trigger a series of chemical reactions with specific components in the wine matrix, leading to the formation of undesirable compounds and a negative experience for the consumer.

One of the most significant effects of oxygen exposure is the reduction in sulfur dioxide (SO_2) concentration, which renders wine susceptible to microbial spoilage and premature loss of freshness and fruit characters, especially during extended storage periods. Attempts to minimise the impact of oxidation typically focus on reducing the availability of oxygen during storage and bottling by the use of inert gas cover and the purging of entrained oxygen from tanks or bottles. Once in bottle, the diffusion of oxygen into wine can be somewhat controlled by the choice of closure, with an emphasis on selecting an appropriate closure with a low oxygen transmission rate (OTR).

Although oxygen plays a central role in oxidation processes, it is now known that the speed and extent of oxidation is dependent, to a large extent, on the concentration of transition metals in the wine. Recent studies by Danilewicz (2003, 2007 and 2011) have shown that, for non-enzymatic oxidation to occur in wine, trace metal ions such as copper and iron must be present to act as a catalyst for the oxidation process. This creates a chain reaction which ultimately results in the quenching of bisulfite ions and an increased risk of wine deterioration.

Although the mechanisms involved in the oxidation process are still a matter of some debate, it is well known that, by removing or limiting the concentration of transition metals present in wine, the oxidation process can be significantly retarded (Cantu et al. 2009; Kreitman et al. 2013; Kreitman et al. 2016a, b). To date, few studies have been carried out to evaluate commercially relevant strategies to make wines less susceptible to the impacts of oxygen by binding or sequestering these metals, although Danilewicz (2007) did show that the removal of iron and copper from a model wine system can have a dramatic effect on SO_2 depletion (see Figure 2 below).

One approach is to use cross-linked polymers (CLPs) to sequester metal ions such as copper and iron to reduce the speed and extent of oxidation reactions, thereby extending wine shelf life. A number of studies (Green et al. 1995; Eder et al. 2003) have shown that the concentration of transition metals present in wine, specifically copper and iron, can be reduced through treatment with specific CLPs.

Most research carried out to date has focused on the use of various ion exchange resins to achieve this; the most notable success has been achieved with polyvinyl pyrollidinone – polyvinyl imidazole (PVP-PVI) copolymers and acidic cation exchange resins with active iminodiacetate groups bound to a macroporous polystyrene matrix. The former has shown effectiveness in reducing transition metal concentration in musts during fermentation (Nicolini et al. 2004) and the latter have been used extensively for reducing heavy metal content in wastewater and in groundwater remediation (e.g. Rengaraj et al. 2001).

For both types of CLPs described, transition metals present in the wine are able to bind to the active groups present in these CLPs,

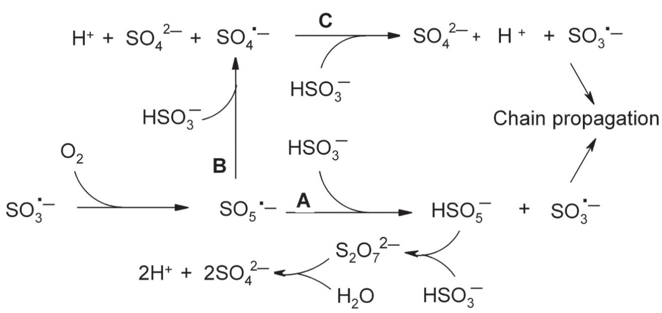

Figure 1. Proposed radical chain reaction scheme for bisulfite oxidation

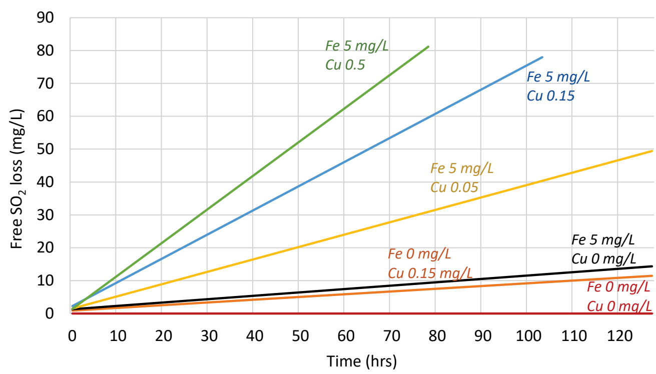

Figure 2. The comparative loss in free sulfur dioxide (SO_2) concentration due to different levels of iron (Fe) and copper (Cu) in a model-wine system at 18.5-20.5°C over 5 days [based on data generated by Danilewicz (2007)]

with selectivity generally being higher for divalent metal ions such as Fe^{2+} and Cu^{2+}. In the case of those resins with active iminodiacetate groups, the metal ions are exchanged for monovalent ions such as H^+ or Na^+, leading to a potential increase in wine pH or salinity respectively, if used in a high mass: volume ratio (Figures 3 and 4).

Some studies have examined the effect of treatments to remove transition metals from wine on other wine components (Mattivi et al. 2000; Benitez et al. 2002; Mira et al. 2007; Kreitman et al. 2013), with most observing a modest reduction in polyphenols (predominantly hydroxycinnamates) and anthocyanins, as well as titratable acidity in the form of tartaric acid. When used sparingly, these studies have shown a negligible impact on the organoleptic properties of the wine.

Materials and methods

A series of experiments were constructed to examine the effectiveness of different CLPs in sequestering copper and iron in white and red wines and the resultant effect of this on wine oxidative markers. Individual experiments were carried out in order to evaluate the following:

- Effect of CLP treatment time on iron and copper concentration
- Effect of copper and iron sequestration on resulting sulfur dioxide (SO_2) concentration, wine colour and phenolic profile.

The experiments included treatment of commercial-grade white and red wine samples with two specific CLPs: Divergan® HM (BASF, Germany; supplied as a fine grade powder); and Lewatit® MonoPlus TP207 (Lanxess Deutschland GmbH, Germany; supplied as a coarse grade powder).

Schott bottles were prepared by pre-purging with nitrogen and filling with 500 mL wine. Two replicate bottles were each treated with 500 mg/L Divergan® HM and 500 mg/L of the TP207 resin. The samples were agitated for 48 hours at 17°C, along with an additional two replicate bottles of each wine that received no resin addition. Two replicate bottles were also filled with each wine sample and left untreated (no resin addition) and unagitated (stored static at 17°C for 48 hours).

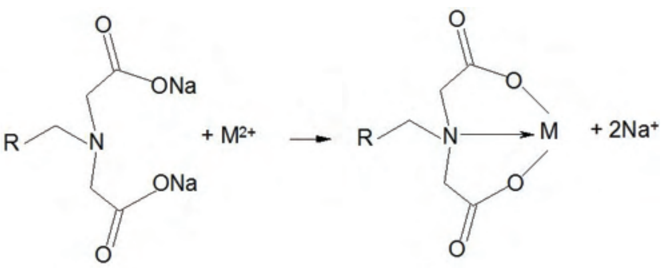

Figure 3. Binding of divalent transition metal ions with active iminodiacetate groups bound to a polystyrene matrix in an acidic cation exchange resin (resin as sodium form)

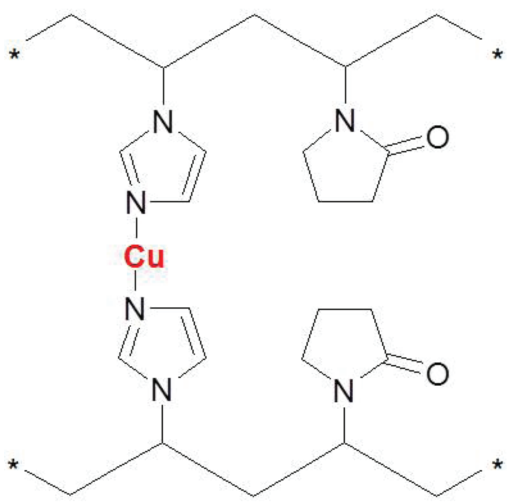

Figure 4. Binding of copper by polyvinyl pyrollidinone – polyvinyl imidazole (PVP-PVI) copolymer

Following filtration to remove the CLPs, samples were analysed for copper and iron concentration immediately following treatment using inductively coupled plasma (ICP) – mass spectroscopy (MS) (Perkin Elmer NexION 350D). Treated samples were also subjected to an accelerated browning test (Singleton and Kramling 1976) to determine the expected impact on brown pigment development during storage.

In order to simulate an accelerated oxidation treatment, all samples were stored at a constant 35°C without inert gas cover in the headspace of the bottles. The wines were sampled at multiple intervals over a 17-day period to monitor changes in SO_2 and colour intensity. The SO_2 concentration was measured using Flow Injection Analysis (Lachat Instruments), and colour measurements were carried out at 280, 320, 420 and 520 nm using a Varian UV/Visible spectrophotometer.

Results and discussion

Sequestering of metals by CLPs

Figures 5a and 5b show the effect of the resin treatment on the concentration of copper and iron respectively in the white wine over the 48-hour treatment period. All data points shown are the average concentrations for each pair of replicate samples analysed.

It can be seen that the Divergan was more effective in reducing the overall concentration of metals in the wine. After 48 hours, the final concentration of iron was approximately 0.4 mg/L in the Divergan -treated wine, compared to approximately 0.8 mg/L in the TP207-treated wine. The faster reduction in metal concentration observed for Divergan may be due to a stronger scavenging potential or the smaller particle size of the fine grade powder sample supplied; an increased contact surface area between the wine and the CLP is likely to enhance binding of the metals to the polyvinyl imidazole functional group of the Divergan.

Both resins were successful in reducing the copper concentration to below its limit of quantification (0.1 mg/L), although the Divergan

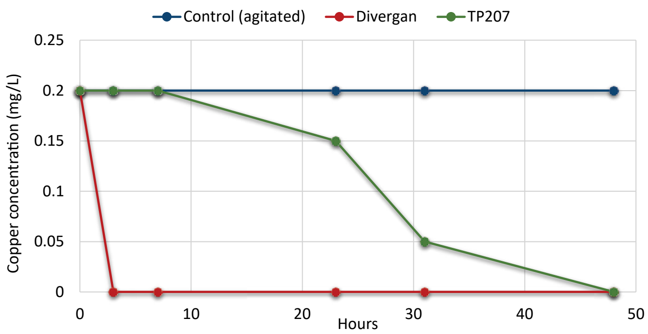

Figure 5a. Concentration of copper in commercial white wine treated with Divergan and TP207 over a 48-hour period. Agitated control wine (untreated) was monitored over the same period.

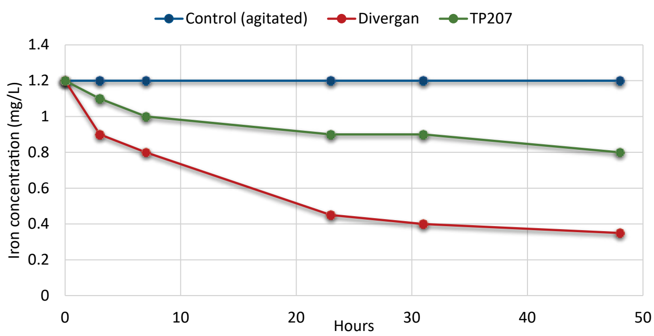

Figure 5b. Concentration of iron in commercial white wine treated with Divergan and TP207 over a 48-hour period. Agitated control wine (untreated) was monitored over the same period.

appeared to sequester the copper far more quickly (within three hours) compared to the TP207.

Similar trends can be seen for the commercial red wine treated with the two CLPs. Figures 6a and 6b show the concentration of copper and iron respectively over the 48-hour treatment period following treatment with Divergan and TP207. Again, the Divergan appears to be more effective in reducing metal concentration, with this effect being more noticeable with iron; after 48 hours, the TP207-treated wine still contains almost 1 mg/L iron, whereas the Divergan-treated wine has no detectable iron present (<0.3 mg/L).

The Divergan was also more effective in reducing the concentration of copper in the wine during the 48-hour treatment period, compared with the TP207. In a similar manner to the white wine, copper concentration was reduced to below its quantification limit relatively quickly (within 7 hours) using Divergan, whereas the TP207 resin took up to 24 hours to remove all copper from the wine.

The differences in iron concentration between the white wine and the red wine, due to the sequestration effect of the two CLPs, may be due to the nature of the compounds the iron is bound to in the two wine matrices. Iron that is strongly bound to other components in the wine may be unavailable for sequestration, depending on the mechanisms involved with the two types of resins. This is an area that warrants further study.

The results of the accelerated browning test carried out on the white wine immediately following treatment with the two CLPs can be seen in Figure 7.

Based on these results, one would expect the white wines treated with the two CLPs to exhibit a lower degree of browning than the control wines during subsequent oxidation, with the TP207-treated wine slightly more susceptible to browning than the Divergan-treated wine.

Impact of metal removal on wine shelf life

The free SO_2 concentration in the wine samples was monitored over the 17-day period of accelerated oxidation. Only data from the first ten days, post-treatment, are included below as free SO_2 concentration reduced rapidly beyond ten days in both wines under these conditions, resulting in SO_2 concentrations below the limit of quantification (4 mg/L) (Figures 8a and 8b). The period from −2 to 0 days represents the 48-hour treatment period when the CLP was applied. Untreated control wines (both agitated and unagitated) were included for each wine, to provide a reference point for the resulting shelf life and to assess the relative impact of the agitation process on the untreated wine samples. All data points represent the average concentration measured for each pair of replicate samples.

The data shows that the wines treated with the two CLPs were able to maintain a higher concentration of free SO_2 over the ten-day post-treatment period, with the Divergan-treated wines exhibiting a slower depletion in free SO_2 during the accelerated oxidation process, for both white and red wines. Although the initial (t0) concentration of free SO_2 present in the control white and red wines was higher in the unagitated than the agitated samples, as expected, the concentration at the end of the ten-day period was the same, i.e. no free SO_2 was detected at that point.

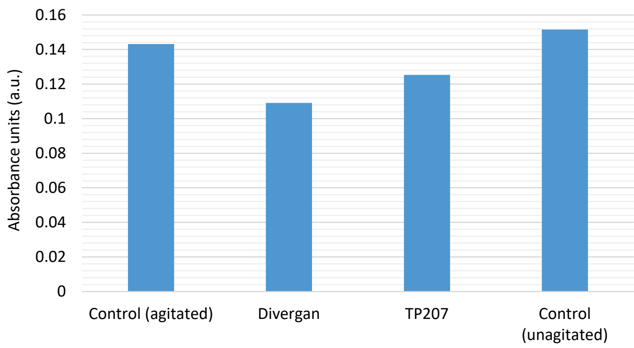

Figure 7. Absorbance values (at 420 nm) for commercial white wine treated with Divergan and TP207 following accelerated browning test. Untreated control wines (both agitated and unagitated) were included for comparison.

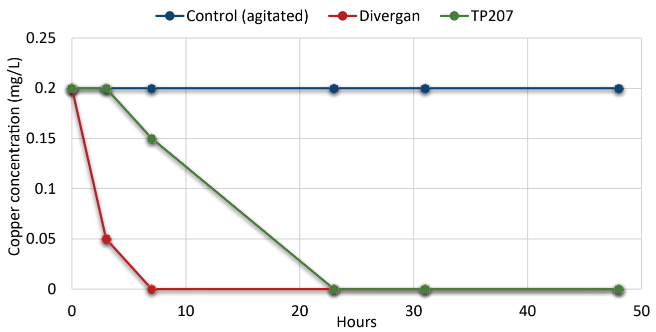

Figure 6a. Concentration of copper in commercial red wine treated with Divergan and TP207 over a 48-hour period. Agitated control wine (untreated) was monitored over the same period.

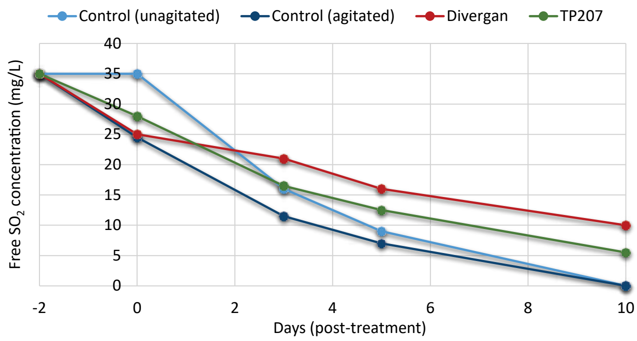

Figure 8a. Concentration of free SO_2 in commercial white wine following treatment with Divergan and TP207 over a 10-day period. Untreated control wines (both agitated and unagitated) were monitored over the same period.

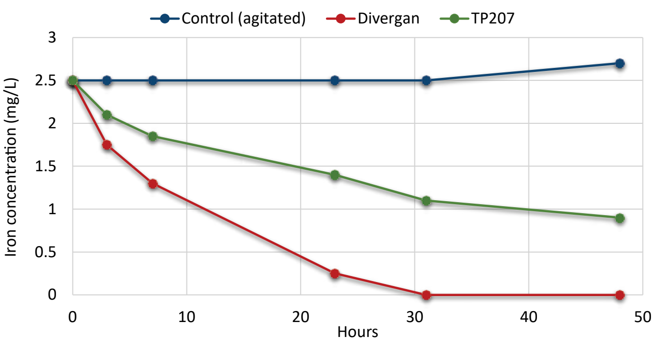

Figure 6b. Concentration of iron in commercial red wine treated with Divergan and TP207 over a 48-hour period. Agitated control wine (untreated) was monitored over the same period.

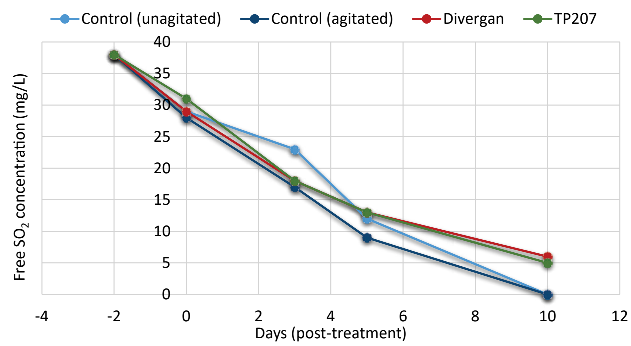

Figure 8b. Concentration of free SO_2 in commercial red wine following treatment with Divergan and TP207 over a 10-day period. Untreated control wines (both agitated and unagitated) were monitored over the same period.

Clearly, the removal of most of the iron and copper from both wines has resulted in a reduction in the speed with which oxidation reactions are able to take place, resulting in a higher residual concentration of free SO_2 after the accelerated oxidation period. The presence of trace concentrations of these two metals, as well as the presence of other transition metals, such as manganese (which was unaffected by the CLP treatment process) appears to provide a mechanism for the continuation of these oxidation processes, although at a lower rate than observed when iron and copper are present at their initial (untreated) concentrations.

The difference in the impact of the CLP treatment between the white and red wines may be due to the greater quantities of natural antioxidants that are typically found in red wines.

Tabulated results for the colour measurements carried out on the white and red wines are provided in Table 1 and Table 2.

Table 1. Colour measurements carried out on control and CLP-treated white wines across 17-day accelerated oxidation period post-treatment

OD280 nm (Total phenolics)	**Pre-treatment**	**0 days**	**3 days**	**5 days**	**10 days**	**17 days**
Control (unagitated)	7.391	7.52	7.14	7.15	7.62	7.55
Control (agitated)		7.60	7.05	7.16	7.46	7.29
Divergan		6.43	5.90	6.14	6.20	6.13
TP207		7.41	7.01	7.32	7.26	7.16
OD320 nm (Total hydroxycinnamates)	**Pre-treatment**	**0 days**	**3 days**	**5 days**	**10 days**	**17 days**
Control (unagitated)	4.43	-	4.51	4.58	4.77	4.76
Control (agitated)		-	4.51	4.59	4.70	4.60
Divergan		-	3.11	3.17	3.34	3.32
TP207		-	4.33	4.48	4.53	4.48
OD420 nm (Browning)	**Pre-treatment**	**0 days**	**3 days**	**5 days**	**10 days**	**17 days**
Control (unagitated)	0.073	0.071	0.065	0.079	0.098	0.115
Control (agitated)		0.074	0.066	0.081	0.096	0.120
Divergan		0.049	0.038	0.050	0.066	0.079
TP207		0.071	0.068	0.069	0.085	0.102

Table 2. Colour measurements carried out on control and CLP-treated red wines across 17-day accelerated oxidation period post-treatment

OD280 nm (Total phenolics)	**Pre-treatment**	**0 days**	**3 days**	**5 days**	**10 days**	**17 days**
Control (unagitated)	49.8	54.9	54.4	53.2	61.0	55.5
Control (agitated)		55.1	54.1	53.5	61.7	56.2
Divergan		51.1	52.0	54.6	59.9	53.1
TP207		53.6	53.2	55.2	63.2	54.1
OD420 nm (Browning)	**Pre-treatment**	**0 days**	**3 days**	**5 days**	**10 days**	**17 days**
Control (unagitated)	2.08	2.17	2.32	2.46	2.88	3.20
Control (agitated)		2.21	2.40	2.82	2.97	3.32
Divergan		2.07	2.19	2.25	2.46	2.68
TP207		2.10	2.24	2.36	2.69	2.92
OD520 nm (Red pigments)	**Pre-treatment**	**0 days**	**3 days**	**5 days**	**10 days**	**17 days**
Control (unagitated)	2.50	-	2.80	3.11	3.78	3.94
Control (agitated)		2.65	3.00	3.58	3.88	4.02
Divergan		2.49	2.68	2.76	3.12	3.33
TP207		2.52	2.76	2.95	3.49	3.65

Compared to the control (untreated) wines, both CLP treated white wines exhibited significantly different optical density measurements during the accelerated oxidation period, although the impact was minimal with the TP207-treated wine. Treatment with Divergan resulted in a significant decrease in total phenolics (OD280) for the white wine, with most of this being due to a reduction in hydroxycinnamic acid (HCA) derivatives (OD320). Both CLPs affected the browning indicator (OD420), although the effect was much more significant with Divergan.

The much lower 420nm measurement of the wine treated with Divergan suggests that the oxidation processes that lead to browning of white wine have been retarded by the removal of a greater proportion of iron during treatment with Divergan, as the copper concentrations were reduced to similar levels by both CLPs. The 420 nm measurement results here correlate reasonably well with those from the accelerated browning test employed immediately after resin treatment.

The impact of Divergan on reducing HCA concentration has been reported previously (Mattivi et al. 2000; Eder et al. 2003; Nicolini et al. 2004). The Mattivi (2004) study indicated that the reduction in HCA was driven by specific removal of trans-caffeoyltartaric acid (CTA), which is typically the most dominant HCA present in wine and active in both enzymatic oxidation and auto-oxidation reactions. Mattivi (2004) also noted a reduction in catechins following treatment, further explaining the reduction seen in the OD280 measurement in these trials.

For the red wine, all optical density measurements for the CLP-treated wines were lower than those for the (untreated) control wines. Again, the OD280 and OD420 measurements were lower for the Divergan-treated wine than for the TP207-treated wine, reflecting a modest reduction in phenolics concentration and browning. The relative impact of the CLP treatment on these indicators was much lower than that seen for the white wine, which, in part, explains the less significant impact that the CLP treatments had on the shelf life (free SO_2 level) of the treated red wine.

The two CLP treatments also affected absorbance measurements at 520 nm, which are indicative of red colour (pigments). Again, this is consistent with observations made by Mattivi (2000), who reported a relatively modest reduction in colour intensity and anthocyanin concentration in wines treated with Divergan. Both CLPs had an impact on the OD520 measurement, with levels slightly lower in the wine treated with Divergan.

The concentration of sodium was measured in the wines prior to treatment with the two CLPs and again following accelerated oxida-

Table 3. Sodium concentration and pH of control and CLP-treated wines prior to treatment and after 17-day post-treatment accelerated oxidation period

Sample	**Sodium conc. (mg/L) pre-treatment**	**Sodium conc. (mg/L) after treatment**	**pH pre-treatment**	**pH after treatment**
White wine				
Control (unagitated)	22.0	23.0	3.30	-
Control (agitated)		24.4		-
Divergan		31.5		3.40
TP207		51.0		3.33
Red wine				
Control (unagitated)	31.0	29.0	3.50	-
Control (agitated)		29.3		-
Divergan		33.0		3.55
TP207		51.5		3.48

tion, along with the pH levels of the wines, pre- and post-treatment (Table 3). As the TP207 used in these experiments was in its sodium form, an increase in sodium concentration would be expected in the wines treated with TP207, due to ionic exchange between the PVP-PVI copolymer and the iron and copper available in the wines. The actual sodium concentration increase observed is far greater than the comparative decrease in copper and iron concentration levels, suggesting that sodium cations have been exchanged with other components in the wines during treatment.

The pH increase in the wines treated with Divergan is small but significant, in comparison with the control (untreated wines). This effect has also been seen in previous studies (Mattivi et al. 2000; Eder et al. 2003). This increase may, in part, be due to a decrease in organic acid concentration that results from the CLP treatment. Studies carried out in 1995 (Green et al.) and 2007 (Mira et al.) showed that titratable acidity decreased in wines following treatment with Divergan; in the case of Green et al. (1995), the effect appeared to be due to a specific reduction in tartaric acid content for both white and red wines. The effect on tartaric acid was only significant for the red wine treated in the Mira (2007) study though, with no impact apparent for the Divergan-treated white wine.

In summary, Figure 9 shows the relative reduction in free SO_2 concentration between the control and CLP-treated wines during the first ten days of accelerated oxidation. This shows that the relative impact of the treatment was greater for the white wine than the red wine and that Divergan had a much bigger impact than TP207 on the shelf life of both wines. Some of this difference may be due to the fact that the Divergan used was a finer-grade resin than the TP207 and therefore had a much smaller mean particle size and a much larger contact surface area. It may also be due to the different affinity that each CLP displays in order to scavenge metals from the wines.

This study also shows that, although the majority of the iron and copper present in the wines can be removed via CLP treatment, the wines are still susceptible to the impact of oxidation, especially with respect to a reduction in SO_2 levels. This is particularly noticeable in the red wine treated with Divergan, which contained no detectable copper or iron post-treatment, but still exhibited a marked depletion in free SO_2 concentration during accelerated oxidation.

A recent study by Danilewicz (2016) indicates that manganese can play a significant role in the oxidation of wine, especially when copper and iron concentrations are high, and can facilitate the oxidation of 4-methylcatechol, in a model wine solution. The degree of impact that the manganese present in the wines used in this study (1.7 mg/L in the red wine; 1.2 mg/L in the white wine) could have during accelerated oxidation is hard to determine. However, it is possible that, even with depleted iron and copper present, the manganese is able to facilitate oxidation reactions which lead to free SO_2 depletion.

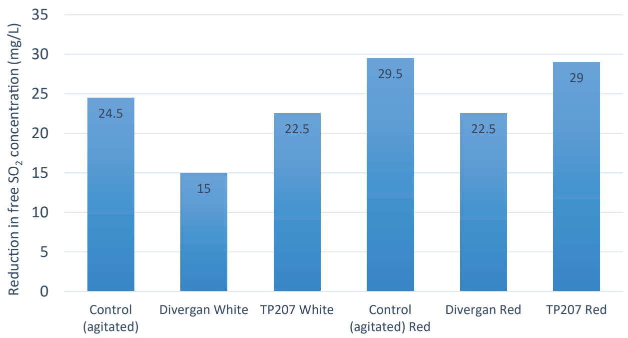

Figure 9. Loss of free SO_2 concentration in white and red wines following treatment with Divergan and TP207 over a 10-day accelerated oxidation period. SO_2 loss in the untreated (agitated control) wines over the same period are shown for comparison.

Summary

Both CLPs evaluated in this study have shown the potential for sequestering both iron and copper from wine, with the majority of both metals being removed from commercial white and red wines within 48 hours. The resulting wines have been shown, through accelerated oxidation treatment, to have a longer shelf life than untreated (control) wines, with a lower free SO_2 depletion rate and a reduced susceptibility to browning.

Further studies into the impact that these types of CLPs can have on metal sequestration and resulting wine shelf life are required to assess the degree of impact that CLP treatment can have on different wine types/styles and the potential risk that these resins may pose to resulting wine sensory attributes.

Acknowledgements

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Next generation yield prediction technologies

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Abstract

Accurate yield estimation is a critical aspect of both viticulture and winemaking as it affects the entire supply chain. Industry standard practice in yield estimation can take substantial resources yet still provide inaccurate results. In order to automate yield estimation methods we developed a method for generating high-resolution relative maps of visible vine parameters such as shoot or bunch density. The method is based on using low-cost cameras as sensors that can be mounted on vehicles that are already travelling through the vineyard. Camera data is processed to give a geo-referenced map without requiring an expensive GPS.

The relative yield maps can be generated from when the first leaves separate. This means that the maps can be used to adjust management practices such as trimming, thinning or mulching during the season. The processed images can also be used to map non-bearing sections of canopy and to identify missing vines, which has the potential to help in the detection of trunk diseases such as eutypa. The relative yield maps are converted into absolute yield maps using estimates from either sampling locations on the ground or historical data. Forecasts using these maps not only provide an overall tonnage for the block, but also an indication of the variation within the block without using a yield monitor.

No paper available, please view this presentation at http://bit.ly/16thWhitty.

Advances in the application of robotics to agriculture

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Abstract

Over the last five years there has been rapidly growing interest in the use of automated machinery and software processes amongst various agricultural and environment groups. The farm of the future will likely involve a 'system of systems' where teams of relatively small robots and sensors work together to collect information and perform mechanical tasks. In this talk, I will present our work in the development of robotics and intelligent systems for improving land and labour productivity of farms, and will provide examples from the broadacre agriculture, tree crop and vegetable industries. With better sensing, data analytics, and real-time control, robots will be able to collect vast amounts of precise information about the health and maturity of crops. This information, along with the automation of mechanical processes, will help to increase the efficiency of farming, leading to better yield and profitability. We will also start to see new capabilities such as variable rate planting and fertigation, minimal (if any) chemical usage, and selective harvesting. Through these advances, agricultural robotics has the potential to transform the way food is grown, produced, and delivered.

No paper available, please view this presentation at http://bit.ly/16thFitch.

Designing wineries for future quality wine production

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Abstract

The opportunities for designing future wineries for the delivery of high quality wines will be in environmental and sustainability considerations, process efficiency, winemaking precision and analysis of vineyard-winery interactions. The environmental aspects will require a deliberate consideration of the energy, carbon, chemical and water footprints of the winery activities and the partial or complete adoption of on-site capture and storage technologies. The production efficiency aspects will range from advanced adaptive process control for lot-specific fermentation and extraction patterns to the adoption of technologies that will enable fewer wine transfers (and therefore fewer tank washings) and of automated cleaning cycles that do not require labour and capture all cleaning solutions and water for reuse. The winemaking precision will come from automated monitoring of fermentation patterns coupled with real-time modelling of fermentation rates (and the corresponding rates of energy and carbon dioxide release) to drive temperature and redox conditions that convert grape chemistry into wine chemistry more effectively (minimising the extent of the need for fining, post-fermentation treatments and blending). The fermentor size will determine the resolution with which vineyard composition can be captured and the degree that the highest value grapes can be retained separately rather than being lost in larger, cheaper, multi-vineyard wine fermentors. Future winery designs will require the development of real-time dynamic models that can be used to test alternative seasonal and daily grape delivery scenarios and to quantify the interaction of the design and its energy and water systems. A number of examples of improved process technologies, such as phone-based density, temperature and colour and phenolic extraction patterns, fermentation control strategies based on sulfide formation or redox potential and process models that can predict future fermentation patterns, the likelihood of stuck fermentations and future heat release, from the first third of each fermentation, could also be introduced into existing winery facilities as well as new winery designs.

Webcast of this presentation available at http://bit.ly/16thBoulton.

Introduction

The design of future wineries will require a radically different approach, one in which the primary objective will be to achieve a chosen water, chemical, carbon or energy footprint or some aggregate weighting of these or other environmental impacts, not just a design based on grape intake or fermentation capacity. As an example, the limits for sodium and nitrates in discharge from a wastewater treatment system might be specified and this would prompt the question of the application of an aerobic treatment technology and ask what the sources of sodium and nitrogen in the waste stream are and where are they are coming from. This would lead to a linkage to the choice of cleaning chemistry, the materials used to remove tartrate deposits from tank walls, the reason why tartrates are on the wall, the choice of coolant temperature in the tank jackets and the practice of washing of yeast and pulp into the wastewater to be treated. This reverse engineering will require a team with knowledge in alternative cleaning and sanitation options, the temperature control of wine in storage, refrigeration systems and alternatives for the removal of solids prior to waste treatment systems, not just the ability to design a waste treatment pond that handles the biological oxygen demand. Such an approach will place less attention on delivery of stand-alone engineered systems that meet winemaking practices or peak loads and more on delivering integrated solutions that will provide minimal or zero environmental impacts, low water and energy intensities. It will include storage systems and more extensive automation but with a smaller operating labour requirement. It will also call for more lateral considerations of the daily dynamic interaction of energy and water loops between winemaking, buildings and utilities than presently exists in peak-load or capacity-based designs. Future design criteria might also include secondary impacts and value functions, even business risk and future benefits based on life cycle rather than the now more common short-term return on investment calculations. Some design choices might even be made to ensure approval by a local, regional, national or international certification program, rather than just achieving an acceptable return on investment or the lowest capital investment solution while meeting regulatory requirements.

The focus on limited water availability will quickly move the design questions to how to capture and reuse cleaning solutions, so that the water is used many times, not just once. This will lead to questions on what cleaning chemistries are required or preferred and what the basis of such cleaning protocols and chemistries are. At present there is a wide range of accepted practices for cleaning fermentors and storage vessels. Future designs will need these choices to be clearly established before considering a separate cleaning water loop in which spent solutions will be filtered and recovered for the next cleaning cycle. These considerations will require much more attention to be given to the chemistry of the starting water instead of just the volume of water required. The water will need to have a low mineral content (hardness) and an absence of silica as these lead to surface deposits on equipment and make water difficult to reprocess.

A future winery might choose to design passive barrel buildings with high thermal resistance in order to get smaller internal temperature fluctuations that will have little or no air conditioning requirement and have higher controlled humidity to provide less evaporative wine losses. All future buildings should have steeper sloping roofs that will be oriented for optimal solar energy capture during harvest and all roofs will be designed for rainwater capture rather than storm water run-off. Rainwater storage tanks will be a common architectural feature and 'wastewater' ponds will be missing.

It should be obvious that future design teams will need to have access to a level of understanding of cleaning chemistry, wireless technology, energy systems, vapour releases and water chemistry, passive buildings, on-site energy and water storage and all aspects of sustainability, far beyond what has previously been required for architecture and engineering design purposes. They will also be challenged to develop comprehensive value functions such as a life cycle view in order to make better choices in systems and their integration.

Water considerations

Future wineries built in many parts of Australia, but also in arid regions of Argentina, Spain, California and China, will have to be built so that they require much less water for operations than is used today. Water will be the dominant driver of such designs and the chemistry of that water will be critical to adopting membrane-based, water recovery systems. Future wineries will probably need to capture and store rainwater for at least six months to a year, so that on-site water tanks, either above ground or below, will become as common as the reservoir or dam in low rainfall vineyards. Future design analyses will require a deeper understanding of the thinking behind wine transfers and the barrel, container and tank washing required due to such tasks, and the water footprint of the cleaning protocol. A design principle of minimising the number of wine transfers will invoke some serious questioning of conventional thinking and winemaking practices. The most obvious alternative is to move towards treatment technologies that can be applied outside of a tank, with untreated wine being drawn from the base of the tank and the treated portion returned and layered onto the wine surface in the same tank. Examples of such treatments would be fluidised-bed potassium bitartrate stabilisation or electrodialysis and regenerable adsorption columns instead of batch additions such as Bentonite fining within tanks (Boulton 2014).

The cleaning protocol will drive the design of the cleaning water loop. Future cleaning water will need to be captured and reused as many as 10 times and recovered at a rate of 80% to 90% in each cycle. The decision to rinse-clean or rinse-clean-sanitise winery vessels and equipment will require a clear standard for surface viable counts of wine organisms, a contact time for solutions and the definition of the solutions including pH, other agents, their concentrations, temperature and possibly redox potential. These conditions will be needed for the design of the recovery membranes, the storage tanks and the monitoring and control components of an advanced cleaning water recovery system. The ease of cleaning by an automated clean in place system will depend on the fermentor/tank diameter and other internal features.

An essential part of the design analysis of water systems will be a value function for water, not simply the cost of water and its initial treatment. Apart from a strategic value, discussed below, this would be a value based on the water chemistry, in particular the absence of dissolved solids and silica. Since the deposits left on equipment surfaces when water has evaporated are proportional to the dissolved solids concentration and certain components such as calcium and magnesium carbonates, and more especially silica, will form crystals on equipment, pipework and on recovery filters. Even if demineralisation by ion exchange is to be used it will generate a new waste stream that is high in salts as a by-product. Clearly the initial water chemistry will influence the extent to which water can be recovered and reused as well as the performance of the membranes to be employed. There should be a value metric that would make water high in dissolved solids, hardness and containing silica expensive to use, and to encourage the use of water with less dissolved solids.

The possibility of eliminating surface deposits by a design choice to use rainwater as the starting water leads to the questions of capture and storage of winter rain until harvest use. Even in dry climates, the rainfall can provide all of the water required for tank cleaning if it is captured and reused several times. The winery at UC Davis has been designed to operate on one million litres of water captured from adjacent building roofs, in a region where the annual average rainfall is 450 mm, with annual episodes only half of this value. This water is essentially mineral free and when filtered to the reverse osmosis level it will be free of all microbes, viruses and toxins as well. The filter resistance (and therefore energy consumption) for reverse osmosis membranes varies with the salt concentration by a power index of 0.37 (Figure 1). As a result, rainwater requires about 20 times less energy to be filtered in recovery than sea water just due to the salt content alone. If the starting water is free of mineral content, the design of a recovery system for cleaning solutions using nanofilter membranes is facilitated and able to provide the recovery of the inorganic mineral buffers used to control the pH of contact conditions (Boulton 2015). The investment in tank storage will then be offset by the ease of cleaning, lower chemical use and discharge, no scale deposits and an ability to use water for at least ten cycles based on a recovery rate of 90%. The low salt content and absence of silica in rainwater are essential for this system to perform efficiently but in the face of limited water it is probably an essential change for future wineries and food facilities to adopt.

Energy considerations

Privatisation of power grids and the growth in solar photovoltaics adoption have resulted in time-of-day billing and real-time pricing of electricity which will be important to include in any design and costing of energy systems. The limitation of solar power has traditionally been that it is only good during daylight hours and requires grid connection so power can be supplied at other times. The design options today include banking of the excess energy produced during the day in one of several forms, to be used at another time typically in the following 16 hours or over the next few days. The circuits for DC power from the panels can be coupled with other intermittent energy generation (such as wind power) into stored energy for later use both for building and process power. The storage can be as DC electricity in lithium ion batteries which is already a reality.

The experience curve for solar photovoltaic panels, sometimes referred to as Swanson's Law, notes that the unit cost ($/W) falls by 20% for every doubling of the cumulative installed capacity (Swanson 2006). This corresponds to about a halving in price every ten years at the current rate of adoption in the US, or 8% pa. Electric vehicles presently display a doubling every two years in the US. The change out of lithium ion batteries from electric cars is now supplying a secondary market for building energy battery storage systems. In 2015 there were 1.2 million electric cars in the US and after a five-year lifetime in vehicles these batteries will provide 25 GWh of storage in the 'second life' market for buildings and industrial storage systems. Germany has set a target of one million electric cars on their roads by 2020, or another 25 GWh of battery storage for future buildings.

The rate of the experience curve for lithium batteries is now approximately 43%, Figure 2. The unit price reductions over the past 4 years have been at close to 8% pa based on two doublings in installed capacity and this is expected to hold or increase with future electric

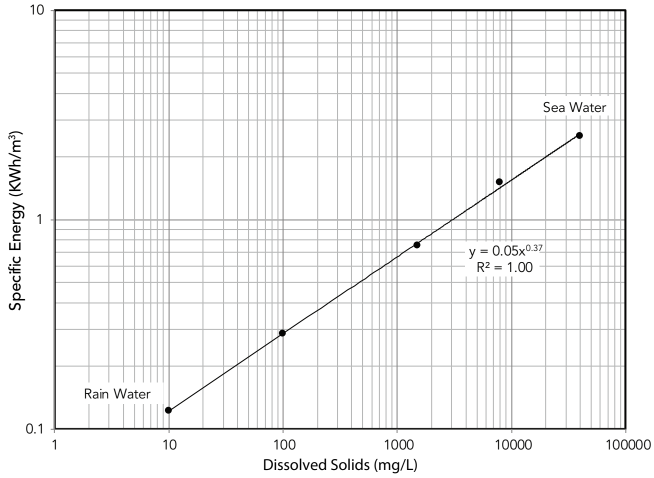

Figure 1. The effect of salt concentration of water on the specific energy consumption of reverse osmosis membrane filters (Boulton 2016)

car adoption. By comparison, the rate of the experience curve for solar photovoltaic panels was close to 35% during the two previous decades (these experience curves have been derived from cross-plots of price versus installed capacity using the original time series data of Savvantidou et al. 2015, Liebreich 2012 and Liebreich 2015). This means that any future winery design should consider a battery option for on-site energy storage at the building and industrial scale since it, like solar panels, will be the rare example of systems whose price will continue to decline into the foreseeable future.

The banking of daily solar energy into captured forms such as electrical (Li batteries), thermal (ice banks), sub-cooling of water storage tanks and pressure forms (compressed air) provides the possibility of capturing rather than returning excess daily energy production and provides a buffer for the intermittent harvest requirements. Subsequent considerations will be the choice of DC motors on systems that would be used for such storage to avoid the transfer losses associated with conversion to AC power. Winery equipment selection will come into play in the adoption of DC-powered air compressors for bladder presses rather than the on-board 220 VAC and 440 VAC units that commercial presses presently employ. The ability to slowly compress large volumes of air over a 24-hour cycle rather than the on-demand high power surges of the current systems, will enable more efficient allocation of energy throughout the day based on other demands. Recent advances in the technology of invertor systems will provide the ability to match the phase angle to that of the loads as well as matching the frequency and amplitude, something that is not possible in the present generation of invertors and is particularly important in three-phase circuits.

Future winery designs should also consider choosing passive solar hot water loops as an alternative to boiler and fuel-based hot water systems. The need for steam should be questioned as unnecessary given that effective thermal killing (five-decade reduction) of organisms such as *E. coli* can be obtained at 60°C in less than five minutes. The benefits of not using hydrocarbon fuels, elimination of flammability hazards, fire code requirements and worker safety training associated with them will need to be included in any economic analysis of such alternatives. A number of commercial solar tube arrays can now cover walls and roofs as a shade layer while at the same time being less conspicuous as roof fixtures.

Chemistry considerations

Future winery designs will probably be expected to operate with smaller releases of chemical components as vapours, wastewater or solid waste. In terms of vapour emissions obviously carbon dioxide is the major release, with smaller releases of ethanol during fermentation and much smaller releases of ethanol and sulfur dioxide during wine storage and transfers. Designers might call for direct vapour capture at fermentors and removal from inside buildings to keep the carbon dioxide and ethanol at their most concentrated levels for possible carbon sequestration as a carbonate salt, and ethanol capture as a soluble acetate product.

The water releases will focus more on sodium, phosphate and nitrate, as undesirable discharges into streams and ground water that are not addressed by conventional water treatment systems designed for reducing biological and chemical oxygen demands (BOD and COD), respectively. The questioning might begin with a component such as nitrate ions and might have to be traced back to its source or sources in the winery, where it is primarily formed from amino and organic nitrogen components as a result of microbial action in aerobic treatment systems. This will prompt further discussions of why an aerobic treatment system should be chosen and additionally why the BOD treatment requirement is so high and why the organic nitrogen is also high. This last part is due to the practice of washing yeast and grape pulp into drains and allowing them into the treatment system.

The next major group of chemical release comes from cleaning and sanitising practices, so the design team will need to address the chemical components, their concentrations, and the temperature and contact time required for a chosen practice. This will require a more rigorous description of death kinetics of winery organisms than exists today. At best, designs could be based on *E. coli* and the next question is what level of elimination, usually measured as decade reduction of viable organisms, is required. Winery practices might be different for 'cleaning' versus 'sanitising' and for fermentors versus bottling tanks. While combinations of chemistries will provide the required cleanliness, the next questions will be: what are their contributions of these to the BOD and COD, and what are their release levels and footprints for sodium, phosphate and nitrate?

If a specific objective is to capture and reuse cleaning solutions, now their interaction with crossflow or nanofilter membranes will need to be a consideration. For cleaning protocols that call for high and low pH solutions, monovalent inorganic buffers can be chosen that are equimolar and become a dilute neutral salt solution when mixed for building use (toilets, landscape) or vineyard use or water discharge. Dilute paired potassium solutions of hydroxide and bisulfate meet these requirements, are fully recovered on a nanofilter with the water they are contained in and make no contribution to BOD or COD loads (Boulton 2015). Such a system would allow the reuse of both water and the solution chemistry many times, not just once, reducing both footprints simultaneously. The same reference provides killing curves that can be used for design purposes for hot water and dilute hydrogen peroxide solutions at acidic, neutral and basic pH conditions.

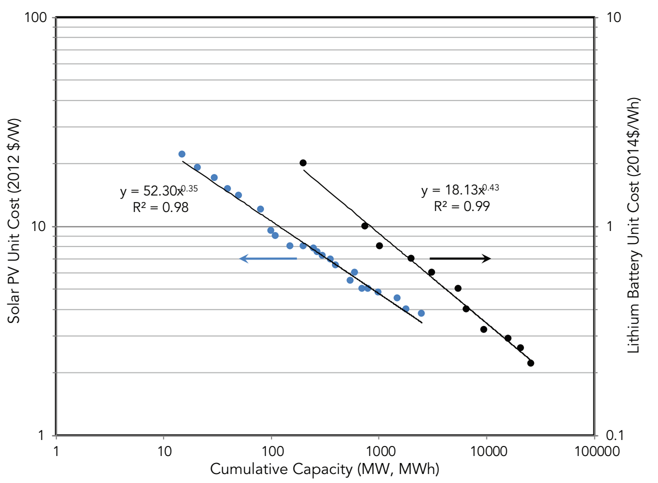

Figure 2. The experience curves of the unit cost for solar photovoltaic panels and lithium ion batteries

Process efficiency considerations

There are some winemaking choices that should be challenged if future winery designs are to be more efficient and more sustainable. The first of these is the choice of the time required to cool grape must or juice on delivery to the winery, prior to a pre-soak or with the onset of fermentation, or during cold stabilisation of wines. The times often specified are not supported by research or data but rather perception, yet they result in the greatest and often most intense load requirements on the winery cooling system. In engineering terms the delivery of such heat transfer rates usually leads to establishing very low coolant temperatures, especially when jackets are used for such actions. As will be seen below, the application of low refrigeration temperature is very energy inefficient and secondarily can lead to the unnecessary deposition of potassium bitartrate salt onto surfaces when that was not the intention. The simple reduction of these surge loads by allowing twice as long for the temperature to be achieved would be a major advance in future winery designs.

A popular design aspect of temperature control of jacketed fermentation tanks is the use of standard control systems in which the coolant flow continues until the juice or wine temperature in the tank has reached the set point. While this seems to meet the objective, it fails due to the limitation of heat transfer being the conduction within the wine and through the wall, not on the coolant flow side. This results in cold or only moderately warmed coolant being returned to the refrigeration system. The lower temperature difference between the coolant and refrigerant reduces refrigeration plant/chiller efficiency. The application of pulsed jacket flows in which only warm coolant is returned can be implemented with a temperature sensor on the jacket outlet that controls the return flow of the coolant. This action, using a rise of three to five degrees, prevents the return of cold or partially warm coolant to the chiller, reducing the heat transfer rate in the chiller and helps to maintain flow and pressure within other sections of the delivery manifold.

The design of future fermentors might also consider connections that facilitate not only automated cleaning such as clean in place, but also the ability to capture the cleaning solutions to make it easier to implement recovery and reuse for water considerations. They should also be fitted for vapour capture or the ability to gather most of the vapours as they are emitted from the vent. This will allow their removal from the work space and deliver them in a concentrated form for sequestration of ethanol stripping or other considerations.

Precision of winemaking considerations

Perhaps the greatest design improvements for fermentors lies in the widespread installation of dedicated pump-over or mixing lines for each fermentor. These will be needed for automated operations but more importantly they will become the location for future sensor systems that will provide the fermentation information that most winemakers could use to take critical actions. The fluid passing through such vertical sensor sections will provide a more representative and reliable estimate of the properties of the wine volume than in-tank wall-based sensors provide.

The instrumental measurement of density using pressure transducers was pioneered more than two decades ago yet few wineries today have such measurement systems, let alone in a form which can be immediately accessed either at the cellar or remotely. The ability to see the evolving fermentation pattern will be a minimum necessity in future winery designs, Figure 3a. The ability to measure this leads immediately to the display of the rate of fermentation from the first derivative of the density curve, Figure 3b. This trace can be used to understand the potential for an incomplete fermentation usually by a third of the way into the fermentation. Its peak represents the point at which the growth rate of the yeast is equaled by their death rate and there can be no higher fermentation rate unless the temperature is raised. This peak rate and the time at which it occurs form the basis of predicting successful or incomplete fermentations. Knowing this and being able to raise the temperature in a timely manner is crucial in preventing an incomplete fermentation and its subsequent handling and a potential loss in value.

The next measures that should be incorporated into future fermentation sensor arrays are yeast cell mass and the redox potential of the fermentation, Figure 4a. Work is in progress to perfect the application of high frequency capacitance measurements for the estimation of yeast cell mass in the presence of bubbles and suspended grape solids. By comparison, the redox potential has been proposed as a determining factor in the formation of hydrogen sulfide when elemental sulfur residues are at significant levels (Rankine 1963). It may also be important in the formation of thiol and thioacetate components during fermentation as well. The wide variation in the depth to which the redox potential curve falls seems to be related to both the yeast strain and the juice composition and measuring it might lead to an improved understanding of the causal conditions. Other sensor measurements that would enable more precise conversion of grape composition into a wine are colour extraction and phenolic extraction curves (Boulton 2014), Figure 4b.

The data collected during fermentations can be used to fit fermentation models which will provide both better fermentation diagnos-

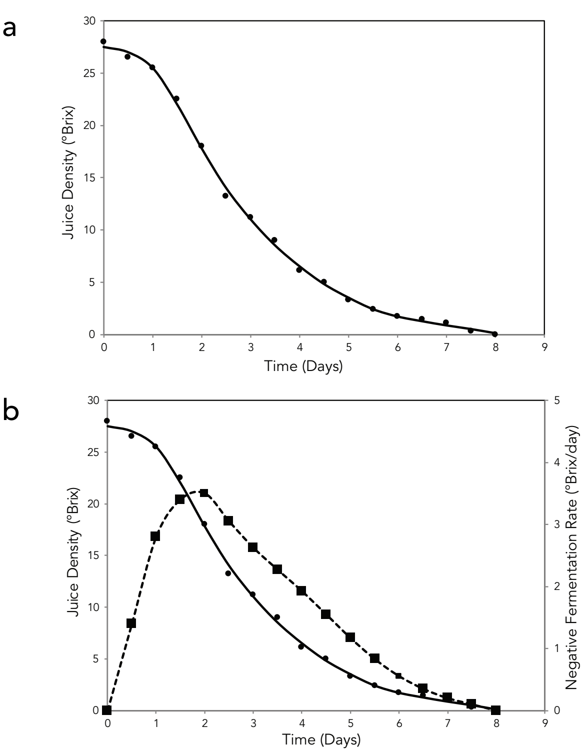

Figure 3. Future real-time fermentation displays for winemakers; a) Density, b) Density (●) and rate of fermentation (■)

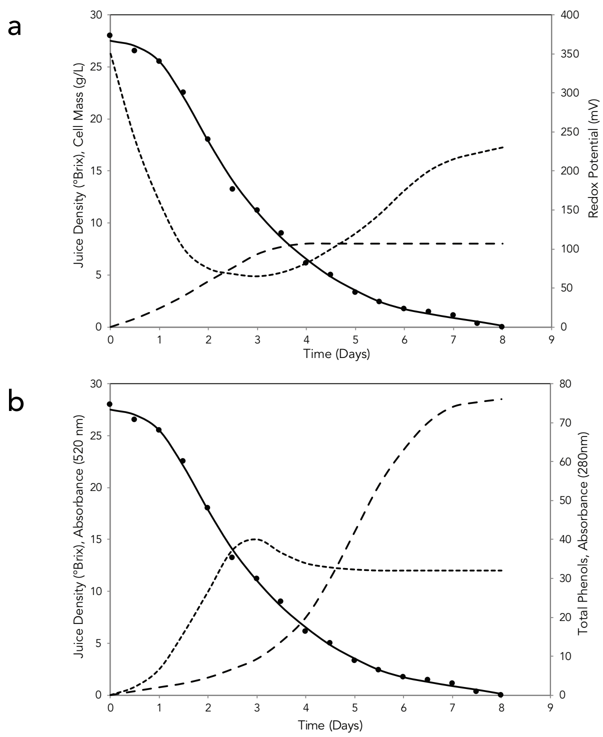

Figure 4. Future real-time fermentation displays for winemakers; a) Density (●), yeast cell mass (- -) and redox potential (--) and b) Density (●), colour (- -) and total phenol content (--)

tics and a prediction of future rates. The rate data could be used in the planning of hourly and daily winery fermentation cooling requirements and this would be needed for smarter allocation of available energy into storage forms. The ability to view such data and fermentation patterns on personal phones, anywhere in the world (with cell reception) has been possible in recent years. In the future winery fermentation patterns will also need to be returned to the fermentor so that it can be viewed by a person standing in front of it, using Bluetooth technology. This avoids the searching of databases or screens for the information of interest and the seamless display of fermentation information within the cellar.

Winery building and barrel room considerations

Future designs of winery buildings might range from barrel and case goods warehouses or entire facilities underground, mostly for energy efficiency and thermal stability reasons. The option to design for less variable temperature and humidity patterns will prompt a discussion of why these occur and that will lead to analysis of thermal insulation of the building envelope. While this is usually addressed by installing an air conditioning system, future designs will place more emphasis on limiting heat gain rather than using energy to remove it after it is in the building. The complication for the high humidity in barrel ageing spaces is that it is the variation in temperature that causes the variation in humidity and that air conditioning systems condense water out as they cool the air. This requires that water be added back usually in the form of a spray or mist to return the humidity to the desired level.

The design of a more energy efficient building will begin with a consideration of the diurnal temperature curves at mid-summer and mid-winter at the site. Many warm climates will have a diurnal range of 20°C or 30°C in summer. The design objective might be to reduce the natural internal air temperature oscillation to 2°C or 3°C, that is, a tenfold attenuation in the outside condition. Such a task can be met with a very high insulation envelope or placing the building underground or below grade or with an earthen wall berm, so that the outside wall temperature is significantly lower than the surface temperature of the above ground version when the solar radiation is included. The second consideration will be the night air temperature minimum as this will determine its usefulness as an early morning air exchange to sub-cool the building.

The Jess Jackson Sustainable Winery Building at UC Davis is an example of a highly insulated envelope that maintains small daily temperature oscillations at 2°C when the outside air is between 35°C to 40°C, without air conditioning, Figure 5. In this example night air is introduced at about midnight, since the outside air is close to the inside temperature of 23°C to 35°C and the fan operates for less than an hour each day. Note that if the intake air was changed to the minimum air temperature, generally 15°C and at 5 am during summer, the temperature could be lowered to 20°C easily or the building could be deliberately sub-cooled to overcome a heat event lasting for several days when the air intake is suspended. The building envelope has a thermal resistance value of RSI=10.6 (R=60) in the walls and RSI=13.2 (R=75) in the roof. It also has an option for a below-grade, rock bed capable of being cooled after the building has been cooled, with the thermal storage in the rocks, at the night air temperature of 15°C.

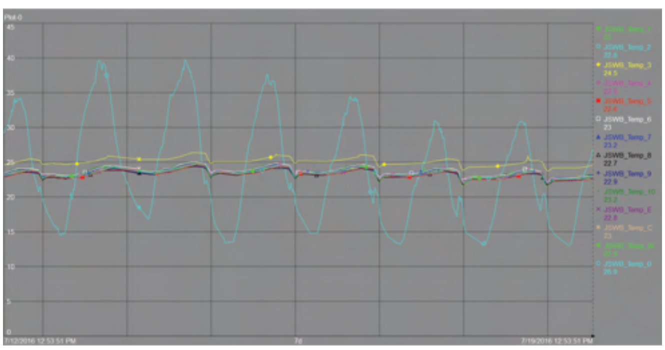

Figure 5. The daily pattern of outside (blue-green) and inside air (all other colours) temperatures in the Jackson Building at UC Davis, over a one-week period in July 2016. The aqua (cyan) curve is the outside air temperature, the cluster of other curves are temperatures in rooms within the building.

Such buildings should become the reference for future barrel ageing and case goods buildings, where little air turnover is required and occupancy is a minimum. If this was to be humidified, the need for rehumidification has been eliminated by not having the water loss associated with an air conditioning system.

A plausible alternative for building modulation in some locations is to use deep soil temperatures as an exchange sink for air or water cooling. This involves an extensive tubing loop of small diameter so that it has high surface area to volume ratio. Such geothermal systems are limited by the soil temperature profile which is in turn a function of the soil type and other features. Figure 6 shows the annual temperature of the air, surface and soil at 6.1 m depth, based on a study of soil temperatures in Griffith, NSW (West 1952). Note that while the summer air, surface and deep soil temperatures are 33°C, 28°C and 20°C, the deep soil temperature actually warms slightly in winter but stays within a 2°C range throughout the year. Any building or geothermal tube system placed at this depth would have such a modulated external condition, and be essentially constant at this temperature all year round.

Other helpful design considerations for winery buildings will be the understanding of the role of outside wall temperature on the heat gain of a building. The surface temperature in direct sunlight can be close to 20°C above the prevailing air temperature and this can account for as much as 25% of the building heat gain. Covering of all exposed walls and roof, by solar panels, passive solar tubes, large overhangs or shading barriers can have a significant effect on reducing the energy required for temperature control of the building.

Cooling and refrigeration system considerations

The phase out of chlorofluorocarbon (CFC) refrigerants was originally called for by the evidence and recognition that Freon 12 (or R-12) and related refrigerants were accumulating in the atmosphere and responsible for depletion of the upper ozone layer, first reported in 1985. This led to the adoption of hydrochlorofluorocarbon (HCFC) refrigerants like R-22 that have a much lower ozone depleting potential on a transitional basis and hydrofluorocarbon (HFC) refrigerants like R-134a that essentially have no ozone depleting potential. Today, the phase out of HFC refrigerants is being mandated due to their global warming potential (GWP). The phase out of HFC refrig-

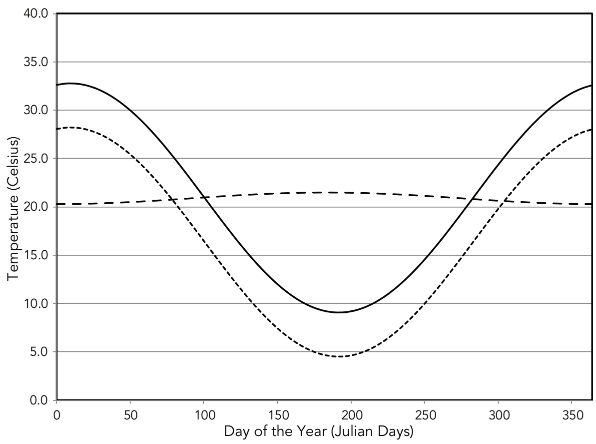

Figure 6. The calculated air, soil surface and deep soil temperatures at an interior site in Australia. Air (—), surface (---) and deep soil (- - -)

erants in the European Union began last year from a baseline of the average between 2009 and 2012. The reduction to 93% of that level is required this year, with 63% in 2018 and 45% in 2021, to 21% by 2030. Other countries are following this move but with other steps and timetables. Future refrigeration systems will likely see a return to ammonia (R-717) with a GWP of 0, carbon dioxide (R-744) with a GWP of 1, propane (R-290) with a GWP of 3.3 or similar refrigerants (Anon. 2016). The hazardous conditions that were previously associated with ammonia and carbon dioxide systems could be eliminated with the advances in gas sensor technologies for leak detection and advanced ventilation pipework or by locating these systems outside. These changes are coming not from considerations of energy efficiency or physical properties of these refrigerants but from the long-term environmental considerations of their chemical properties in the atmosphere.

The performance of a refrigeration system depends on the temperature at which the refrigerant evaporates at the intake of the compressor and the temperature at which the compressed gas is condensed back to a liquid in the condenser. The measure of this efficiency is the coefficient of performance (COP) and this relates the electrical energy consumed by the compressor in proportion to the energy removed by the cooling system. The evaporation temperature is generally chosen to deliver a coolant temperature that is desired for process conditions, such as the specified juice or wine temperature. The energy efficiency decreases and the power requirement increases as colder coolant is required, typically by 3% per degree Celsius. A similar decrease in efficiency is seen as the condensation temperature increases. Design choices of a 5°C warmer coolant and a 5°C cooler condenser should yield electrical energy reductions of 30% and can be compensated in heat transfer rate by higher flow rates and pulsed-jacket cooling control.

Future winery designs which will call for improved energy efficiency and a smaller power requirement will have to give renewed consideration of water-cooled condensers using a water loop rather than a cooling tower and heat removal from this loop will typically be by exchange with stored water tanks. The thermal mass of such tanks will eliminate evaporative water losses from the cooling towers and the delivery of temperatures similar to, or cooler than, the wet bulb temperature of the air as current designs employ.

They will also have to consider operating at warmer coolant temperatures in winery operations so that the refrigerant can evaporate at a warmer temperature and be more energy efficient. Currently the widespread use of glycol (or other aqueous-organic mixtures) coolant temperatures of zero to −5°C creates a number of related issues in wineries. The first is that these temperatures are the cause of significant crystallisation of potassium bitartrate on the inside walls of jacketed tanks and related pipework, even if the wine is at a warmer condition. The second is that the energy losses to ambient air are higher than need be and the third is that the energy consumption of the refrigeration system is greater than it needs to be, simply due to coolant temperature choice.

The tartrate deposition problem on the inside wall due to low temperature coolant will show up in the cleaning procedure where hot water or high pH sodium hydroxide are used to clean the surface. These indirect impacts will appear in the chemical use for cleaning, the sodium concentration of the wastewater and the energy requirement of the hot water system. All of these familiar conditions can exist in wineries because they are out of the realm of normal considerations of a heat transfer calculation to establish a cooling capacity for juice or wine chillers or tank jackets.

The adoption of water as the coolant fluid operating at 3°C to 5°C will also allow part of the daily peak electrical generation from a solar array to be banked in the form of an ice bed and retrieved by direct heat transfer with the coolant water when needed. Ice has a very dense thermal storage capacity because it includes the energy of latent heat in it. A bed of ice cubes will enable water to flow through the bed and offers faster heat recovery than solid block ice banks.

Real-time modelling of delivery and activity patterns

In the future, the design process of an efficient winery will have to develop an event-driven simulation model capable of doing hourly (or faster) analysis of the changing conditions of all systems. Such models will have to be capable of analysing the expected impact of various grape delivery patterns by day, week or a season. Such analysis will be required to understand the equipment size and number choices for both primary and secondary impacts on fermentation, pressing and receiving, all energy, water and cooling systems, and staff resources.

With the ongoing development of autonomous scanning of vineyards for crop level and grape maturity, the next phase of such data collection will be the sharing of the associated harvesting information in real-time and the incorporation of that into the daily simulation model at the winery. This will be needed to create a more accurate daily schedule with real-time updates of activities, resources and tank availability for both short-term needs and decisions but also expected scenarios several days out. These models will be linked to the energy management system that will allow the distribution of on-site energy into secondary storage systems on an hourly basis.

It is assumed that all grape delivery will be captured by RFID, Bluetooth or scanners so that identity, source, vineyard location and timestamp are captured for traceability, linking to analytical information, and winemaking decisions and grape contract payments.

Strategic, financial and enterprise costs in winery design evaluations

In order to design wineries for present and future environmental conditions, the investment analysis will need to use more advanced financial models than are commonly employed. The present situation of environmental risk to wineries can be seen as a direct result of an overemphasis on the short-term ROI approach and the inability of this practice to result in designs that can address or adapt to ever-changing and business threatening conditions such as drought and water scarcity, unpredictable energy expenses and sustainability issues such as carbon dioxide in the future.

Alternative methods of analysis such as life cycle costing and risk assessment analysis can be used but the challenge arises how to express them on an annual cost basis especially when other financial allocations usually have much shorter timelines. One possibility is the concept of insurance where an annual insurance premium would be expensed as the staggered investment to avoid some risk. Most businesses pay insurance for fire, floods, earthquakes or maybe drought and crop loss without questioning the probability of such events, only the coverage of the policy. Such an approach would allow for the design of wineries based on the risk of business losses associated with a drought (cancelling grape contracts, not producing the expected wine volumes, loss of shelf space and possibly loss of brand value) to support the investment of rainwater storage, on-site energy systems and capture and multiple-use cleaning water systems. In reality the loss to the enterprise of a water limitation is usually a multiple of the savings that would be used to justify an investment in water savings. At present, design justifications ignore the probability of drought and use the return on investment based on annual cost savings of water purchases, pumping, treatment and labour with capital depreciation over three or five years.

Another factor in a design approach that uses investment return is that it cannot see the loss of value due to downgrading of high value grapes due to a shortage of fermentors to keep them as separate lots.

Due to the economy of scale, larger fermentors will always be cheaper per litre than small ones and that the highest return of a design will always be fewer, larger fermentors for a given wine production. The problem is that when high-value grapes cannot be kept separate, the enterprise loses its highest value wine, but it probably will not show up in the financials immediately, or ever be traced back to a shortage of fermentors capable of capturing them, which was actually a design choice, driven by the return on investment. The resolution to which a winery can keep high value grapes separate is the precision to which they can convert their best grapes into their best wines. This further supports the notion that there is a need for a more comprehensive value function that can be used when design decisions are being made about the size and number of fermentors.

In some quarters, the compounded decline in unit cost of solar panels and lithium ion batteries is a difficult concept to accept in a world where costs typically increase due to inflation and or scarcity. This can become an outdated attitude and some people have limited ability to embrace these changes in a constructive manner in critical design deliberations. This human factor can lead to poor design choices and only heightens the need for a more comprehensive financial analysis method.

Finally, publicly traded companies are now being asked to make more disclosure with not only a sustainability report in their annual reports, but also an assessment of risk to the business due to sustainability forces. In the future, all companies, even small family wineries, will have to begin posting their annual energy, water, carbon and chemical footprints in order to retain credibility. As sustainability has matured, it has entered a new phase in which certificates and claims are beginning to become so common that they are losing their impact. Future wineries need to be designed with this in mind so that it becomes an imbedded aspect of the design.

Acknowledgements

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Designing and implementing efficient production systems

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Abstract

Accolade is the fourth largest wine company in the world, with around 40 million (9 litre equivalent) cases sold in 2014. It is the largest wine company in the UK and Australia and is the custodian of brands from key New World wine-producing regions. It has the largest wine bottling, warehouse and distribution centre in Europe and the largest cask packaging facility in Australia. In Australia it has seven winery sites in four states, and has a presence in 20 premium winegrowing regions.

When introducing new systems at Accolade, we always start with the same question: what are the 'needs and wants' of our customers (both internal and external) that we are trying to achieve? With this in mind as a starting point, a cross-functional project team is developed and the scoping process begins, using the following techniques:

- *Describe the problem/opportunity*
- *Whom does it impact?*
- *How does it impact?*
- *When does it impact?*
- *What are the impacts from this problem?*
- *Where is the problem manifesting itself?*
- *Why should the problem be fixed?*
- *What will happen if the problem is not fixed?*
- *What are the key benefits from fixing the problem?*
- *Outputs delivered/objective achieved*

As well as enhancing the quality of the wines we produce, all the projects that we have completed help to run the business more efficiently, while significantly reducing the impact on the environment.

Some examples of projects include:

- *Improved benchmarking scheduling and planning of all winery operation from intake of fruit until bottling*
- *Improved focus on workplace training and succession planning with employees in all departments*
- *Improved packaging technology and efficiency, including in-line check weighing and total dissolved oxygen analysis.*

No paper available, please view this presentation at http://bit.ly/16thHodgson.

Australian wine in 2050

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Abstract

2050 is only 34 years away. Many of today's wine drinkers will still be enjoying a glass or two of wine with their friends and family over a good meal. Wines from the 2016 vintage will still be in some of their cellars - as the wines of the early 80s are today. But can we assume that the wine style of 2050 will be similar and recognisable to the 2016 wine drinker, give or take a bit of fashion?

34 years is a generation. It's almost enough time to plant a vine and for it to become old. It is enough time to discover, plant and popularise a new variety or wine style. It is certainly enough time to greatly increase our fundamental knowledge of what is happening in the environment, the vineyards, the vine, the cellar and the bottle (or whatever container we might be using to package wine).

If we assume that the Australian wine industry will continue to discover, learn and apply knowledge at an ever-increasing rate, what could we know by 2050? Can we assume that our social and natural environments will let us continue to evolve? Will we still have a social licence to make wine? Will there be a place for viticulture in a world with a global population of 9.6 billion and a changed climate? If so, how big will the Australian wine industry and its key players be, how will it be structured and how will we be engaging with our competitors, customers and consumers? The answers will of course depend on many factors, some of which may be out of our control. When we look back from a buoyant and exciting 2050 it will be because we have been responsible, efficient, curious and clever, fostered strong relationships and dared to dream. But will we still have a corkscrew in the kitchen drawer?

Webcast of this presentation available at http://bit.ly/16thRose.

What was happening in 1982?

For those that can remember 1982 it is worth a quick reflection on what was happening then; what was influencing society, science and technology and how we communicated with each other.

- In 1982 Argentina invaded the Falkland Islands and the United Kingdom went to war over it. The CD player was sold for the first time (The People History 2016), the first biosynthetic (genetically engineered) human insulin became commercially available (American Diabetes Association 2012) and the *Time* Man of the Year was The Computer (Friedrich 1983) heralding the beginning of the information age.
- In Australian politics, Malcolm Fraser was the Australian Prime Minister, Neville Wran the Premier of New South Wales and Joh Bjelke-Petersen the Premier of Queensland. During 1982 John Bannon was elected as Premier of South Australia and in Tasmania protests about the Franklin Dam had begun (Anon. 2016).
- Random breath testing was introduced for the first time in Australia in 1982 in New South Wales (Homel 1986). We were watching *The Man from Snowy River* and listening to the *Eye of the Tiger* by Survivor as television channels Nine, Seven and the ABC conducted stereo test transmissions for the first time (Anon. 2016).
- In Australian sport The South Melbourne Swans moved to Sydney, Carlton defeated Richmond to win the VFL premiership, the Commonwealth Games were held in Brisbane and Gurner's Lane won the Melbourne Cup (Anon. 2016).
- It was the beginning of the internet as we know it as TCP/IP became the protocol for ARPANET (Zimmermann 2012). While we've had car-mounted mobile phones since 1981, Australia's first hand-held mobile phone call wasn't made until 1987 (Moses 2013).

The Australian wine industry in 1982

- In 1982 South East Australia was in severe drought. The Jimmy Watson Trophy was won by Hamilton's Ewell Vineyards Mildara JW Classic Coonawarra Cabernet Shiraz 1981 (Jimmy Watson's) and Terry Lee was the Managing Director of the Australian Wine Research Institute.
- The *Australian Grapegrower and Winemaker* magazine had features on grafting of vines, cover cropping and 'T' trellising. White wines were in demand; the irrigated Riesling price increased to $270 per tonne compared to Shiraz which fell to $145 (Hesketh 1982) and Americans hadn't yet 'discovered' Australian Shiraz – confusing it with Petite Sirah (Anon. 1982).
- In the innovation arena Brown Brothers had just released their first Tarango – a variety bred specifically for Australian conditions. Delta Airlines were serving wine in aluminium cans (Hesketh 1982).
- In 1982 the industry was working towards an oversupply situation, and was only a couple of years away from the Federal Government's financed vine-pull scheme (Anderson 2015). Australia had 42,457 bearing hectares and crushed 499,777 tonnes of wine-grapes (Anderson 2015).

Snapshot in 2016

Since 1982 Australia has changed from having a mostly domestically focused wine industry, to a globally significant wine producing country. In 2016 Australian wineries crushed an estimated 1.81 million tonnes (Battaglene 2016).

In the last 34 years, we have thought about, researched and adopted innovations such as: partial rootzone drying; mechanical pruning and harvesting; trellising; and rootstocks and precision agriculture through remote sensing technologies. We now know much more about wine yeast and bacteria and have a plethora of them to choose from; research into flavour chemistry has explained and identified flavours in grapes and wines such as terpenes and thiols as well as other influences of wine quality such as *Brettanomyces* and smoke taint. We have also spent a lot of time learning about consumer preferences and tried to make wines that they enjoy (Wine History 2015).

The few listed subjects above, and hundreds more, have been highlighted, discussed or disseminated at ASVO (Australian Society of Viticulture and Oenology) seminars and these Australian Wine Industry Technical Conferences (AWITC) over the last 34 years, and most of the concepts, or what has followed on, are fundamental – often taken for granted in our day-to-day winemaking lives today.

Some of the most recent work has been that around the 'discovery' of the genomes of the organisms that we work with: humans, grapevines, yeast and bacteria, the significance of which is now being revealed. The understandings that we will get from these discoveries will shape much of the next 34 years….

2050

In 2050 there will be a buoyant and exciting Australian wine industry. The following discussion looks back from 2050 and covers at a very high level some of the things that the author thinks we will have had to do to get there. It's not an exhaustive discussion by any means, and has drawn heavily on the other presentations – taking the opportunity to make this paper a non-chronological summary of the 16th AWITC. It is deliberately optimistic – the risks and threats for the future can be inferred if we don't achieve this end.

The author believes that we can be a buoyant and exciting Australian wine industry in 2050, part of a strong global community. We are already an incredible industry, full of passionate people. Over the next 34 years we will have shown ourselves to be responsible, efficient, curious, clever, a little cunning, collaborative and among those who dare to dream.

What does a successful wine industry look like in 2050?

We have maintained our social license. We have been involved in the wine and health debate and have contributed to evidence-based alcohol regulation. We have embraced and worked with a changing climate and we are growing grapes efficiently and in places where we have earned our place in agriculture, understanding that the planet has 9.6 billion people that need to be fed, 2.4 billion more than now (United Nations Department for Economic and Social Affairs 2013) although Jamie Goode in his discussion about the social license of wine wasn't quite so optimistic (Goode 2016b).

In 2016 Joanna Andrew in her introduction to Session 1 at the 16th AWITC told us to 'challenge convention' and we have. Michael O'Brien (O'Brien 2016) said to 'respect the past, but know it's the knowledge that we take to the future that's important', and we listened.

Sue Bell (Bell 2016) reminded us that we are intrinsically connected to country, to respect our special places and to work with the land, not on it – we thought about it and acted accordingly.

The Australian wine industry has reached 2050 with long-term strategies. Some of these were difficult in the short term, but now our industry strategies and plans are longer than terms of office or government cycles. We have worked through tough times with compassion and helped those who were disadvantaged or whose lives have changed.

We worked together – the big and the small, the growers, the makers and the marketers, all of the value chain. We listened to Senator Anne Ruston at the opening of the 16th AWITC when she said 'I am your voice in government' but for her to be this she said 'industry unity is the singularly most important thing.

We listened to Michael O'Brien (O'Brien 2016) who said that with 'Give and take together we can become one', and we worked hard to become a united industry, with powerful lobbying ability that governments take seriously and trusts. Importantly through these relationships we have achieved sensible and responsible legislation and long-term support from governments including appropriate research, development and extension (RDE) funding and marketing support.

Many of the silos and departments of the past have gone. Scientists talk about art, marketers talk about science and grapegrowers are winegrowers, intrinsically and actively involved in the planning, crafting and promoting of the wines.

We have worked with the world in RDE to tackle and answer the big questions, and our RDE system and scientists are still the envy of the world.

We are profitable – everyone in the value chain is valued, knows what they contribute and is rewarded fairly.

We are efficient. We celebrate diversity but we are lean in bureaucracy; we collaborate when we can and compete when we need to; we have minimised waste in all parts of the value chain and made what was once considered waste a productive by-product in many instances.

We make a product that is relevant to the consumer.

What do our consumers look like in 2050?

Those in the wine industry in 2016 who were born after 1982 will shape what 2050 looks like. These people will fulfil the expectations expressed in this paper. Along with their friends around the world they are known as the millennials.

Danny Brager (Brager 2016) told us how important millennials are already and how they will become large consumers of wine. He correctly predicted that our consumers in 2050 want instantaneous everything, but they want to learn and they want to share. Mark McCrindle (McCrindle 2016) reiterated this in his closing address.

Our consumers in 2050 demand transparency and authenticity, they want something that's been made for them not for everyone else and that is made by someone they respect. They make health-based decisions and buy what they value. Many speakers at the 16th AWITC spoke about value, including Ryan Hodgson (Hodgson 2016).

Communication is immediate and entertaining. Mass marketing is dead as Tim Merchant (Merchant 2016) suggested it would be. Now it's all about intelligent customer engagement.

After the breakthroughs in the transport and energy industries it is now easy to travel around the world quickly and efficiently, so tourism is important and we have created a suite of commissionable travel products as Mark Wilsdon (Wilsdon 2016) suggested. Our consumers expect to be able to see the people behind the stories in their market too: virtually, digitally, or in the flesh.

We now understand much more about our consumers and what makes them tick. We understand the complexities of how they taste, why they like what they like and why they are different from each other. We understand the genetic basis of consumer wine preferences and anosmia, can match consumers with styles, and segment markets by the way people taste. Mango Parker (Parker 2016) gave an example of this when she explained the complexity of the tasting of glycocides. Terry Acree (Acree 2016) and John Hayes (Hayes 2016) introduced us to the complex genetic differences between people and why no amount of training (or telling) can make up for genetic differences. We have used this knowledge to redefine how we judge and assess wine as well as how we learn about our consumers.

Where are we selling wine in 2050?

Wherever we sell wine we are doing it responsibly.

Mark Wilsdon (Wilsdon 2016) and Leslie Norris (Norris 2016) both told us that wine should be part of lifestyle – combined with art, music, food, family and friends.

Leslie also talked about creating a sensory experience to include wine, which in turn creates an emotional attachment with the wine. We can now recreate our cellar door experience, with all its smells and ambiance for our remote customers.

Wine is available widely to complement other experiences or as an experience of its own, to touch and buy or consume. E-commerce is also very important for wine sales.

The traditional wine markets still have a place but now wine is a part of many more cultures – as Brett McKinnon (panel presentation Session 1 at the 16th AWITC) prophesied 'the growth was where we didn't exist' in 2016.

As Tony Battaglene predicted at the same event, we now have Free Trade Agreements and standardisation of market access across the globe. After much work from Steve Guy (Guy 2016) and Wine Australia we now have risk-based international wine trading that has given us greater freedom and reduced regulation, common analytical platforms and agreed analytical measures – meaning it's easier and simpler to export wine.

China lived up to expectations as a major market for Australian wine, although it was a bit rocky for a while, and it now has in excess of 150 million regular wine drinkers. Brazil, Korea, Japan, Indonesia and India are all major wine markets for Australian wine.

Importantly we have transport and distribution systems that fully preserve wine quality.

What are the wines of 2050 like?

Many wine drinkers still have wines in the cellar from 2016 that are relevant and enjoyed; there hasn't been a complete change to the meaning of what wine is but there has been evolution as we have remained relevant to our consumers.

In typical Australian style we have done what many predicted we couldn't – everything (well lots of things anyway!). As Andrew Weeks (Weeks 2016) wanted we have breached the perception of just being 'sunshine in a bottle' and finally after much soul searching and collaboration, hard work, education and perseverance, and a bit of government funding, we got the story about our fine and great wines to the world. Dan Jago (Jago 2016) is proud to know that Australia now has a number of 'wines of pedigree' that the world fights over to obtain when they are released.

But that's not all we have done. Seizing opportunity quickly and on a larger scale we created a uniquely Aussie offering, a palette of wines differentiated from the rest of the world by their captivating stories and images. We didn't get there fighting to the bottom on price because there is demand for the wines as they are unique. This wasn't a foreign concept in 2016 as Fiona Donald (Donald 2016) told us – we have always had some uniquely Australian wines and styles. We understand that not every wine is for everybody or every market, so we have been strategic where each is targeted. Many of our customers and consumers think they are getting something that has been made just for them and have been willing to build partnerships with us to achieve this.

There are many things that differentiate these wines and the stories attached to them. Some are about the people and communities who grow and make the wines; many are about their place and regional identity which includes the 40,000 years of history and culture and the beautiful and textured multicultural society of the present.

Brian Croser (Croser 2016) said that 'to drink wine is to drink nature', and now, with breakthrough science we know so much about the complex array of factors that influence the way a vine expresses itself and the final wine.

Some regions have recreated themselves, with a unique selling point and an all-out focus to be something. Sue Bell (Bell 2016) predicted that the Riverland could be the home of Australian Rosé. Some wines are made with new varieties in blends and styles such as those that Kim Chalmers (Chalmers 2016) talked about. Some are using different clones that we now can identify and optimise by region, site or wine style, with the tools that the work of Mike McCarthy (McCarthy 2016) and Simon Schmidt (Schmidt 2016) have given us.

Other wines are being made with heightened targeted flavours, such as thiols through the work that Olivier Geffroy and Remi Schneider (Schneider 2016) have done.

All wines are made with our improved understanding, if not direct application, of what drives varietal, clonal and regional differentiation and what regulates them. Examples are the marker compounds rotundone and cineole that Guillaume Antalick (Antalick 2016) told us about.

While we are making a lot of wines in Australia in 2050, most wines are differentiated by something unique, they are in demand because of this and we are able to charge a premium for them.

Despite the best efforts of many over the years, our wines have not been successfully counterfeited due to the policies and strategies that we put in place after listening to John Spink (Spink 2016) and the clever technology we have implemented after listening to Tim Merchant (Merchant 2016).

Thanks to the work of Martin Day (Day 2016) and those who followed him with their 'Rolls Royce analytical machines' we have the analytical ability to authenticate wine to brand, country and place with 99.9% accuracy.

How are the wines of 2050 made?

In many ways we can tailor our wines now, not with genetic engineering but by breeding microorganisms and vines to maximise the traits that we want. The tool box is big. Projects like Microwine and Wildwine (Chambers 2016) and what followed have given us huge amounts of information that we have used to choose how to ferment our wines.

We have worked on many levels to overcome the pressures of more rapidly developing phenology and compressed vintages as a result of climate change that Paul Petrie (Petrie 2016) told us about.

We have techniques to ferment reds in less time and varieties and clones and vineyard practices to spread out ripening. We have clever techniques to slow the vine's ripening such as sunscreens, film forming agents, different pruning times, or targeted manipulation and canopy management; techniques such as those that Everard Edwards (Edwards 2016) told us about.

We have winemaking practices to lower sugar and alcohol and we have the ability to ameliorate wines when something out of our control has gone wrong, such as smoke taint.

Inspired by the years of work by people like Roger Boulton (Boulton 2016) we have production systems that allow for minimal wine movements. We have 'one-tank winemaking' options.

Where are the vineyards of 2050?

Hans Schulz (Schulz 2016) told us that by 2050 we would need another 125 million hectares of land to feed the world, and at least 11% more water than we currently use or have in agriculture. We have continued to develop state of the art irrigation, recycling and reclamation systems. The use of cheaper and smarter renewable energy allows us to efficiently use desalinated water.

Some of our vineyards have been converted to food production, but many remain, and others are now growing in more marginal country, where our knowledge of how to overcome the challenges of these sites has been used. We have new varieties, clones and rootstocks and we have focused on environments where pest and disease threats can be minimised. We have planted in new inland regions where nights are either cooler or warmer and have overcome the issues that Hans Schulz (Schulz 2016) told us about associated with warmer nights and changes in gene expression. We have also planted in areas that were previously too cold – although there is not a lot further south that we can go!

What do the vineyards of 2050 look like?

We have comprehensive data about the national vineyards. VinSites, Entwine, and other complementary sustainability programs work together and we benchmark within regions and with other regions, and continually improve.

We are leading the world in best practice, low input and sustainable viticulture and this is now one of our competitive advantages and stories, so much so that other agri-industries have worked with us to adopt similar systems as we have led the country in protecting our natural resources and 'caring for our country'.

We still talk about terroir, regionality and place, and have agreement about what these words mean. We know that, however each winemaker chooses to define them, these concepts are fundamental to differentiating our wines from each other and those around the world.

We had the 'intelligent debate' that Jamie Goode (Goode 2016a) told us to have, and have a credible scientific base upon which to mount terroir claims.

Now we have many authentic stories to tell the world about why our wines taste like they do made from the grapes that they are.

We understand that terroir is a living thing; terroir has evolved and is as relevant now as it was 34 years ago.

After Peter Bissell (Session 4 chair) reminded us how small we are in the universe, Paul Chambers (Chambers 2016) told us that we are just as insignificant, by numbers anyway, on a planet where there are more microbes than stars in the universe. We now know the important role that microbes, yeast and bacteria play in defining terroir.

In many of our regions and vineyards we still have the same varieties (and vines) that we did in 2016. With lots of knowledge and some clever ideas we have been able to adapt the vines' microclimate (and micro terroir) as the macro climate has warmed. We have also used the warming climate to evolve the styles of many of the wines and it hasn't been an issue – fashion even in this industry moves more quickly than climate change!

We have a raft of effective management tools in our tool box for preventing and overcoming diseases. We have more targeted strategies as we have a much better fundamental understanding of the causes and actions of the pests, and the vines are healthy.

We are getting the most out of our vineyards. We have asked and answered the question – what is the ultimate potential of a grapevine for both yield and quality, and we know how to unlock this potential. The days of automatic crop thinning to get yields down for quality in many vineyards are gone.

We are able to estimate and predict yields – to within 5% and really understand the reasons for variability and diversity. The work of the Smart Robotic Viticulture Team at the University of NSW (Whitty 2016 and Liu 2016) has changed the way that we measure variables such as crop and canopy of the vineyard, as well as how we control pests.

Now that we can measure and understand so many of the aspects of quality and style, objective measures are the basis of fair trading of grapes and payment. Keren Bindon (Bindon 2016) gave us a taste of this.

Rob Bramley (Bramley 2016) isn't surprised that we are using precision viticultural systems such as irrigation and selective harvesting, and that there are robots in our vineyards: driverless tractors and automated vineyard management tools for weeding, pruning, canopy manipulation, and even 'hand picking'. Robert Fitch (Fitch 2016) told us that robots could be doing much more for us that we had previously imagined and now they do – saving cost and allowing us get the timing right for best results.

Some things haven't changed in 2050

We are still curious about what we don't know, and we have a whole new suite of 'known unknowns' that were not even thought about in 2016. The Australian wine community still get together to share our knowledge and experiences to continuously improve, as we plan for the challenges that will face us in 2085.

To finish

There was much that could have been and was not addressed in this paper, including competitors, social media, packaging and industry size and scale. What was addressed shows the bias and interests of the author, what was not addressed are areas that are beyond her expertise. This simply highlights how important it will be to get the right people, with the right mix of skills 'around the table' to make the long-term plans for the Australian wine industry and to cover all the issues (and then some) so well summed up by Roger Boulton at the beginning of his inspiring presentation (Boulton 2016).

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The social licence of wine

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Abstract

Wine is a luxury that many of us enjoy daily, but those of us in the wine trade have to acknowledge that there are positives and negatives associated with this culturally rich beverage. In ancient civilisations, wine played a pivotal role in the daily lives of the population. Agricultural land ill-suited to crops often proved ideally suited to vines. In the modern world, there is some discussion about whether, with increasing pressure on food supplies, viticulture is an expensive luxury when vines are planted on more fertile soils. There is also the dark side of wine: many people struggle with alcohol addictions. There is also debate about the level of wine consumption where the health and safety/welfare of drinkers is impacted, as well as the health and safety/welfare of society per se. In many Western societies, there is the re-emergence of a neo-prohibitionist movement where government-sponsored bodies see even a modest level of alcohol consumption as potentially harmful. The chaotic pattern of drinking among young people, with weekend binge drinking the norm, is leading to a legislative agenda that stigmatises all alcohol consumption, with potentially significant impacts on the wine industry. At the same time, there is convincing evidence from meta-analysis of published results that moderate alcohol consumption is actually healthy for the general population, with corresponding lower levels of cardiovascular disease a robust finding from research studies. The wine trade is a significant employer, and for Australia, its wine industry takes the role of a global ambassador. It is therefore important for the wine industry to enter into a discussion of the social licence for wine, and to show to the government that it is able to self-legislate, at the same time as championing its huge social benefit as a beverage that brings a safe, pleasurable and life-enhancing richness to those who participate wisely in its enjoyment.

Webcast of this presentation available at http://bit.ly/16thJGoode.

Wine's privileged place

Sometimes we in the wine trade are guilty of taking the place that wine has in society for granted. For thousands of years wine has played an important part in many cultures. This has largely been in countries of wine production, but other nations have also had a thirst for wine: for example, the UK has been important in the development of the wine industries of Bordeaux, Jerez and Porto. Emigrants from wine cultures have taken their wine culture with them as they have travelled: for example, the Dalmatians at the heart of the New Zealand wine industry and the Silesians of the Barossa Valley.

In Western societies, wine currently enjoys a privileged place compared with other alcoholic beverages. This seems entirely normal to us because we are in the trade, and are familiar with this situation. If, however, we take a step outside the wine trade bubble and look inwards, it becomes an unusual situation. This privileged place is in some ways a historical artefact, and one that we should work hard to preserve.

Wine has a privileged place on the table. In restaurants worldwide, wine and food have been linked to the point that when diners are seated they are offered a wine list. This will include other drinks, but it is wine that enjoys primacy. Food and wine matching is seen as an intrinsic part of fine dining. This is something the wine trade should celebrate, but something we shouldn't assume will continue forever.

In this paper, I will be looking at some of the broader ideas surrounding the social licence of wine. This is an important discussion for the wine industry, but it's also a complex one.

Alcohol and health

As I write I'm on a plane on the way to Australia. I remember the first time I flew to Australia, back in March 1996. Trying to find the cheapest flight, I opted for Olympic Airlines, Greece's national carrier. It seems strange to think about it, but back in those days (20 years ago, now, but it seems quite recent) people used to smoke on planes. As we checked in, with our paper tickets, we were assigned seats in the smoking section. Our protests fell on deaf ears, and we had to fly at the back of the plane, where 80% of the flight (mostly Greeks, practically all of whom smoked) congregated to puff away. It was pretty grim. Now of course, no one smokes on planes. It just seems a stupid idea. No one smokes in bars. No one smokes in the office. Advertising of smoking is severely restricted in many countries, and the display of cigarettes at point-of-sale is also commonly banned. This is a massive societal change, and from my perspective, as a non-smoker, it's brilliant. Society has quite rightly become very anti-smoking.

Worryingly, though, society has also become somewhat anti-drinking, too. This change is not as dramatic, but for those of us in the wine trade, it's certainly something we should be concerned about. The argument in favour of drinking is that there is a safe level of consumption, whereas any level of smoking is hazardous. This distinction is one that is now being disregarded in the UK by public health authorities, with the message being one that any level of drinking carries with it risk. Many in the public health sector would like to do to alcohol what they have done to tobacco.

The evidence against alcohol is mounting. The abuse of alcohol is widespread, and attempts have been made to quantify it. These reports make for sobering reading. Of course, there's no way of knowing how real the figures quoted are (and, just as with many grant proposals which begin with an assessment of the cost of the problem that the research is intended to solve, the numbers seem awfully big), but these sorts of figures are likely to influence policy decisions.

The Institute of Alcohol Studies published a report in 2015 titled Alcohol's Harm to Others (Gell 2015). This is a quote from it:

In the UK, the cost of alcohol's harm to others was estimated in 2004 at up to £15.4 billion including £1.4-1.7 billion to the health service, up to £7.3 billion in crime and public disorder costs and up to £6.4 billion in workplace related costs. Further, there are costs to family and social networks that cannot be quantified using available data, for example the cost to children affected by parental alcohol problems. More recent figures calculated for the European Union place the societal costs of alcohol consumption in 2010 at € 155.8 billion (£115.4 billion). In Australia, the tangible costs per year resulting from other's alcohol consumption are estimated at AUS $14.2 billion (£7.2 billion) and the intangible costs at AUS $6.4 billion

(£3.3 billion). Given limited government resources, this alcohol-related spending reflects a large opportunity cost in terms of other areas of healthcare or government spending sacrificed.

To governments in Western nations, figures like this mean that the appeal of restricting alcohol availability through higher taxation, or limiting retailing or advertising, is irresistible.

Binge drinking among teenagers and younger adults is a huge problem in the UK. Chaotic drinking patterns are resulting in serious liver damage – a hepatic surgeon I spoke to said that he's regularly seeing patients with end-stage liver failure in their late 20s. City centres on Friday and Saturday nights are turning into dangerous places.

Against this backdrop, the UK Chief Medical Officer, Dame Sally Davies, has recently initiated a consultation looking to revise safe drinking guidelines. This consultation began in January 2016. It was proposed that recommended drinking levels should be set at 14 units per man/woman per week, and in addition announced that there was no truly safe drinking level. This reduces the recommended level from 21 to 14 units for men, while leaving the level for women the same. It has been pointed out that these guidelines are based on a relative risk set at 1%, which is the equivalent of one hour of television watching per week, or two bacon sandwiches over the same period. The most controversial element is the statement that there is no safe drinking level.

Davies went on the important BBC Radio 4 flagship program 'Today' and said this: 'There's an old wives' tale that we were all brought up on - that a glass of red wine protected the heart.' This contradicts the clear message from the scientific literature that alcohol has a protective effect against cardiovascular disease when consumed in moderation by the general population. In Western populations moderate drinkers live longer than non-drinkers, who in turn live longer than heavy drinkers. This is called the 'J-shaped curve'. It's a consistent finding in what is known as 'epidemiological' studies—those that look at the incidence and distribution of diseases, and their causal factors.

The J-shape refers to the curve on a graph you get if plot mortality (the risk of dying) against alcohol consumption. Moderate drinking increases life expectancy, mainly through its protective effects on the cardiovascular system. Heavy drinkers also enjoy this benefit, but their risk of death starts to increase because they are more likely to suffer from the various conditions related to heavy drinking, such as cirrhosis of the liver, high blood pressure, stroke, certain cancers, and increased risk of accidental or violent death. It is a pretty robust finding that has been replicated in countless studies to the degree that it is no longer controversial. It's also quite a significant effect: research spanning back 25 years on the subject indicates that moderate drinkers cut their risk of heart attack by as much as one-quarter (Di Castelnuovo et al. 2006).

This message was reinforced by two papers published in the British Medical Journal in 2011, both from William Ghali and colleagues. These papers represented what is known as a meta-analysis, which is a study that attempts to bring together all published evidence on a particular subject from the medical literature in order to draw a more robust conclusion. In the first paper (Brien et al. 2011), Ghali carried out a review of the literature looking at studies that had examined the effect of alcohol consumption on biomarkers of coronary heart disease. They screened almost 5,000 articles, and included the results from 44, which were the relevant studies that met their criteria for suitable data. Overall, 13 biomarkers were included in the analysis. Alcohol was shown to significantly increase high-density lipoprotein (HDL) cholesterol, with a dose–response relationship, and it decreased fibrinogen levels. It didn't change triglyceride levels but it increased adiponectin and apolipoprotein A1. All of these changes are reported to be cardioprotective. The authors noted that these changes are 'well within a pharmacologically relevant magnitude', meaning that alcohol is acting as a prescribed medicine might. They point out that the degree of HDL cholesterol increase is better than can be achieved with any single therapy. Alcohol, consumed moderately, seems to be acting as a good drug.

The second paper (Ronksley et al. 2011) looked at selected cardiovascular disease outcomes. It examined 4,235 studies, and 84 turned out to be suitable for inclusion in the meta-analysis. The results examined the relative risk of dying for drinkers versus non-drinkers, and once again came up with some significant results. A moderate drinker has 0.75 risk of dying of cardiovascular disease compared with a non-drinker, and 0.71 risk of incident coronary heart disease. An alcohol consumption of 2.5–14.9 g/day (roughly one or two drinks) results in a 14–25% reduction of risk of cardiovascular disease compared with abstainers. Both studies together suggest that alcohol may be having a causal role here: there is a dose–response relationship, and the association is specific, in that alcohol is not uniformly protective for other diseases, such as cancer.

How do we respond?

So what is the response of the wine industry to government advice like this? On the one hand, the wine industry wants to be a sensible, self-regulating body, and seen as such, and in the past wine companies have supported efforts by public health bodies to encourage moderate consumption of alcoholic beverages. For example, many retailers have the government safe drinking guidelines printed on the back labels of their bottles. It has now got to the point, however, where these limits don't seem supported by good evidence. It is understandable that governments need to do something about excessive and chaotic drinking patterns in young people, but the acceptance by the drinks industry of these recommendations cements them into societal narrative on alcohol. If they are printed on the back label of wine bottles, then eventually people will believe them to be true. The fact that they are more-or-less plucked from the air, or generated with a relative risk set almost absurdly low, is completely lost on the population.

The wine industry must self-regulate and behave responsibly, and be seen to do so, but must contest misleading presentations of science. This is a very difficult balance to get right, and in the current climate it seems people are afraid to speak out. We need to move beyond the age gauge – that annoying and completely useless sop to public health found on most winery websites, where viewers have to enter a date of birth in order to read about wine.

One area where the wine industry could take a lead is in discussions about taxation. This is currently a pertinent topic in Australia, where changes to wine taxation are being proposed. The current situation, with the Wine Equalization Tax, is a mess. It's a bolt-on solution to an unintended consequence of a change enacted some time ago. There's currently a discussion about whether a move to a volumetric tax might make more sense. This is a complicated discussion, but it's widely stated that one way to reduce alcohol consumption and therefore reduce societal harm is to increase its price (e.g. Wagenaar et al. 2009; Sheron 2016). A volumetric tax would also help those in the wine industry producing more premium products. In the UK, there was a recent discussion about the introduction of minimum unit pricing, which would wipe inexpensive alcoholic beverages off the shelf. In the UK there are currently cheap high strength ciders and lagers that are favoured by binge-drinking teenagers. These are irresponsible products. Most wine would be unaffected by the proposed minimum unit pricing, but it would take these cynical products off the shelves. Sadly, the wine industry in the UK fell in line with the rest of the drinks business, and this opportunity to do societal good was lost. This only puts wind into the sails of the neoprohibitionist lobby.

Viticulture and food security

While alcohol and health is the big battle ground, there's another aspect to the social licence of wine. This is food security. With growing populations, arable land is becoming a scarce resource as the need to feed people presents challenges to agriculture.

Global population is predicted to grow and then plateau at 9 billion. Feeding these people will be a challenge and there will be competition for land, water and energy (Godfray et al. 2010). At the same time there will be increased pressures to protect the environment from further degradation. Add into the mix substantial climate change and we have a problem that will need some smart solutions.

In the future, it is likely that vineyards that are planted on land suitable for other crops may find their legitimacy criticised. Can we make a case for wine being more than just a frivolous luxury that has to vacate its place at the table in order that more may be fed?

Conclusion: seizing the narrative agenda

People don't listen to facts. Facts don't change minds: stories and emotions do. As a wine industry we need to seize back the narrative agenda, which we have currently allowed public health professionals to dominate. We need to begin telling the story of wine. Is wine just another alcoholic beverage? If not, why is wine different? We should emphasise its cultural and historical significance. There are positive associations with wine, such as gastronomy and beautiful places. Most importantly, good wine has a link with a place. There's a connection between what is in the glass and where it comes from that's compelling and separates wine out from other drinks. Wine is not just another drink. If we do nothing, then we will face increasing problems with our social licence, and we should act now as we see the storm clouds gathering, rather than wait for the first drops of rain to fall. Above all, we must hold fast to the Roman saying *abusus non tollit usum* – abuse does not disqualify the legitimacy of correct use. It's important that as a wine industry, we don't take our social license for granted.

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Scientific opportunities that will disrupt

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Abstract

Many would agree that one of the most repulsive practices in the world is that of organ theft. How to stop such terrible practices? Policing and penalties? Try this instead:

1. *Isolate a skin cell from a patient in need of a new organ*
2. *Reprogram this skin cell into an organ-specific cell type and grow it into a functional organ*
3. *Surgically replace the failed organ with the freshly grown and immune-compatible organ.*

A decade ago this would have sounded like an illusion but this may no longer be so. In 2015 scientists from the University of Queensland published a paper in Nature *in which they reported the generation of mini-kidneys from human stem cells in a sterile dish. The wine industry must come to accept equally wildly disruptive ideas in due course. At the extreme, these could include the ability to produce 'wine' tailored to the genomes of individual consumers.*

In the more immediate term one could imagine seeing drones hovering over vineyards performing pruning based on previous harvest data from individual vines, and perhaps even harvesting grapes based on their spectral characteristics. Over the medium term, might we encounter high-tech, 'smart' vineyards and wineries with no people in sight? Finally, could vineyards become obsolete as all grape components will be produced from cell cultures followed by fermentation from synthetically-tailored yeasts?

All this sounds far-fetched, but the first synthetically grown meat has already been turned into a burger with meat-like characters. It is not a matter of whether these opportunities will present themselves but whether we are prepared to grasp them and, if so, when and how. This presentation will try to illustrate some of the disruptive forces coming our way and argue that we need to be at the leading edge of this in the wine industry.

No paper available, please view this presentation at http://bit.ly/16thHoj.

Changing times, emerging trends: a snapshot of the changes transforming the Australian wine industry now and towards 2025

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Abstract

Only occasionally in history do massive demographic changes combine with huge social shifts, ongoing generational transitions and unprecedented technological innovation so that within the span of a decade society altogether alters. Australia is currently in the midst of one such transformation. While such change impacts everyone and every organisation, the leaders that will future proof their organisations are those who understand the times, influence the trends and shape the future.

In this presentation social analyst Mark McCrindle will help the leaders of the Australian wine industry navigate through the megatrends transforming Australia and deliver a snapshot of 2025. He will discuss the key implications of these trends on the changing business and consumer landscape.

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