

Stream 2.5: Protecting the quality and integrity of Australian wine

1. Abstract:

Technical problems which arise during grapegrowing and wine processing have the potential to cause widespread loss of wine quality and integrity, and result in direct and indirect economic losses to grape and wine producers. This stream used a proven platform which supports the knowledge and skills base of Australian wine industry personnel, to provide pre-competitive solutions to grape and wine production, composition and processing problems. The activities of the stream also provided an early warning system for industry-wide trends and emergencies, generating information which was applied to the prioritisation of solution-focused research activities. The stream enabled Australian grape and wine levy payers to access the AWRI's diverse technical knowledge and capabilities, in order to identify and address problems in a strictly confidential manner and to ensure these issues did not reoccur. Cases were dealt with in an efficient time-frame which, wherever possible, allowed problems to be mitigated, and maximum grape and wine value to be retained or recovered. The adoption of the advice and solutions provided by these activities also prevented sub-standard or tainted fruit and wine entering the supply chain. In instances where grape or wine value was not recoverable, the technical reports ensure that any potential legal and insurance proceedings have access to robust technical information. The capabilities provided by this stream are highly valued by the grape and wine sector, and the AWRI is considered the 'go to' organisation for 'helpdesk' services for grape and wine producers. This was evidenced by over 10,000 winemaking, viticultural, wine and health-related and regulatory queries confidentially answered by helpdesk services staff, and greater than 1,400 winemaking investigations conducted and reported upon during the life of the AWRI's 7-year RD&E Plan.

In short, this stream provided:

- a rapid helpdesk service in the areas of oenology, viticulture, health and regulatory control;
- a problem solving service in the areas of oenology, viticulture and regulatory control;
- a small-scale, practical research trial capability when gaps in the current knowledge base were identified; and
- novel method development for the increased understanding of unknown taints and contaminations.

2. Executive summary:

The activities in this stream have been critical in identifying and resolving technical issues and provided knowledge across areas including grapegrowing, winemaking, packaging, regulatory and trade-related issues. The specialised helpdesk support services provided considerable benefit and value for the industry as rapid help was available on a wide area of topics. The activities in this stream contributed to the resolution of issues which, if left unresolved or repeated by different industry practitioners, incur losses or unnecessary costs, create inefficiencies, and potentially damage brands.

An independent economic assessment of the industry impact of the resolution and avoidance of selected, common taints and faults achieved through the AWRI's activities was conducted by Econsearch (www.econsearch.com.au) in 2012. It was found that the AWRI's activities resulted in a net present value ¹(NPV) of ~\$264 million on an incremental RD&E investment of ~\$8.6 million, comprising of:

• *Brettanomyces* management and avoidance from 1998 to 2009 (~\$4.9 million¹ on an incremental RD&E investment of ~\$3.2 million);

¹ The calculated figures represent the approximate cost to industry had this service not been available.



- halophenol taint mitigation and avoidance from 2004 to 2011 (~\$124 million¹ on an incremental RD&E investment of ~\$1.5 million);
- smoke taint mitigation and avoidance from 2003 to 2011 (~\$41 million¹ on an incremental RD&E investment of ~\$1.5 million); and
- general helpdesk services (\sim \$50 million¹ on an incremental RD&E investment of \sim \$2.4 million).

Over 10,000 winemaking, viticultural, and regulatory queries were confidentially answered by the AWRI helpdesk services staff and greater than 1,400 winemaking investigations were conducted and reported upon during the investment agreement (AWRI annual reports 2006-2012). This service has broad uptake and was consistently used by producers of all sizes and across all regions in managing and preventing quality loss during production. On average, approximately 15% of queries received turned into investigations where samples were requested and further analysis performed to ascertain and fix the problem(s).

The AWRI staff maintain constant interaction with vineyard and winery personnel and other key stakeholders, and monitor the AWRI's Commercial Services laboratory. This provides an insight into the changing technical, scientific and regulatory issues being encountered on the ground, both industry-wide and on a state and regional level. This activity provided early warning of issues that could potentially have a large-scale negative impact on the quality, integrity and economic viability of Australian wine. One such example of this is taints and contaminations: a problem that continues to cause concern for all grapegrowers and winemakers and has a large potential for significant quality and financial loss.

The bushfires in Victoria in 2009 provide another example of how the activities in this stream provided support for an issue that had serious consequences, and had affected a large number of wineries and grapegrowers. The initial touch point with the AWRI was through the specialised helpdesk services where many calls, in a short period of time, were received about fire damaged vineyards and wineries damaged from heat and smoke exposure, smoke drift and smoke tainted fruit. Producers were assisted through a coordinated approach involving GWRDC, DPI-Victoria and the AWRI which facilitated a large number of grapes being tested and the results interpreted in a very short period of time.

Another important aspect of the touch point with industry is the ability to identify key areas of industry need, which can be applied to the prioritisation of research activities. In 2007, the AWRI was contacted about a *Eucalyptus*-like character in a number of different wines made in the vicinity of a few *Eucalyptus* trees. This query formed the catalyst for further research into this character in wine, resulting in a routine method being developed for Eucalypt character.

This pre-competitive levy-funded service provides an international competitive advantage to Australian wine producers through fast resolution of technical, regulatory and trade issues. The dissemination of learnings throughout industry and the development of new analytical methods help to prevent the same issue occurring elsewhere.

Being solely funded by levies matched with government funding enabled the activities in this stream to be carried out in an independent and impartial manner, with no third-party potential influence, protecting the integrity of the results and providing greater confidence to stakeholders. The activities of this stream have, however, benefitted greatly from interactions with a number of wine industry and closely aligned organisations whose involvement assisted with queries and investigations, which included wineries; Wine Australia; CSIRO; wine industry associations; key industry suppliers of equipment, yeast, winemaking additives and processing aids; universities; viticulture and wine consultants; legal representatives; and insurance and loss adjusters.

3. Background:

The Australian wine industry maintains a reputation as a producer of wines of high quality and



integrity, and the projects in this stream have a history of protecting and enhancing that reputation by pro-actively addressing technical problems and issues that pose potential threats.

The principal rationale behind the founding of the AWRI in 1955 was to elucidate solutions to technical problems being faced by the wine industry, which continues to be a major function of the AWRI. The provision of technical support through this stream is considered to be an important contributing factor to the development of a wine industry that is recognised as one of the most technologically advanced in the world. Examination of the extensive records and databases developed within this stream covering several decades indicates that many prominent Australian wine businesses and winemakers have been, and continue to be, reliant on the technical support offered by the AWRI.

Over time, the nature of the problems being addressed, and the technology potentially available to investigate them, become more complex. The AWRI's Problem Solving teams increasingly rely on the AWRI's Mass Spectrometry facility to elucidate the causes of taints and contamination. Stream staff maintain constant interaction with industry personnel which provides invaluable insight into the changing technical issues being encountered industry-wide or on a state or regional level. Staff respond to early warnings of issues that could potentially have large-scale negative impacts on the quality and integrity of Australian wine. The stream had demonstrable success in developing and implementing industry-wide solutions to widely encountered technical problems, and the small-scale research projects conducted provided a continual flow of new knowledge on the composition of Australian wine and the winemaking process.

This stream also encompasses the industry's front-line emergency response team to address cases of potentially widespread contamination of grapes and wine.

4. Stream objectives:

The objectives for this stream were:

- Protect and enhance the reputation of the Australian wine industry for high quality and high integrity.
- Develop state-of-the-art analytical methods for the analysis of potential contaminants.
- Generate new knowledge related to the winemaking process and to the composition of Australian wine from information generated by research trials conducted at the AWRI under this and other Streams, and elsewhere.
- Use this new knowledge to provide technical support to the Australian wine industry to optimise and maintain the quality and integrity of Australian wine.
- Protect and enhance the technologically advanced status of the Australian wine industry.
- Provide early warning of issues that could potentially have large-scale negative impacts on the quality and integrity of Australian wine.
- Actively transfer the knowledge generated by the stream to the Australian wine industry in a manner that facilitates its rapid adoption and application.
- Develop the technical abilities of personnel employed in the Australian wine industry in order to facilitate the rapid adoption of new knowledge generated by this and other streams.
- Facilitate continual and incremental improvement in the quality and affordability of Australian wine and the profitability of the Australian wine industry.
- Supply technically accurate information for promulgation by the media.
- Assist industry bodies to deal with technical issues that pose a threat to them or to the image of the Australian wine industry.
- Act as a multiplier on the returns from previous investments in research by more widely and actively disseminating outcomes from that research in a manner that favours their adoption.



5. Methodology:

The AWRI has been providing technical problem solving and emergency response services to the Australian wine industry since its inception. As such, it is acutely aware of the resources required to effectively assist producers to identify and overcome technical issues.

The activities in this stream were undertaken by four experienced winemakers, a mass spectrometrist specialist, two scientists and support staff. This team maintained a 'helpdesk' and responded to industry requests for technical assistance and, when necessary, formed the front line emergency response unit.

Industry personnel were able to contact the helpdesk via a variety of ways (phone, email, visit in person or samples delivered with accompanying notes) to seek advice and ask questions about particular problems being experienced with wine(s), grapes, regulatory issues, packaging or other issues associated with the production of wine. The nature of the enquiries varied considerably; all advice provided was recorded on a database against a set of key words and linked to the stakeholder's details allowing trends to be monitored and identified. When required, onsite visits by helpdesk staff facilitated communication with the industry practitioners, leading to clearer and expedited understandings of the issue. One-on-one interactions also led to more rapid delivery of solutions, thus enhancing adoption. Where problems were unable to be resolved at the initial contact, the enquiry turned into an investigation which was managed on a case-by-case basis. Solutions to the problems were provided to the stakeholders through a variety of means (by phone/in person, email and formal reports). These too were recorded on a database linked to the stakeholder's details allowing trends to be monitored and identified.

Capabilities are maintained in this stream to respond to industry emergencies and a procedure is in place for effective response. Once the AWRI is alerted to the emergency (for example, through a number of mechanisms, including stakeholders contacting the AWRI on a similar issue in a short period of time; constant two-way interaction with stakeholders from all over Australia, via a monitoring approach with the AWRI's Commercial Services laboratory; as well as interaction with peak industry bodies) the AWRI brings it to the attention of the industry's Crisis Management Team (comprised of the Grape and Wine Research and Development Corporation, Wine Australia, Winemakers' Federation of Australia, Wine Grape Growers Australia and the AWRI). If it is agreed that the AWRI would responded on the industry's behalf, the AWRI to address the industry issue. This necessarily involves the AWRI staff 'downing tools' on other projects to direct the required skills to address the issue. This work is prioritised until the emergency issue is resolved. Fortunately, no industry technical emergencies arose during the investment agreement, although the advent of the bush fires in 2007 and 2009 did require marshalling of diverse and significant resources within the AWRI to assist the industry to minimise losses.

Often complex taint and contamination type investigations involved identification and quantification of trace amounts of unknown contaminants via the analytical technique of mass spectrometry. State of the art analytical methods were developed, for some of these unknown and more prevalent contaminants which were then utilised for future investigations. This enabled better understanding of the potential levels of these compounds in wines, processing aids and additives. New analytical methods developed during the investment agreement include: the varietal differentiation and pathogenesis-related (PR) protein methods; the method for detecting chlorophenols bound to tartaric acid; a method for skatol; the geosmin method; the development of the free and bound smoke taint assays; the developed air sampling technique for chloroanisoles and chlorophenols; and the development of the new laccase analysis (Hayasaka et al. 2008a, Hayasaka et al. 2008b, AWRI Annual Report 2009, AWRI Annual Report 2011, AWRI Annual Report 2012).

Through active interaction with grapegrowers, winemakers, regional and state associations, valuable insight has been developed into the issues that have the potential to cause reduced quality in Australian



wines. This insight was further enhanced also through monitoring and analysing the trends from the queries taken and the investigations performed, which were captured on a database. The data generated were then applied to the prioritisation of research and extension activities, for example, through the development of the next roadshow workshop themed 'Adapting to difficult vintages'.

6. Results and discussion:

The helpdesk services staff responded to over 10,000 winemaking, viticultural, and regulatory queries and greater than 1,400 winemaking investigations were conducted and reported upon during the investment agreement (AWRI annual reports 2006-2012). The majority of the queries were received from wine companies and suppliers closely aligned with the wine industry (approximately 95%), whilst the remaining queries were made up from government organisations (4%) and students (1%). This service was consistently used by producers of all sizes and across all regions in managing and preventing quality loss during production. On average, approximately 15% of queries turned into investigations where samples were requested and further analysis performed to ascertain the problem and how to remediate the problem(s).

Winemaking	Viticulture	Regulatory
Taints and contaminations	Agrochemical	Export and domestic regulations
Analysis methods	Viticulture – general	Wine standards
Haze/deposit	MRL and 'Dog book'	Consumer information
Fermentation advice	Chemical trespass	Additives and Processing aids
Sulfur dioxide	Fungal and Insect pests	Allergens
Brettanomyces	Vine nutrition	Contaminants
Sensory assessments	Varieties	

Table 1. The most requested topics for information for financial years 2010/2011 and 2011/2012.

The type and nature of the queries taken vary considerably, and monitoring these does allow an insight into the types of problems the industry is experiencing. For example, during the 2011 vintage which was dominated by wet weather across south eastern Australia, one in every five queries during the vintage months was related in some way to moulds and botrytis. Similarly, during the hot and dry vintages of 2008 and 2009, spikes in queries relating to these conditions were observed and included heat stressed fruit, very ripe fruit, stuck and sluggish fermentations, excessively high sugar levels, high alcohols and smoke taint from bushfires. A snapshot of some of the queries and the key findings from the knowledge gained are presented in Table 2.

One of the major on-going issues during the reporting period was smoke taint resulting from bushfires, especially two major fires during 2007 and 2009 which saw a spike in queries received on this issue. In the past, a single volatile phenol (guaiacol) in grapes was used as a marker for smoke exposure. However, this single marker proved to be unreliable in many cases, especially for grapes affected by the 2009 bushfires. Work conducted during the investment agreement identified that numerous glycosidic metabolites are formed from smoke compounds and act as a pool of precursors from which smoky aromas, in the form of volatile phenols, can be released. As a result of method development work, 40 markers have now been identified as indicators of smoke exposure (Hayasaka et al. 2009, Hayasaka et al. 2010a, Hayasaka et al. 2010b, Hayasaka et al. 2010c).

Support was provided to regions when insect pest outbreaks occurred. Issues with apple looper, 6-spotted mite, plague locusts and fruit fly were encountered. Each required identification of appropriate control strategies, consultation with industry and sometimes permits to be sought from the Australian Pesticides and Veterinary Medicines Australia.

Assistance for issues related to health and regulation was commonly provided as a result of changes to, or differences in, Australian and international legislation. These changes included those to the minimum alcohol content of wine; permitted sources of wood for barrels; approval of new additives



and processing aids, such as sodium carboxymethyl cellulose; changes to analytical limits for wine constituents such as 2,4-D, cobalt, manganese, natamycin, phthalates and phosphorous acid. The assistance provided also included communication to wine industry stakeholders to facilitate wine exports to avoid rejection and return to Australia with economic imposts. In addition, changes to allergen warning statements, nutrition content claims and health claims in Australia, Canada, EU and USA had the potential to impact on trade in wine as they were not consistently adopted domestically and by export destinations.

A snapshot of some of the key findings and outputs from the activities conducted in this stream is presented in Table 2.

Table 2. Some of the key issues, solutions and knowledge advances made during the period July 2006 – June 2013.

Year	Issue	Outcome		
2006	Changes in EU agrochemical tolerance	The existing Australian policy and practices established around the use of captan and carbaryl agrochemicals in viticulture wer determined to be sufficient to meet the new EU regulatory requirements.		
	Major chlorophenol taint problem affecting several wineries	A liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) method was developed to determine the presence of chlorophenols in tartaric acid. This method was one of the crucial outcomes of a major 'plastic taint' investigation, as it was used to prove that a contaminated batch of tartaric acid was the source of the chlorophenol compound responsible for the tainting.		
	Variable, post-bottling growth of 'Brett' yeast	The results of investigations conclusively proved that post- bottling growth of the spoilage yeast <i>Dekkera/Brettanomyces</i> ('Brett') is responsible for observations of variable amounts of the compound 4-ethylphenol (4-EP), which is responsible for the 'bandaid' character associated with 'Brett' spoilage, in different bottles of the same wine. Results showed that 'Brett' could produce approximately 1000 μ g/L of 4-EP from assimilation of approximately 300 mg/L (0.3 g/L) of residual sugar (glucose plus fructose).		
	DL tartrate deposits spike	Calcium-DL-tartrate instability traced back to the use of a racemic processing aid - DL-KHT seed crystals. Key finding is that the cause of this type of instability had not previously been seen before.		
2007	Eucalyptus in wine	Queries and investigations relating to ' <i>Eucalyptus</i> ' flavours in wines led to a research project and method development. A key finding of the investigations was that proximity of grapevines to <i>Eucalyptus</i> trees was correlated with the concentration of eucalyptol in wines made from the grapes from the vines.		
	Origin of taint in tartaric acid	By using a custom built sampling device and statistical analysis of the analytical results, the newly developed LC-MS/MS method for detecting chlorophenols in tartaric acid (see above) was used to prove that the chlorophenol contamination in a batch of tartaric acid was uniformly distributed throughout the bags. This information was used to conclude that the contamination occurred before the bags were packaged. This information was crucial for clearing the winery involved of any allegation that the contamination might have occurred whilst the tartaric was held in storage at the winery.		
	Faecal-like taint	A new LC-MS/MS method was developed for the compound skatole in order to investigate a possible cause for a 'faecal-like'		



Year	Issue	Outcome
		taint. Quantification of the compound indole was also included in
		the method, so that both compounds could be determined in one
		analysis run.
	Earthy taint	A gas chromatography-mass spectrometry (GC-MS) method to
		semi-quantify the compound geosmin was developed in response
		to a taint investigation, where an earthy taint was found not to be
		due to either chloroanisoles or bromoanisoles.
	Minerals in wine	A survey was undertaken on the bromide and chloride content of
		Australian juice and wine. A key finding is that the content in
		juice and wine clearly reflects the content in the vineyard soil and
		irrigation water used. These results were presented at the OIV
		expert group meetings to demonstrate that the mineral content of
		Australian juice and wine is naturally higher than that of other
		countries, and should not be used as a technical barrier to trade.
	Smoke taint from	Following major bushfires that resulted in the tainting of grapes
	bushfires	and wine as a consequence of exposure to smoke, work was
		initiated to investigate the existence of smoke taint precursor
		compounds using LC-MS. It was thought that smoke-derived
		volatile compounds such as guaiacols might accumulate as
		glycoside derivatives and then be later released during
		fermentation through enzyme activity.
2008	Heatwave affects vintage	Winemakers struggled to deal with the heatwave effects in
	leading to a spike in	maintaining wine quality. Almost double the usual number of
	queries	queries related to stuck fermentations during the year, the
		majority of which were most likely related to a heat wave that
		affected much of southern Australia in the first half of March
		2008.
	Varietal identification	A method for the detection of PR-proteins in juice using LC-MS
		was developed which can be used to help differentiate varieties
		based on their protein profiles.
	Stuck fermentation	 Key finding from 'hot' vintage: high must sugar
	queries spike	concentration was the most important factor leading to the
		stuck or sluggish fermentations.
	Musty taints	Wine quality loss avoided by identifying and understanding more
		sources of potential musty taints. Sources of musty compounds
		are not limited to just cork. Winemaking additives, processing
		aids, water used during winemaking and environmental sources
		are all capable of tainting wines with musty like taints.
	Tartrate deposits continue	Insufficient cold-stabilisation of wines prior to bottling or the
	to occur	application of an inappropriate cold stability test is perceived to
		be the major cause of these instabilities.
	Copper related hazes	Uptake in use of screwcap closure linked to increase in copper
	increasing	related hazes post-bottling.
	Smoke taint precursors	Experiments where grapes were exposed to smoke showed that
		the formation of guaiacol glucoside in grapes increases
		remarkably as a consequence of exposure to smoke.
2009	Heatwave affects vintage	Vintage again endured a record heatwave event in late January
	C	and early February with potential for wine quality losses two
		years in a row.
	Sensory assessments	Wineries assisted through solutions provided for a variety of
	spike	sensory related problems. These included: reductive characters,
	F	tainted wines, deterioration post-bottling, variability post-bottling
		and various microbiological-related spoilage characters.



Year	Issue	Outcome
	Stuck ferments spike during vintage heatwave	Again high sugar levels were experienced and strategies to mitigate the flow-on effects during fermentation were provided. Number of queries were not as high as previous year suggesting uptake of advice had been successful.
	Smoke taint from bushfires	 Understanding of smoke taint and the compounds responsible was considerably improved with the development and implementation of various methods: An air sampling technique was developed so that smoke could be sampled in the field and then taken to the laboratory for analysis. A method for quantification of guaiacol glycosides as guaiacol precursors in wine was developed using HPLC-MS/MS. Methods for the quantification of free and bound volatile phenols including phenol, isomeric cresols, guaiacol, methylguaiacol, vinylguaiacol, syringol and methylsyringol were developed using HPLC-MS/MS and GC-MS.
2010	What is the significance of the presence of low concentrations of smoke taint compounds in grapes? Phosphorous acid	 A comprehensive survey of the baseline levels of volatile phenols and their glycosides in samples of grapes was conducted in order to establish 'normal' levels of these analytes. The results helped winemakers make more informed accept/reject decisions about grapes with low levels of smoke taint compounds. Initial investigations indicated that products used as foliar
	agrochemical detected in some wines, even though the agrochemical was not used in the vineyard	 sprays, fertilisers and diammonium phosphate (DAP) could be potential sources of phosphorous acid (PA). Results of a scoping study that followed showed that PA present in DAP products used during vinification does contribute to measurable PA in finished wine. In addition, 19 out of 25 viticultural fertiliser products tested contained PA. Wineries were therefore informed that the use of these products might have the potential to contribute to measurable PA in finished wine.
	Natamycin in wine	In collaboration with the South Africa National Department of Agriculture, an LC-MS method was developed to determine the concentration of the fungicide natamycin in wine, which enabled winemakers to confidently export wine to those countries imposing a maximum limit for natamycin.
	Calcium DL-tartrate deposits due to use of tartaric acid containing D- tartaric acid isomer	A high performance liquid chromatography (HPLC) method for the detection and quantification of the amount of D-tartaric acid present in 'L-tartaric acid' was developed. This method can be used to determine if any D-tartaric acid 'impurity' is present in tartaric acid.
2011	Locusts outbreaks threatening pre-vintage	Strategies that were satisfactory to grape purchasers and allowed land managers to meet their obligations to control locust outbreaks were developed, providing options when 'hoppers' were encroaching vineyards.
	Wet vintage – Queries relating to Botrytis spike	Integrity and wine quality protected by providing advice to wineries during wet vintage. 20% of calls received during vintage were related to botrytis infection and managing botrytis infected fruit from a winemaking perspective. A large proportion of the information sought was about laccase testing and a key finding was that the laccase test kits currently available can be difficult to interpret when testing red juices and wines with low laccase



Year	Issue	Outcome activities.	
	Laccase test kit results difficult to interpret with some red juices and wines	An alternative laccase method was developed. The method uses a different substrate to that utilised by the currently available laccase test kits. The colour change resulting from the reaction of laccase with the substrate is more easily observed than the colour	
		change associated with the currently available laccase test kits. Use of this method for red juices and wines with low suspected laccase activities should decrease the number of problems encountered with results interpretation.	
	Flood-affected wine	The potential for human pathogenic bacteria in flood-affected wine and wine cellars was assessed. Viable pathogenic bacteria were not recovered from wine after approximately an hour depending on the type of wine, and any risk from flood-affected wine can be reduced by a rigorous sanitation regime with isopropyl alcohol.	
	Sparkling wine becoming tainted with	Methods for air-sampling and analysis were developed for determining the concentration of chlorophenols and ableroepiecles contained in the atmosphere of the storage	
	tetrachloranisole during storage in a warehouse	chloroanisoles contained in the atmosphere of the storage warehouse. The results were used to show that the mechanism of tainting was aerial contamination followed by migration of tetrachloroanisole through the crown seals during storage.	
2012	Manganese in wine	A survey of the manganese content of 392 Australian and international wines was undertaken which showed that the manganese content varies considerably within countries and regions reflecting variability in soil and water manganese content and the permitted use of pesticides during grape growing. Approximately 10% of all wines are above the Chinese maximum limit for residual manganese in wine and cannot be exported to China.	
	2,4,D	Tissue samples were analysed and low levels of 2,4-D residue were identified as being present in regions where spray drift damage had been observed. The residue levels recorded were found to be below the level of the common analytical determination and not an MRL issue.	
	Hydrocarbon taints	Wine quality loss avoided by identifying and understanding more sources of potential taints. Aromatic hydrocarbons were identified in wine transported in a flexible bulk wine container. Key findings: environmental contaminations during transport are possible in both wine and wine related additives and processing aids.	
	Gluconic acid analysis required to test possible correlations with % Botrytis infection of grapes and/or laccase activity in juice	An existing enzyme method for gluconic acid was modified so that it could successfully be used for juice analysis. Gluconic acid levels were reasonably (exponentially) correlated with Botrytis infection, however, there was a poor correlation between gluconic acid concentration and laccase activity.	
2013	Database	Knowledge was developed and captured in a central database which helped the understanding and monitoring of the issues occurring on a regional, state and national level. By acting on the issues identified, the Australian wine industry is provided with a competitive advantage through identification of problems and avoidance of sub-standard or tainted wines from entering the market place.	



Year	Issue	Outcome	
	Requirement to measure	Both LC-MS and GC-MS analytical methods were developed to	
	levels of phthalates in	determine the content of particular phthalates in wine, which	
	export wines	enabled winemakers to confidently export wine to those countries	
	-	imposing a maximum limit for phthalates.	

The development of new analytical methods is an important outcome associated with many of the issues that arise for the wine industry, as detailed in Table 2. New methods are often required to elucidate the compounds responsible for issues associated with taint, where the presence of a tiny amount of a compound (or compounds) can have a large negative impact on the sensory acceptability of a product. However, a method can also be required to detect the presence of a compound that has the potential to cause a wine instability problem, such as the calcium DL-tartrate and laccase issues referred to in Table 2.

A timeline of all the new methods developed during the period July 2006 – June 2013 is presented in Figure 1.

2006

- LC-MS varietal differentiation of juice method
- LC-MS/MS tartaric acid chlorophenols method
- 2,4-D method improved (lower detection limit)

2008

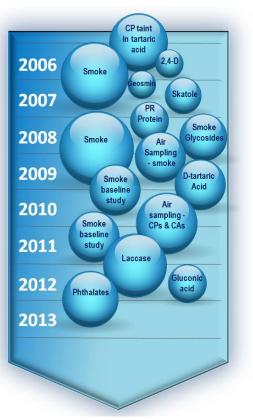
- PR protein method developed
- Formation of guaiacol glucoside as a consequence of exposure to smoke confirmed

2010

- HPLC method for separation of tartaric acid isomers (solid tartaric acid)
- Methods used for smoke taint background level study

2012

- New laccase method developed
- Enzyme method for gluconic acid in juice



2007

- Skatole method (including indole)
- Geosmin method (semi-quantitative)
- Smoke precursor method development started

2009

- Air sampling technique for smoke taint developed
- LC-MS/MS method for quantification of guaiacol glycosides as guaiacol precursors in wine developed
- quantification of free and bound volatile phenols developed using GC-MS & LC-MS/MS

2011

• Air sampling method for chlorophenols (CPs) and chloroanisoles (CAs)

2013

- GC-MS method developed for measuring phthalate levels
- LC-MS/MS method also developed for measuring phthalates

Figure 1: Timeline of method development July 2006-June 2013. Bubble size is approximately proportional to the size of the issue indicated in the bubble.

7. Outcome and Conclusion:

One of the main aims for this stream was to provide a rapid helpdesk service for stakeholders across the value chain including grapegrowers, winemakers and industry personnel to assist with technical, regulatory and scientific issues. The stream also sought to pre-empt and address any industry emergency issues as they arise, as well as developing novel analytical methods for unknown contaminants via the analytical technique mass spectrometry.

The Australian Wine Research Institute





Queries and Investigations

The many users of the helpdesk service have overcome their technical problems and had their technical knowledge enhanced through interacting with the AWRI. Over 10,000 winemaking, viticultural, and regulatory queries have been confidentially answered by helpdesk services staff and greater than 1,400 winemaking investigations conducted and reported upon during the investment agreement (AWRI annual reports 2006-2012). The service was consistently used by producers of all sizes and across all regions in managing and preventing quality loss during production. On average, approximately 15% of queries turned into investigations where samples were requested and further analysis was performed to ascertain the problem and how to remediate the problem(s).

Smoke taint

Valuable assistance was provided to grapegrowers and winemakers in dealing with smoke taint. There have been a number of major bushfires in recent years and, from the constant interaction and work performed, there has been a number of significant outcomes, this includes the development of a number of analytical methods to assist producers understand the extent of smoke damage in fruit and wine. In addition, a comprehensive survey of the baseline levels of the major volatile phenols involved in smoke taint, and their glycosides, was conducted using the new GC-MS and HPLC-MS/MS methods developed. The results of this survey revealed that grapes not exposed to smoke naturally contain low levels of the smoke taint compounds. Consequently, the clearer distinction between non-smoked and smoked samples revealed by the new methods is now being used as a reliable diagnostic strategy to identify smoke exposure and to assess the impact of smoke exposure in grapes and wine.

Value to industry assessed

An independent economic assessment of the helpdesk service, was conducted by Econsearch (www.econsearch.com.au) in 2012which focused on the industry impact of the resolution and avoidance of selected, common taints and faults. It was estimated that the AWRI's activities resulted a net present value (NPV) of \sim \$264 million¹ on an incremental RD&E investment of \sim \$8.6 million, comprising of:

- *Brettanomyces* management and avoidance from 1998 to 2009 (~\$4.9 million¹ on an incremental RD&E investment of ~\$3.2 million);
- halophenol taint mitigation and avoidance from 2004 to 2011 (~\$124 million¹ on an incremental RD&E investment of ~\$1.5 million);
- smoke taint mitigation and avoidance from 2003 to 2011 (~\$41 million¹ on an incremental RD&E investment of ~\$1.5 million); and
- general helpdesk services (\sim \$50 million¹ on an incremental RD&E investment of \sim \$2.4 million).

Another significant outcome is that the helpdesk service is a pivotal connection with industry that can provide valuable input into future research needs. For example, *Eucalyptus* character in wine has led to a research project, as has smoke taint, with both issues being raised through the helpdesk service initially.

Phosphorous acid

Due to the inadequacy of chemical options for disease control during the wet 2011 vintage, the AWRI, working with a diverse range of stakeholders, successfully lobbied regulators for emergency use permits to provide options for growers. A review of the permissions relating to the use of phosphorous acid (PA): a chemical that is effective but not recommended for trade reasons was also undertaken. The review highlighted the need for research that would support appeals to international markets to set maximum residue limits for this chemical. To this end, fruit was collected from vineyards where known applications had been made and levels of PA were tested in the grapes and resultant wine. Coupled with a survey of PA levels in local and foriegn wines, the final report was used by Wine



Australia and Winemakers' Federation of Australia in negotiations with key international trading partners for the purpose of establishing an MRL for PA in grapes.

Novel analytical methods

New methods developed as part of investigations have enabled the industry to improve their understanding of the product being tested – whether this is for identifying a problem or modifying a technique for a desired outcome. Some examples of outcomes resulting from the use of the developed novel analytical methods are provided below:

- The varietal differentiation and PR protein methods have been useful tools in the settling of disputes about varietal identity, and so avoiding lengthy and costly legal proceedings.
- The method for detecting chlorophenols bound to tartaric acid was instrumental in proving the mode of tainting during the halophenol investigations during 2006/2007.
- The development of a method for skatol has allowed confirmation, or otherwise, of the compound responsible for faecal-like taints in wines, thus providing further understanding of the mode of tainting and therefore ways of possible avoidance in the future.
- The geosmin method has been used to identify musty/eathy-like taints in wines where chloroanisoles were suspected to be responsible, but were not detected as part of initial analyses.
- The development of the free and bound smoke taint assays has allowed the measurement of smoke exposure in grapes prior to winemaking, consequently enabling winemakers to make critical decisions early (when to harvest, whether to purchase grapes or bulk wine etc.). It has also allowed winemakers to quantify the effect of different amelioration processes to reduce the taint.
- The developed air sampling technique for chloroanisoles and chlorophenols allowed elucidation of the mode of tainting. Knowing the mode of tainting, the winery involved was able to change the way sparkling wine was stored in order to avoid re-occurrence of tainting in the future.
- The development of the new laccase analysis will allow easier observation of the reaction colour change, consequently reducing the chances of incorrect interpretation of the laccase activity results.

8. Recommendations:

Extend the 'helpdesk' service to address vineyard and regulatory-related issues.

The successful model of the helpdesk service has been proven in its effective resolution and potential recurrence of technical problems for winemakers, which can extend to assisting producers to manage insurance claims. There is, however, unrealised potential for this type of service to be provided to resolve vineyard and regulatory-related issues to help maintain the integrity and quality of Australian wine. This could be performed using the current rapid helpdesk support service platform, which is considered sound and robust for delivering a high value to all users of the service, (to be addressed in Project 4.1.2 of AWRI's new R,D&E plan).

Maintaining the emergency response capability is also considered an important function within the stream, (to be addressed in project 2.3.1 of AWRI's new R,D&E plan).

Assist winemakers to deal with climate change. The extreme variable nature in vintages, notably the past five vintages, which were all climatically different and each presenting their own unique challenges, presents an important opportunity to assist wineries adapt to climate change. Vintages in recent times have been remembered for droughts, hot and dry, heatwaves, floods and very wet seasons. This area will become a focus not only in vineyards and for grapegrowers, but also for winemakers dealing with the end product from extreme seasonal variability issues such as those experienced in the 2008, 2009 and 2011 (see results section), (to be addressed in Project 4.1.2 in the AWRI's R,D&E plan 2008-2013).



Eliminate bulk transport effects on wine quality. Other areas of unrealised potential includes the understanding of the effects on wine quality through bulk transportation, and developing better impermeable materials for transporting wine to prevent contamination from external (environmental) sources. Many queries and investigations have been conducted on wine having being contaminated during bulk transport during this investment agreement. With approximately 50% of Australian wine exports now shipped in bulk and packaged outside of Australia, the reputation of the Australian wine industry is at stake should tainted or contaminated wine end up in the market place, (to be addressed in Project 3.6.1 of the AWRI's R,D&E plan 2008-2013).

Guaranteed authenticity. Assistance was provided to wineries during the investment agreement on authenticity of their products. This is an area where further work needs to be undertaken and is considered another unrealised potential for future research, (to be addressed in Project 2.2.2 of the AWRI's R,D&E plan 2008-2013).

Being one step ahead. Prediction of future research needs and issues can potentially be gained through mining the data accumulated from the helpdesk services. Being one step ahead, will provide great benefit and value for the Australian wine industry as well as research organisations through thought leadership in potential wine related issues, (to be addressed in projects 4.1.2 and 5.1.1 in the AWRI's R,D&E plan 2008-2013).

Pesticide sprays and the effect in wine. Based on several issues addressed by the AWRI, there is a need for further method development and research into the cause and effect relationship between pesticide sprays in vineyards and the increasing background concentrations of chlorophenols in juice and wines, (to be addressed in Project 1.3.3 of the AWRI's R,D&E plan 2008-2013).

Using new products with confidence. Many discussions have been had with winemakers and wine industry personnel seeking reassurance from the AWRI whether a product new to the industry is suitable for use. Wineries with significant resources do their own testing, however the majority of the wineries in Australia do not have the capability to do their own research, (to be addressed in Project 4.2.1 of the AWRI's R,D&E plan 2008-2013).



9. Budget reconciliation:

Financial Year	Receipts / Income 0	Outgoings / Expenditure @
Year 1: 2006/2007	\$723,894	\$723,894
Year 2: 2007/2008	\$625,799	\$625,799
Year 3: 2008/2009	\$748,741	\$748,741
Year 4: 2009/2010	\$845,266	\$845,266
Year 5: 2010/2011	\$779,809	\$779,809
Year 6: 2011/2012	\$970,696	\$970,696
Year 7: 2012/2013	\$782,929	\$782,929
TOTAL	\$5,477,134	\$5,477,134

• Note that the GWRDC – AWRI Investment Agreement budget was established and approved at an aggregate level, with variances to budget (i.e. annual overspends and underspends) reported and considered at that same aggregate (i.e. whole of agreement) level. The receipts / income relating to a Stream for any year therefore equate to the outgoings / expenditure within that Stream for that year, as any variances between total Investment Agreement funding received and total funds expended were considered at the whole of Agreement rather than individual Stream level.

2 Includes a pro-rated share of Theme 5 *Executive management and administration*.

I hereby certify that this statement is true and accurate.			
Signature of duly authorised representative.			
Chris Day	Group Manager - Corporate Services	.29/ 11/2013	
Name:	Title:	Date:	



10. References:

The AWRI's R,D&E plan 2013-2018; http://www.awri.com.au/about_the_awri/rde-plan/

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