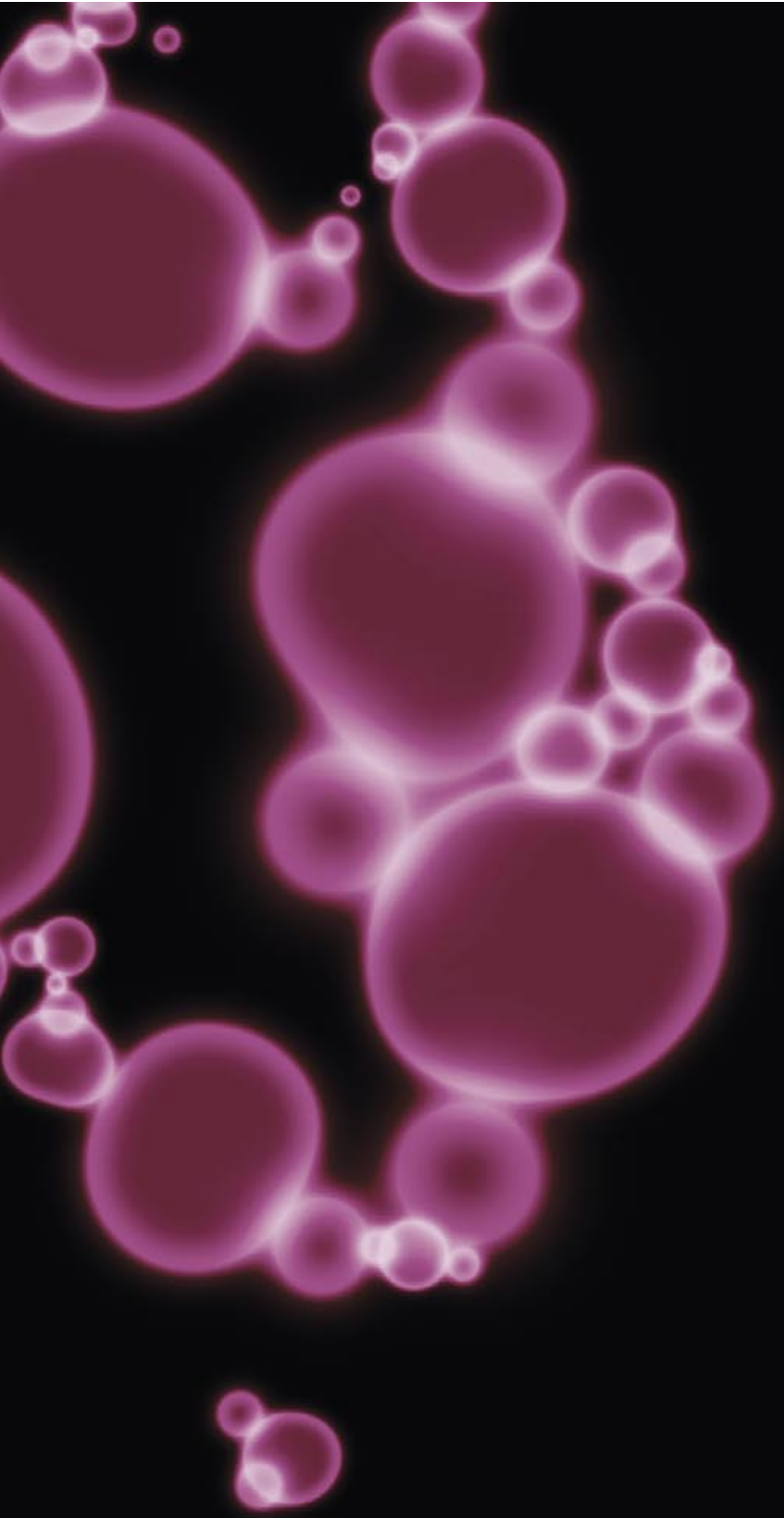


The Australian Wine Research Institute
Annual Report 2004

50th Annual Report

Presented to the Australian wine industry 30 June 2004

Chairman's report

During the past year, the AWRI has significantly increased its outreach with an increase in extension activities on a number of fronts.

AWRI staff members gave 137 presentations around Australia and the world during the 2003/2004 year. This included seven Roadshows held in Wodonga, Hunter Valley, Griffith, Mudgee, Canberra, Bunbury and Manjimup. Twenty-eight further presentations were given within workshops, with a record number of ten *Trouble free winemaking – causes and prevention of common wine instabilities* workshops being presented, in three states. Thirteen posters were presented. AWRI staff members gave 96 lectures to undergraduate students, and supervised 21 postgraduate students. Forty-two staff manuscripts were published in refereed and non-refereed industry journals during the year. Staff members responded to 59 approaches from Australian and international media to comment on technical matters. Further, AWRI staff members responded to 6,979 requests for information from the Australian wine industry and others (this does not include the number of problem investigations undertaken [1,262], or the work undertaken by the Analytical Service).

Not only do these activities provide an important means of demonstrating the AWRI at work but they offer a valuable snapshot of the industry's problems on which our research scientists can focus. The cosmopolitan mix of 13 nations which constitutes our staff and students proves continually that cultural differences recede behind more important goals – in their case a focus on endeavour and scientific excellence. Our staff members are to be commended for the diligence they apply to their tasks under conditions of limited space. During the year, a great deal of progress has been made in developing plans and costings for a number of building options, and the co-operation of our key partners in progressing these plans gives a clear indication of the importance they place on developing a critical mass research facility.

In last year's report, mention was made of two important areas where practical analytical approaches of considerable impact had been developed from seemingly unrelated lines of scientific investigation. The genesis of a readily accessible chemical index of *Brettanomyces* activity as a spin off from wood flavour research, and the development of juice fingerprinting techniques to verify varietal composition from the work on protein haze are both excellent examples of the serendipitous breakthrough being completely impossible to plan for in a highly organised programmatic research environment. What might prove to be an even more spectacular breakthrough is currently dawning from the work being conducted on volatile thiols. As a result of following a lead provided by some research reporting bacterial production of some obscure enzymes, the capability of individual yeasts to produce different profiles of very potent volatile thiol flavour is now being detailed. This work has the potential to reveal an important part of the story surrounding the modification of varietal flavour during the process of alcoholic fermentation. This process probes deep into the very substance of wine, unravelling the reasons why wine has a mystical complexity compared to juice or must, which merely tastes sweet. We can probably expect some practical benefits in the form of an additional important tool in the choice chest of the winemaker and if the work proves to be truly pivotal, some enhancement of the Australian and AWRI influence might be a secondary benefit.

Stories such as this do, however, prompt other questions. If our researchers are able to demonstrate a handful of groundbreaking findings, with our research program focussed on delivering predicted outcomes within short time lines, what would the true potential be if a greater emphasis on strategic research was put in place?

In this context it is important that the very clear distinction between strategic and fundamental research be understood. Fundamental research is usually characterised by little immediate application, great expense and very varied productivity and, as such, could hardly be expected to be a popular choice for industry levy based funding. Strategic research based on areas of key expertise and targeted towards general goals of clear benefit to the grape and wine industries would be both much less risky and more predictably productive. In the current climate where 'bang for the buck' is paramount, short time lines for predicted outcomes are the norm.

There is a very strong case to argue that the time is now appropriate to increase the strategic content of the work at the AWRI in key strength areas such as phenolic chemistry and flavour chemistry with a likely improvement in overall output.

It is interesting to note that the *Brettanomyces* project, which was essentially non-existent three years ago, has recently been compared more than favourably to work emanating from much longer established research projects in several other countries. This benchmarking highlighted the delivery of useful protocols to facilitate control of *Brettanomyces*.

Robin Day
INSTITUTE CHAIRMAN

It is very pleasing for the AWRI, as a supporting institution, to be able to congratulate the Australian Society of Viticulture and Oenology on the success of the *Australian Journal of Grape and Wine Research*. The journal recently received an SCI 'impact factor' rating in excess of that enjoyed by many much longer established journals, and in fact leads all the other wine research journals. We are also pleased to learn this year, on the subject of journals and ratings, that an AWRI paper entitled 'Screening for potential pigments derived from anthocyanins in red wine using nanoelectrospray tandem mass spectrometry' by Yoji Hayasaka and Robert Asenstorfer was one of the most cited papers published in the *Journal of Agriculture and Food Chemistry* in 2002.

The Managing Director, Professor Peter Høj, has in the latter part of this year managed the bid process for a Co-operative Research Centre for Wine. This project, which differs fundamentally from the previous two CRCs for Viticulture, is very market oriented in nature. If the bid is successful (it goes to final selection in October 2004), it will bring about a significant gearing up of research programs with direct market outcomes for all participating research providers; the AWRI being the major one. The very strong linkage between wine compositional research and consumer sensory studies will mean an increase in emphasis on sensory work at the AWRI. The ability to use advanced sensory techniques to investigate and prove flavour relationships has the potential for substantial value adding and cost benefits in industry.

At a time of financial stress in some sections of the grape and wine industry, it is not so surprising to see some of the recent opposition to proposed R&D levy increases. It should be obvious to anyone who has comprehensively examined the benefits of R&D expenditure, that this is a very short-sighted approach. It is perhaps indicative of the fact that management within the wine industry is not always fully aware of the benefits delivered by investment in R&D.

There have been a number of attempts to calculate the benefits of grape and wine R&D. One detailed study conducted by the GWRDC three years ago took a sample of 12 million dollars worth of research and calculated the benefit expected. Even after a savage discount for risk and slippage of 79% is applied, this investment calculated to deliver over 100 million dollars of benefit.

Where else can one obtain a nine-fold return on investment?

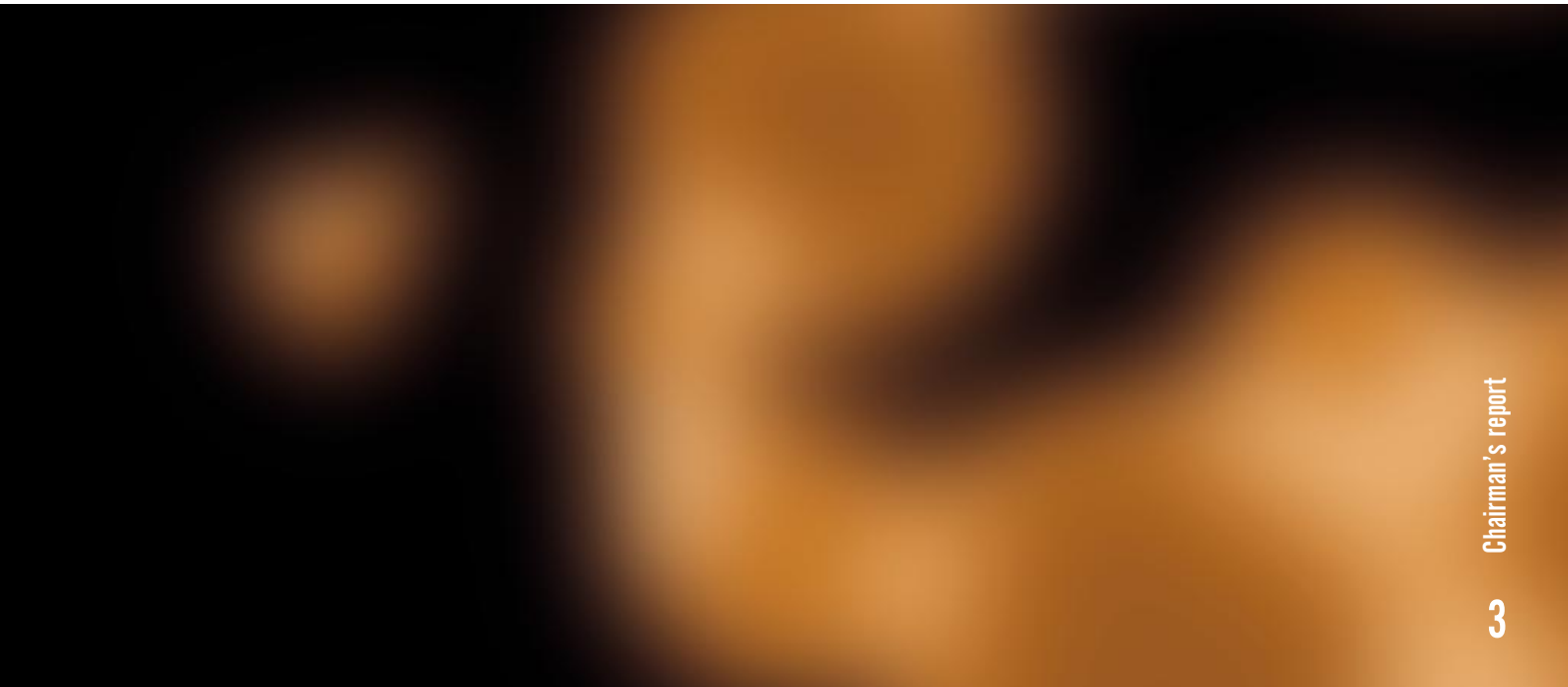
The task for technicians is to explain to their management and their financial scorekeepers that investment in R&D is the best investment their businesses will ever make.

Throughout the year, the AWRI has worked closely with the GWRDC to develop an even closer and more transparent relationship aimed at maximising the delivery of quality research outcomes for the whole of the grape and wine industry. The support we receive from the GWRDC is absolutely critical to our continued existence and, while this is obvious within the AWRI, it is important that it is understood more widely throughout industry.

I would like to acknowledge the continued excellent contribution made by Peter Høj and the staff of the AWRI this year. The increase in targetted extension activity and interaction with the Australian wine industry are maximising the outcomes of our research.

Finally, I would like to recognise the continued support and wise counsel emanating from both the elected and appointed members of the Council of the AWRI, whose inputs are critical for the delivery of valuable industry relevant research outcomes.

Robin Day
Chairman



Council notes

Chairman
At the Council meeting held on 28 October 2003, Mr R.E. Day was elected Chairman of Council.

Members of the Executive Committee
Mr R.E. Day
Professor P.B. Høj
Professor S.D. Tyerman
Mr T.W.B. James
Mr G.R. Linton

Deputy Members of Council
Mr N.P. Blieschke
Mr L.P. Deans
Dr P.R. Dry
Dr. A. Kolutnow (from 25 May 2004)
Mr D.J. McWilliam
Mr J. Northey (until 24 May 2004)
Mr A.N. Sas (from 25 May 2004)
Dr. N.S. Scott (until 24 May 2004)

Audit Sub-Committee
Mr R.E. Day
Mr P.J. Dawson
Mr T.W.B. James

Meetings
Ordinary General Meeting
The 49th Ordinary (Annual) General Meeting was held on 28 October 2003.

Council
The Council of the AWRI met on the following dates: 29 July 2003, 28 October 2003, 24 February 2004, 24 May 2004. Executive members of Council met on 4 December 2003.

Funding
The Council of the AWRI acknowledges the continuing financial support of the Grape and Wine Research and Development Corporation.

Appreciation
The AWRI acknowledges the assistance and cooperation of the following organizations throughout the year:

Australian Wine and Brandy Corporation
Charles Sturt University (National Wine and Grape Industry Centre)
Commonwealth Scientific and Industrial Research Organization (CSIRO)
Cooperative Research Centre for Viticulture
Department of Agriculture, Fisheries and Forestry Australia
South Australian Wine and Brandy
State Departments of Agriculture
State Government of South Australia
The University of Adelaide
Winemakers' Federation of Australia Inc.

Staff

Peter Bordier Høj, MSc, PhD, *UCopenhagen,* Managing Director
Isak Stephanus Pretorius, BSc(Agric)(Hons), MSc(Agric), PhD, *Orange Free State,* Director of Research

Research

Paul Anthony Henschke, BSc(Hons), PhD *UAdel.*, Principal Research Microbiologist
Mark Aidan Sefton, BSc(Hons), PhD *UWA,* Principal Research Chemist
Elizabeth Joy Waters, BSc, PhD *UAdel.*, Principal Research Biochemist

Robert George Dambergs, BSc(Hons) *UAdel.*, PhD *UQld*, Senior Research Chemist
Miguel Antonio de Barros Lopes, BSc *Oregon*, PhD *UC (Santa Barbara)*, Senior Molecular Biologist (concluded 28 May 2004)
Ian Leigh Francis, BSc(Hons) *Monash*, PhD *UAdel.*, Senior Research Chemist
Markus Johannes Herderich, PhD *UWuerzberg*, Senior Research Chemist

Yoji Hayasaka, DipEng(IndChem) *Tokyo I.T.*, MPharm *Vic. Col. Pharm.*, CertIntBusMgt *Monash*, Manager—Mass Spectrometry Facility

Eveline Jutta Charlotte Bartowsky, BSc(Hons), PhD *UAdel.*, Research Microbiologist
Daniel Cozzolino, AgricEng *Uruguay*, PhD, *Aberdeen*, Research Chemist
Gordon Michael Elsey, BSc(Hons), PhD, *Flinders*, Research Chemist
Alan Percy Pollnitz, BSc(Hons), PhD *UAdel.*, Research Chemist/Computer Systems Officer
George Kyriakos Skouroumounis, BSc(Hons) *Flinders*, PhD, GradDipOenol. *UAdel.*, Research Chemist
Paul Alexander Smith, BSc(Hons), PhD *Flinders*, Research Chemist
Patrik Raymond Jones, BAgSc, PhD *UAdel.*, Research Chemist
Jan Hendrik Swiegers, MSc, PhD, *Stellenbosch*, Research Molecular Biologist (commenced 1 February 2004)

Kenneth Frank Pocock, BAppSc *UAdel.*, FAIFST, Senior Chemist

Dimitra Capone, BAppSc, AssDip(Chem) *USthAust.*, Chemist
Kate Alexandra Lattey, BSc *Canterbury*, Chemist/Sensory Analyst
Tangerine 'Mango' Parker, BSc *Flinders*, Chemist
Tracey Ellen Siebert, BSc *UAdel.*, Chemist

Christopher Daniel Curtin, BSc(Hons) *Flinders*, Microbiologist (commenced 5 January 2004)
Jeffrey Mark Eglinton, BSc(Hons) *UAdel.*, Microbiologist/Senior Computer Systems Officer
Cristian Andres Varela Cabrera, BBiochem, MBiochem, PhD *Catholic Uni Chile*, Microbiologist (commenced 3 May 2004)

Leslie Joseph Janik, AssDipIndChem *USthAust.*, MAppSc *USthAust.*, Technical Research Officer
Simon Justin Dillon, BSc(Hons) *Flinders*, Research Assistant (concluded 16 January 2004)

Gayle Ann Baldock, BSc(Hons) *Guelph*, Technical Officer/Casual Analyst
Wieslawa Cynkar, BSc, PhD *Wroclaw,* Technical Officer

Helen Elizabeth Holt, BAgSc(Hons), PhD *LaTrobe*, Technical Officer (commenced 7 October 2003)
Stella Kassara, BSc(Hons) *UAdel.*, Technical Officer (commenced 6 April 2004)
Maria Jolanta Kwiatkowski, MSc *Gliwice*, Technical Officer
Holger Gockowiak, BSc(Hons) *UAdel.*, Part time Laboratory Manager
Kevin Herbert Pardon, AssDip(AppChem) *SAIT*, Laboratory Technician

Jennifer Rose Bellon, Technical Assistant
Jane Melissa McCarthy, AdCertMedLabSc *USthAust.*, CertVetNurs, CertAnimHand *TAFE*, Technical Assistant

Merran Alida Daniel, BTech, BSc(Hons) *Flinders*, Postgraduate Student
Agnieszka Cox, BSc(Hons) *Flinders*, Postgraduate Student
Maria Josephine de Sa, MSc *Nottingham*, BSc(Hons) *Brunel*, Postgraduate Student
Jennifer Gardner, BSc, *UAdel.*, Postgraduate Student
Antonio Grimaldi, MSc (equiv.), *Florence*, Postgraduate Student
Anthony John Heinrich, BBiotech(Hons) *Flinders*, Postgraduate Student
Kate Susan Howell, BSc(Hons) *UNSW*, Postgraduate Student
Oenone Jean Macintyre, BSc, BE(Chem)(Hons), *UAdel.*, Postgraduate Student
Richard Anthony Muhlack, BE(Chem)(Hons), *UAdel.*, Postgraduate Student
Carolyn Jane Puglisi, BSc *Flinders*, BSc(Hons) *UAdel.*, Postgraduate Student
Heather Eunice Smyth, BSc(Hons) *UAdel.*, Postgraduate Student
Kerry Leigh Wilkinson, BSc(Hons) *Flinders*, Postgraduate Student

Rachel Christine Brown, BTech(For's & AnalytChem) *Flinders*, Honours Student
Matthew Carlyle Caldersmith, BSc(Hons) *UAdel.,* Honours Student
Jennifer Cartwright, BAgSc(Hons) *UAdel.*, Honours Student
Julia Stephanie Crossman, BTech(For's & AnalytChem) *Flinders*, Honours Student
Melissa Fettke, BSc *UAdel.*, Honours Student
Jaromir Guzinsky, BBiotech *UAdel.*, Honours Student
Nicola Renee Sleep, BTech(For's & AnalytChem) *Flinders*, Honours Student

Mathias Klein, Visiting Student (commenced 7 October 2003, concluded 7 January 2004)

See Appendix 3 for details of all students supervised by AWRI staff

Industry Services*

Peter William Godden, BAppSc(WineSc) *UAdel.,* Winemaker/Manager Industry Services
Mark Gishen, BE(Chem)(Hons), MEngSc(Chem) *UMelb.,* Quality Liaison Manager
Sally-Jean Bell, BSc(Hons) *UWA,* GradDip(Wine) *Roseworthy,* PhD *UWA*, Viticulturist
Adrian Dermott Coulter, BSc *Flinders,* GradDipOenol, *UAdel.*, Oenologist
Geoffrey David Cowey, BSc(Hons) *UAdel.*, Chemist
Ella Margaret Clare Robinson, BA, BSc(Hons) *UAdel.,* Chemist (concluded 21 January 2004), Editorial Officer (commenced 22 January 2004)
Matthew Grant Holdstock, BSc *Flinders,* GradDipOenol *UAdel.,* Analytical Service Supervisor – Laboratory (until 21 January 2004), Chemist (from 22 January 2004)
Creina Standish Stockley, BSc(Hons) *UAdel.,* MSc *Flinders*, MBA *USthAust.*, Health and Regulatory Information Manager
Narelle Elizabeth D'Costa, Administrator – Industry Services (commenced 1 September 2003)

*Several members of the Industry Services Team lead or take part in a number of research projects.

Communication and Information Services

Raelene Joan Blair, CertAppMgt(Marketing) *AIM*, Communication and Publicity Manager/Personal Assistant to the Director/Conference Manager
Catherine Grace Daniel, BA *ANU*, GradDip(Lib) *RMIT*, Librarian
Ingrid Betty-Maud Oats, DipLibInfo *Adel. Tafe*, Library Technician
Melissa Elizabeth Francis, BA *UMelb.*, DipEd *Melb. State Col.*, Library Assistant

Administration

Hans Engelbert Muhlack, BEc *UAdel.*, CPA Aust., Company Secretary
Rachel Lee Edwards, Accountant
Heather Margaret Donnell, Secretary to the Director
Rhonda Irene Packer, Administration Officer
Pauline Jorgansen, Administration Support
Julie McConnell, Administration Support
Emma-Kate White, Receptionist (concluded 11 June 2004)
Renee Parsons, Receptionist (commenced 21 June 2004)
Jelena Jovanovic, Laboratory Assistant/ Function Support

Analytical Service

Peter Charles Hans Eichinger, BSc(Hons), PhD *UAdel.*, Manager – Analytical Service

Sandra Margaret Lloyd-Davies, BA *Flinders*, Customer Service Manager
Maria Concettina Mills, Customer Service Officer

Randell Leith Taylor, BSc(Hons) *UAdel.*, Trace Analysis Laboratory Supervisor
Gregory Andrew Ruediger, BAppSc *SAIT*, GradDipOenol *UAdel.*, GLP Supervisor

David Rolfe Boehm, BSc *UAdel.*, Technical Officer
Carol Jean Sigston, BAgSc *UAdel.*, Technical Officer (commenced 7 October 2003)
Daniel Scott Tynan, DipAppSc, *USthAust.*, Laboratory Technician (commenced 10 November 2003)
Amanda Louise Cook, AdvCert(Lab Tech) *Mackay*, Senior Laboratory Technician (concluded 31 October 2003)

Slavko Bekavac, Casual Laboratory Technician
Belinda Bramley, Casual Laboratory Technician
Heather Mandy Brooks, Casual Laboratory Technician
Anna Catalano, Casual Laboratory Technician (concluded 12 December 2003)
Matthew James Cream, Casual Laboratory Technician
Danielle Kylie Leedham, Casual Laboratory Technician
Athina Massis, DipAppSc *USthAust.*, Casual Laboratory Technician (concluded 31 October 2003)

Highlights of the year

A method to quantify the relatively low levels of oxygen permeating through closures in wine bottles was developed. The assay uses a water-soluble compound to trap singlet oxygen and should allow us to estimate both oxygen permeation rates and the initial amount of oxygen in the headspace of a wine bottle within six to eight weeks.

A red wine closure/storage study, which included screw caps with different air headspace volumes, has shown that there was no significant sensory difference, and only marginal differences in phenolic composition, between the natural cork-sealed wine and the wine bottled under screw cap with an intermediate headspace volume. The effect of a small headspace volume under screw cap was to enhance slightly a struck flint/rubbery 'reductive' aroma, while storage under a very large headspace volume conferred a slightly elevated oxidative character to the wine, which was evident at 12 months' post-bottling.

Studies of bentonite absorption in collaboration with the School of Chemical Engineering, The University of Adelaide, have indicated that there might be potential for enhanced adsorption, increased sustainability and reduced wine losses through the application of alternative contacting systems which are more efficient than the current batch method.

An electronic library of UV/Vis spectra has been developed to assist identification of grape and wine phenolic compounds by routine HPLC analysis. More than 50 spectra of phenolic compounds and pigments commonly found in red wine have been added, including spectra of commercially available compounds, and reference standards that have been isolated or synthesised by the Tannin team such as malvidin-3-glucoside-6'-coumarate and Vitisin-B. Various approaches for the simple and efficient measurement of tannins by spectroscopic techniques have been assessed, a standard protocol for a prototype method is currently being developed and will be trialed in cooperation with Industry in the 2004/05 vintage.

A number of polar grape skin pigments have been isolated by MLCCC and the mass spectrometric characterisation of the polar grape skin pigments has now been completed: ESI mass spectra have revealed that these pigments are anthocyanin oligomers and the presence of small amounts of such anthocyanin self-condensation products in grape skin and red wine has been demonstrated for the first time.

A broad range of commercially available oenotannins, including grape-derived condensed tannins as well as hydrolysable tannins from oak and galls, has been sourced from Australian and overseas suppliers. The phenolic composition of these oenotannins has been profiled by HPLC analysis and, not unexpectedly, significant differences between the products have been observed. The oenotannins are subject to ongoing characterisation and serve as reference material for HPLC and spectroscopic measurements.

The sensory assessment and analysis of phenolic compounds of red wine made in 2002 and 2003 by the Tannin team at the Hickinbotham Roseworthy Wine Science Laboratory has been completed. The data allowed us to robustly demonstrate the effects of yeast strains on red wine colour and pigment composition, and to characterise the mouth-feel effects caused by the addition of a particular grape-derived oenotannin.

Work performed in collaboration with the School of Agriculture and Wine, The University of Adelaide through the CRC for Viticulture, has shown that fungal infection of grapes influences the protein content and extent of the potential protein instability problems of wine. Powdery mildew infection of grapevines increased protein content in Chardonnay juice and wine. In contrast, infection of Chardonnay or Semillon grapes by *Botrytis cinerea* in the vineyard resulted in decreased levels of proteins in the juice.

A major study investigating consumer preference for a set of Riesling and Chardonnay wines was completed, which showed the diversity of consumer liking for these wine styles and provided data regarding the sensory properties of the wines that most influenced acceptance. Working with Provisor, the sensory analysis capacity available for AWRI research activities and also for wine industry clients has been substantially expanded.

The compound responsible for a 'fungal must' taint evident in industry assessments of wine corks has been identified as 2-methoxy-3, 5-dimethylpyrazine (MDMP). MDMP is an extremely potent compound with an unpleasant, musty, moldy aroma and an aroma detection threshold in a white wine of 2.1 ng/L. We have also prepared a deuterium-labelled analogue of MDMP and developed a stable isotope dilution assay for measuring MDMP in wines and cork extracts. Whilst the contribution of MDMP to the frequency and intensity of cork taint in bottled wine has yet to be established, it has been assessed by some wine industry personnel as second only to TCA as a cause of cork taint in Australian wine.

Several grape and wine components have been identified as precursors to 2,3,6-trimethylphenylbutadiene (TPB) which has been identified by us as a new wine flavour compound derived from grape glycoconjugates. Informal sensory assessment of TPB indicated that it gave complex 'bottle aged' aromas to white wines. A new stable isotope dilution assay for TPB has been developed and used to analyse more than 100 wines. TPB was found at concentrations above threshold in older, compared to younger, wines. It was found mainly in Semillon and, to a lesser extent, in Chardonnay wines.

Two isomeric glucosidic precursors to damascenone, have been synthesised and their conversion to damascenone studied. Both are converted to damascenone rapidly and in high yield at wine pH and room temperature. The formation of damascenone from these glucosides is slower than from the corresponding aglycons, but takes only a few days.

A novel method, which can quantify 11 important volatile sulfur compounds in a single gas chromatography-atomic emission detection (GC-AED) run has been developed. The method is rapid, accurate and precise, and gives levels of detection 1-2 orders of magnitude better than published methods.

AWRI Honours student, Julia Crossman, was awarded the Flinders University Medal.

The paper entitled 'Screening for potential pigments derived from anthocyanins in red wine using nanoelectrospray tandem mass spectrometry' by Yoji Hayasaka and Robert Asenstorfer was one of the most cited papers published in the *Journal of Agriculture and Food Chemistry* in 2002.

The new ThermoFinnigan LCQ *Deca XP Plus* mass spectrometer with *Surveyor* HPLC Pump, PDA detector and autosampler was purchased by Provisor and installed at the Waite Campus Mass Spectrometry Facility, within the AWRI, on 13 November 2003.

A study on the effect of sample preparation methods and storage (frozen for up to 12 months) on the laboratory assays for total soluble solids, pH and the concentrations of total anthocyanins, and total phenolics in red grapes was completed and published, thus providing industry with simple laboratory protocols supported by sound objective validation data.

A proposed industry 'standard' laboratory method for the measurement of the concentration of total anthocyanins in red grapes was prepared.

Continuing investigations into alternative sample presentation modes for NIR scanning confirmed the feasibility of whole fruit analysis, offering the potential to speed up greatly the testing of red grapes by such techniques.

The NIR team commenced the development of training and teaching of the principles and use of multivariate analysis techniques (chemometrics) to other AWRI staff members as well as for industrial and external research partners.

Improved calibrations for the FOSS *WineScan* were developed by the inclusion of Australian wine samples to enhance the calibrations based on European wines that are provided with the instrument. This will be of assistance to Australian producers who have a core need for such technology, in-house.

Optimising the Yeast Assimilable Nitrogen (YAN) content of grape juice to promote fermentation conditions which enhance the production of wine with a clean, fruity aroma profile has been demonstrated. Investigations are continuing with different yeast strains and fermentation conditions to define better the optimal range of juice YAN content.

The first data to indicate that three yeast strains characterised by micro-scale vinification (1 kg) can similarly affect the colour properties of unoaked red wines of, at least, up to eight months of age, made on a pilot winery scale, have been obtained from a red wine fermentation trial conducted in the Hickinbotham Roseworthy Wine Science Laboratory during 2003 by the Lallemand-AWRI Wine Microbiology team collaboration. The micro-scale vinification methodology, which is being used to study the impact of other fermentation parameters on wine colour properties, could expedite optimisation of red wine fermentation.

Intervention strategies for overcoming sluggish fermentation in a difficult-to-ferment Chardonnay, inoculated with a non-conventional *Saccharomyces bayanus* yeast strain, were found not to further detrimentally affect wine aroma by sensory assessment and chemical analysis. This finding suggests that the winemaker has flexibility in the choice of intervention strategies, which, in this work, included (i) sequential inoculation with *Saccharomyces cerevisiae* AWRI 838 at approximately the half-way point of fermentation, (ii) aerobic handling (pump over and 'splash') when fermentation slowed, or (iii) sequential inoculation with *S. bayanus* AWRI 1375 when fermentation had stopped.

Sensory assessment of red wine in which the basic organic acid composition of post-MLF wine had been restored to pre-MLF conditions has provided the first objective evidence that MLF can modify red wine palate or mouth-feel, independent of the pH and titratable acidity. Work in progress to establish the chemical nature of the MLF-induced palate change, might provide a strategy for enhancing the effect.

Data sourced from the AWRI Analytical Service commercial database confirmed that downward trends in mean volatile acidity and bound SO_2 concentrations in Australian wines have continued, coincident with strategies to improve these measures implemented across a range of Industry Services' projects.

A record number of ten *Trouble free winemaking – causes and prevention of common wine instabilities* workshops were presented, in three states.

Data sourced from an Industry Services' targeted survey confirmed that the mean concentration of the *Dekkera/Brettanomyces* yeast-derived compound, 4-ethylphenol, has continued to decline in Cabernet Sauvignon-based wines sourced from five regions. This is coincident with the implementation of strategies developed under the AWRI '*Brettanomyces* project' that were aimed at assisting wine producers to make wines with lower concentrations of this compound than previously.

It was demonstrated there is a large genetic diversity between isolates of *Dekkera/Brettanomyces* yeast from Australian red wines, and apparent large differences in the SO_2 tolerance between genetically similar groups of these isolates.

The AWRI Viticulturist responded to 430 enquiries. The majority (76%) were regarding the use of agrochemicals for pest and disease control, the persistence of residues through winemaking and their effects on fermentation, and issues related to maximum residue limits in overseas markets.

Eleven thousand copies of the AWRI's annual publication, *Agrochemicals registered for use in Australian viticulture 2003/2004* were produced and duplicated on the Institute's website. The booklet was distributed with the *Australian Grapegrower and Winemaker, Technical Review* and in the *Research to Practice*™ IPM and Spray application manuals. The tables were featured in *Australian Viticulture* and *The Grapevine Management Guide 2003/2004* (Somers et al., 2003).

The Viticulturist and Jelka Software developed an agrochemical database. This database is similar to the currently existing MRL database except that it contains all the information related to and presented in the publication, *Agrochemicals registered for use in Australian viticulture 2003/2004*. The ultimate aim is to provide a searchable database to replace the static retrieval of agrochemical information currently available from the AWRI's agrochemical website.

A common spray diary format was developed in conjunction with industry for the 2004/2005 season and was placed on the AWRI website. Along with this format are explanations of the spray diary terminology and other relevant information.

Staff of the John Fornachon Memorial Library responded to 3,788 requests for information during 2003/2004.

Over 4,000 new records were added to the web-accessible database of the Library (available only to Australian winemakers and grapegrowers) during the year, making a total of over 29,500 records available for searching, 24 hours per day, 7 days per week.

AWRI published 42 papers on AWRI activities in refereed and non-refereed publications.

AWRI staff members gave 137 oral presentations, conducted 12 workshops (including 28 presentations within 10 *Trouble free winemaking – causes and prevention of common wine instabilities* workshops presented in three states) and presented 13 posters.

AWRI staff members presented 46 lectures and coordinated a six week subject to undergraduate students.

AWRI staff members supervised/co-supervised 21 postgraduate students.

AWRI staff members recorded and responded to 6,979 requests for information during the 2003/2004 year or, to put the statistics into perspective, 28 people contacted the AWRI seeking information on every working day of the year. This figure does not include the amount of problem samples investigated (1,262) or the request for work through the Analytical Service which conducted >56,000 individual analyses during 2003/2004.

Readers are strongly encouraged to read the report in detail rather than relying on the brief details above for information.

Peter Høj
INSTITUTE DIRECTOR

Staff activities

In addition to undertaking research and other projects described in this report, the AWRI performs a large number of external activities in support of the Australian wine industry.

Information on seminars, talks and poster papers given to outside organisations, academic lectures delivered, graduate students supervised, media interviews given and the papers published is tabulated and can be found in Appendices 1–6 of the Annual Report. Activities in addition to those in the Appendices are described below.

Peter Høj is a member of the following:
Prime Minister's Science, Engineering and Innovation Council
Premier's Science and Research Council (South Australia)
Premier's Wine Council (South Australia)
Wine Industry Technical Advisory Committee (AWBC/WFA)
Provisor Pty Ltd Board
Cooperative Research Centre for Viticulture II (CRCVII) Board
Wine Committee (Royal Agricultural and Horticultural Society of South Australia)
Waite Campus Management Committee
Committee of Management, Viticultural Publishing, publisher of *Australian Journal of Grape and Wine Research*
Editorial Board of the *Journal International des Sciences de la Vigne et du Vin*
Conference Planning Committee of the Twelfth Australian Wine Industry Technical Conference (24-29 July 2004, Melbourne) (Chair)

He is also the AWRI's representative on The University of Adelaide's School of Agriculture and Wine's Advisory Committee and the Management Committee. Professor Høj is the current holder of the Australian Wine Industry Chair of Oenology at The University of Adelaide.

Sakkie Pretorius is a member of the Editorial Board of the following journals: *American Journal of Enology and Viticulture, Annals of Microbiology, FEMS Yeast Research* and *Yeast*. He is also the Co-Chair of the Program Sub-Committee of the Twelfth Australian Wine Industry Technical Conference, and member of the Conference Planning Committee. He is also an Affiliate Professor of the University of Stellenbosch and The University of Adelaide.

Hans Muhlack is the Public Officer of the Australian Wine Industry Technical Conference Inc.

Rae Blair is a member of the Conference Planning Committee for the 12th AWITC and is the Treasurer and Conference Manager of the Australian Wine Industry Technical Conference Inc.

Catherine Daniel is a member of the ALIA Special Libraries Section (SA) Branch.

Creina Stockley is an Affiliate Senior Lecturer at The University of Adelaide. She is further a Member, Wine Industry Technical Advisory Committee (as Technical Liaison), Member, AWBC Legislation Review Committee, Member, Wine Industry National Environment Committee, Member, Eco-efficiency Working Group (Sub-committee of South Australia Wine and Brandy's Environment Committee), Member, Australian delegation to the Office de la Vigne et du Vin, Vice-President, Nutrition and Wine Expert Group of the Office de la Vigne et du Vin, Member, National Drug and Alcohol Research Centre's Young People and Alcohol Project Advisory Group, Member, Scientific Committee, Vindaba Wine and Health International Congress 2005, International Consultant, Center for Wine and Cardiovascular Health, University of Alabama, Chair, Waite Campus Executive Committee. She is also a Board Member of The University of Adelaide's Children's Services.

Elizabeth Waters is an Associate Editor for the *Journal of Agricultural and Food Chemistry*, Manager of Program 1 of the Cooperative Research Centre for Viticulture II (CRCVII) and is a member of the Program Sub-Committee of the Twelfth Australian Wine Industry Technical Conference.

Mark Sefton is on the Editorial Review Board of the *International Journal of Vine and Wine Sciences* and is the project leader of project 1.3 of the CRCVII. He is also an Affiliate Senior Lecturer with The University of Adelaide.

Leigh Francis is an Editorial Board member of the *Journal of the Science of Food and Agriculture* and is also an Affiliate Lecturer with The University of Adelaide.

Markus Herderich is Leader of Project 1.2, the 'Tannin project', of the CRCVII, Affiliate Associate Professor at The University of Adelaide and he is a member of the Advisory Board of the *Journal of Agricultural and Food Chemistry*. He was also a member of the scientific committee, International Workshop on Anthocyanins, Sydney (held 29 January 2004).

Paul Henschke serves on the Editorial Review Board of the following journals: *Australian Journal of Grape and Wine Research; Food Microbiology; Mitteilungen Klosterneuburg;* and the *South African Journal of Enology and Viticulture*. He was the Chair of the *3rd Yeast: Products and Discovery Meeting*, held 4-6 April 2004, Nuriootpa, SA, and is a member of the International Conference of Yeast Genetics and Yeast Molecular Biology committee.

Eveline Bartowsky is a member of The University of Adelaide's School of Agriculture and Wine Occupational Health and Safety Committee and is an Affiliate Lecturer at The University of Adelaide.

Peter Godden is a member of the 12th AWITC Conference Planning and Program sub-Committees and is the Workshop Coordinator of a program of 70 workshops to be held at the Twelfth Australian Wine Industry Technical Conference. He is also an Associate Judge at the Royal Adelaide Wine Show (since 2001).

Mark Gishen is leader of project 1.4 of the CRCVII, and is the AWRI's representative on the Winemakers' Federation of Australia (WFA) Legal Metrology Group.

Matthew Cream serves on the Interwinery Analysis Group Committee.

Visitors to the Institute

Australia

Marta Bellapart, Oliver Crawford, Josephine Horn, Andrew Marks, Paul Milton, Ian Shepherd, Sue Franke, Rachel Coverdale, Randall Cummins, Emma Dal Broi, Peter Gago, Andrew Koerner, Andrew Locke, Steve Roden, Vikki Neldner, Alida Pisano, Tom Barnes, Simon Fischer, Priscilla Buckley, Wayne Falkenberg, Steve Goodwin, Michelle Heagney, Brian Pietsch, Malcolm Ray, Brett Sharp, Kym Schroeter, Andrew Baldwin, Greg Clayfield, Peter Gajewski, Matt Koch, Joanna Marsh, David Matthews, Matthew Pick, Greg Jarratt, Matthew Johnson, Crista Cowell, Chris Dix, Andrew Fleming, Jane Gilham, Justin Knock, Ian Long, Louella McPhan, Kevin Miller, Marie Pearce, Charles Whish, Allen Hart, Briony Hoare, Sue Hodder, Glenn James, Atsuko Kobayashi, Steve Lienert, Peter Taylor, Stuart Pym, Corrina Rayment, John Ballard, Michael Christophersen, Arthur O'Connor, Peter Hayes, Richard Hamilton, Greg Pearce, Gioia Small, Mark Smith, Chris Malcolm, Nick Radford, Steve Jackman, Southcorp Wines (7-9 July 2003).

Dr John Stocker, Chairman, Grape and Wine Research and Development Corporation (17 July 2003)

Michael Eyles, Executive Chair, Agribusiness and Health Group, CSIRO (18 July 2003)

Rachel Lucas, Director Science and Innovation, South Australian Department of Further Education Employment Science and Technology (22 July 2004)

Morris Herman, STO Dept of Defence (28 July 2003)

Jean Bourjade, CEO Australasia, Oeneo Australasia (4 August 2003)

Nick James-Martin, Area Manager-Wine, Air Liquide Australia Ltd (4 August 2003)

Tony Royal, Seguin Moreau (4 August 2003)

Craig Fowler, Executive Director Science, Technology and Innovation Directorate, Department of Further Education, Employment, Science and Technology, Government of South Australia (5 August 2003)

Dick Wilson, Chairman, Drug and Alcohol Services (SA) (1 September 2003)

Terry Kavanagh, Regulatory Affairs Manager of Carlton and United Breweries (8 September 2003)

Michael Perkins and a group of students from Medicinal Chemistry, Flinders University (25 September 2003)

Gayle Manning, Southcorp Wines (25-26 September 2003)
Rachel Walker, Serv-Ag Research Tasmania (1 October 2003)

Russell Johnstone, Joy Dick, Jan Dearden, Stephen Guilbaud-Oulton, Inca Lee, Alistair Dinnison, Martin Wirper, Rachel Reid, Matthew Partridge, Michelle Crowe, Louise Eather, Kirrily Rimmer, Angus Davidson, Orlando-Wyndham Group (14-16 October 2003)

GWRDC Priorities Reference Group: Brian Sainty, Jim Campbell-Clause, Len Schliefert, Jim Lawrie, Emma Jamieson, Trevor Drayton, Graeme Ray, Geoff Knights, Paul Mathews, Kerry deGaris, Richard Hamilton, Samantha Hellams, Erin McCarthy, Philip Manson , Tony Battaglene, Andrew Miller (23 October 2003)

Anton Silove, Orica Chemnet (11 November 2003)

Andreas Betzner, Australian National University (24-27 November 2003)

Guy Adams, Langhorne Creek Winegrape Growers Association; Max Arney, Limestone Coast Wine Industry Council; Tony Baggio and Bruno Brombal, Wine Grape Marketing Board/WGCA; Brenton Baker, Hardy Wine Company; Chris Byrne, Riverland Winegrape Growers Association/WGCA; Paul Clancy, Winetitles; Guy Darling and Philip Engelfield, Victorian and Murray Valley Winegrape Growers' Council/WCGA; Chris Dundon, FABAL; Graeme Fechner, Barossa Valley grower; Ron Fraser, Margaret River Wine Industry Association; Ben Gibson, Orlando Wyndham; Alan Gilgen, McGuigan Simeon Wines; Steve Hampel, Riverland Winegrape Growers Association/WGCA; Peter Hayes, Southcorp; Don Heylen, Adelaide Hills Wine Region; Tony Hoare, Wirra Wirra Vineyards; Philip Laffer, Orlando Wyndham; Rhett Marlow, Winegrape Growers' Council of Australia; Paul Mathews, McLaren Vale Grape, Wine and Tourism Association; Leo Pech, SA Farmers Federation – Winegrapes Section/WGCA; Bruce Phillips, Victorian Wine Industry Association; John Posshingham, SA Farmers Federation – Winegrapes Section/WGCA; Roger Rowe, Adelaide Plains Wine Region; Brian Simpson, Wine Grapes Marketing Board/WGCA; Mike Stone, Victorian and Murray Valley Winegrape Growers' Council/WGCA; Stephen Strachan, Winemakers' Federation of Australia; Jerry White, Southern Fleurieu Vignerons Association (9 December 2003)

Nick Bulleid; Garry Baldwin, Wine Network Australia; Bernard Hickin, Orlando Wyndham; Tim James, Wirra Wirra Vineyards; Tony Royal, Oeneo Australasia; Tim Knappstein, Lenswood Vineyards; Phillip John, Hungerford Hill Wines; Brian Walsh, The Yalumba Wine Co; Neville Rowe, Mitchelton Wines; Chris Hatcher, Beringer Blass; Ian McKenzie; Sue Bell, Hardy Wine Company (16 December 2003)
Daniel Shaw, J. Lohr Winery, USA (13 January 2004)

Paul Anderson, Paul Anderson & Associates (19 January 2004)

Ewa Goldys, Macquarie University (25 March 2004)

Alex Stanko, Lastek (25 March 2004)

Geoff Knight and the Corporate Executive team, Primary Industries and Resources SA (2 April 2004)

Dr Mark Downey, Department of Primary Industries, Mildura (21 April 2004)
James Porter, Piper Alderman (Hon. Consul. Germany) (29 April 2004)

Rob Hunt, Boar's Rock, McLaren Vale (12 May 2004)

Bill Schumann, Department of Agriculture, Fisheries and Forestry (18 June 2004)

Arduino Magnoni, Senior Lecturer, Department of Clinical Pharmacology, Flinders University of South Australia (18 June 2004)

Visitors to the Institute

International

Denmark

Jacqueline Jensen, Journalist, Wines and Spirits – Gastronomi, Denmark (5 March 2004)

Chile

Danilo Sturiza, Deputy Director of TodoChile Program, Director Program for investment attraction in regions of O'Higgins, Antofagasta and Magellanes; Alejandro Soto, Regional Director, CORFO Maule Region; Veronic Godoy, Investment Executive in charge of wine industry, Todo Chille O'Higgins Region. Chile (8 July 2003)

Cristian Varela, Pontificia Universidad Catolica de Chile (Santiago de Chile) (26 & 27 July 2003)

Gonzalo Francisco Pérez Abarzúa, Agrícola y Forestal Arco Iris S.A. (Viña Anakena); Andrés Sánchez Westhoff, Viña Tabontinaja S.A.; José Luis Fuenzalida Izquierdo; Felipe Müller, Santa Teresa S.A. (Viña Santa Inés / De martino); Eduardo Gustavo Reinero Cerda, Agrícola Salve S.A. (Casa Donoso); Clorindo Retamal Bravo, Sociedad Vitivinícola Sagrada Familia S.A.; Cristobal Ossa Prieto, Viña La Rosa Limitada; Rodrigo Miranda Moyano, Viña San Isidro S.A.; Tomás Alejandro Wedeles Pacheco, Viña Santa Rita S.A.; Stefano Gandolini, Sur Andino S.A. / Santa Rita S.A.; Mario Nuñez Ramírez, Frutícola Agua Dulce Limitada; Beatriz Montesinos Iroume, Viña El Rosal S.A. (Viña William Cole); William Cole, Viña El Rosal S.A. (Viña William Cole); Francisco Bravo, Tersainox S.A.; Manuel Madrid, Corporación Chilena del Vino; Mario Perez, CORFO – Fontec, Chile (25 November 2003)

Denmark

Mai Nygaard, Anders Broe-Bendtson, Tom Riisom, FOSS, Denmark (20 April 2004)

France

Gerard Epin, Chairman and CEO, Oeneo, France (4 August 2003)

Philippe Guillomet and Tertius van der Westhusizen of Laffort Oneologie, France (13 October 2003)

Pascale Bruno and Jose Luis Martin Boguillard, Pernod Ricard, France (20 November 2003)

Jacques Rousseau, ICV, France (18 March 2004)

Bruno Tisseyre, Cemagref, Montpellier, France (7 June 2004)

Germany

Dieter Heckel, Scharfenberger, Germany (18 July 2003)

Christian Pfitzner, Federal Agricultural Research Centre, Braunswieg, Germany; (8 September 2003)

Professor Thomas Baaken, University of Applied Sciences (Fachhochschule Münster), Germany (19 January 2004)

Thomas Kessler, Consulate General of the Federal Republic of Germany, Germany (29 April 2004)

Italy

Federico Castellucci, Director of the Italian Winemakers Association, and Director General Elect of OIV, Italy (3 December 2003)

Erik Dogliotti, Dogliotti; Giuseppe Tedesco, Enologo Giuseppe Tedesco; Giuseppe Lamona, Frontenac S.P.A.; Enzo Mecella, Enzo Mecella; Calogero Statella, Feudo Arancio; Filippo Buttafuoco, Settesoli; Fabio Ongaro, Enoimpianti; Dora Marchi, Enosis S.R.L.; Enrico and Michele Moschetta, ColSandago; Pietro Montalto, Cantine Paolini Soc. A.R.L.; Mauro Musso, Intermediazioni vini; Giovanni Chiarle, Cantina di Nizza S.C.A R.L.; Paola Manera and Vincenzo Muni, Sinergo Soc. Coop A.R.L.; Paolo Cardu, Consorzio Fruiticoltura; Giuliano Boni, VinIdea.net, Davide Rolla; Erica Barbieri; Domenico Greco; Tonino Guzzo; Tommaso LaSala; Vincenzo Muni; Ricardo Reina Rutini, Italy (27 April 2004)

Japan

Mari Kurano, TV Tokyo Corporation, Japan (20 November 2003)
Masatoshi Morikawa, Kirin Brewery Ltd, Japan (15-17 March 2004)

The Netherlands

J Han de Winde, DSM Bakery Ingredients, Delft & Kluyver Laboratory of Biotechnology, Delft University of Technology, Delft, The Netherlands (24-27 November 2003)

New Zealand

Richard C. Gardner, Professor of Molecular Genetics, and Dr Laura Nicolau, Lecturer in Wine Science, University of Auckland (August 2003)

Philip Mason, New Zealand Winegrowers and Andy Frost, Montana Wines, New Zealand (9 and 10 December 2003)
Andy Frost, Montana Wines, New Zealand (19 April 2004)

South Africa

Johan van Rooyen, South African Wine and Brandy Company, South Africa (9 December 2003)

Spain

Mireia Torres Maczassek, Miguel Torres S.A., Spain (31 March 2004)

United Kingdom

Martin Hall, Director of Food Science Division and Nick Byrd, Chromatography Section Manager in the Department of Chemistry and Biochemistry, Campden & Chorleywood Food Research Association Group (CCFRA), Gloucestershire, UK (28 October 2003)

Ray Marriott, Botanix Ltd, UK (18 November 2003)

Robert Falconer, Cambridge University, UK (10 May 2004)

USA

Tim Ryan, E. & J. Gallo, USA (24 July 2003)
Phil Williams, PDK Grain, USA (9 September 2003)

Dr Russell Molyneux, USDA, USA (18 September 2003)

Drs Nancy Irelan and Tom Pugh, E. & J. Gallo, USA (11 November 2003)

Dr Steve Price, ETS Laboratories, USA (24 and 26 November 2003)

Jerry, Steve and Carol Lohr, J. Lohr Winery; Jeff Meier and Steve Carter, UC Davis; Scott Williams, Fresno State and Cal Poly, USA (13 January 2004)

Dr Gary Martin, Pfizer Corporation, Kalamazoo, Michigan, USA (20 February 2004)

Vanessa Stockdale, E. & J. Gallo Winery, Modesto, USA (24 March 2004)

Susan Stewart, US Tax and Trade Bureau, USA (26 March 2004)

Steve Kupina, Canandaigua Wines, Constellation Wines, USA (19 April 2004)

Defining, measuring and controlling important volatile aroma and flavour compounds in grapes and wine

Staff: Rachel Brown, Dimitra Capone, Agnieszka Cox, Merran Daniel, Dr Gordon Elsey, Dr Leigh Francis, A/Professor Markus Herderich, Kate Lattey, Kevin Pardon, Mango Parker, Dr Alan Pollnitz, Carolyn Puglisi, Tracey Siebert, Dr George Skouroumounis, Heather Smyth, Katryna van Leeuwin, Kerry Wilkinson and Dr Mark Sefton

Formation of important wine flavour compounds from grape-derived precursors

2,3,6-Trimethylphenylbutadiene (TPB) has been recently identified at the AWRI as a new wine flavour compound derived from grape glycoconjugates (AWRI publication #751). Informal sensory assessment of TPB indicated that it gave complex 'bottle aged' aromas to white wines. We have now completed a six-step synthesis of a deuterium-labelled analogue of TPB and used this analogue to develop a new and more accurate and sensitive stable isotope dilution analysis (SIDA) method to determine TPB in wine. To date, more than 100 commercial wines have been analysed. TPB was found at concentrations above threshold, mainly in Semillon, and to a lesser extent in Chardonnay wines. More TPB was found in older, compared to younger wines. No TPB was found in any of the red wines examined, confirming an earlier trend observed with a smaller number of samples.

Several grape and wine components that were predicted to be precursors of TPB have been synthesised and a series of hydrolytic studies with these compounds has been undertaken. All compounds gave a high concentration of TPB at wine pH. A series of stability studies has also been completed. TPB decomposes relatively slowly in model wine, white wine, or phenolic-stripped red wine but much more rapidly in red wine or in model wines to which grape skin or seed tannins had been added. The data explain why TPB is found only in white wines despite the presence of precursors in both red and white grapes.

Analysis of a young Semillon wine aged in bottle and also in glass ampoules under identical conditions has shown that the concentration of TPB in the ampoule-stored wine was some 40% higher than the wine under cork closures. While, it is possible that oxygen ingress through the closure might have contributed to loss of TPB in bottle, it is more likely that partial absorption (scalping) of TPB by the cork closure is responsible for this loss. A similar level of absorption has been observed for structurally similar compounds (naphthalene, TDN) in the flavour scalping trial undertaken recently and reported last year.

Beta-damascenone is an important grape-derived aroma and flavour compound in wine. It has a powerful 'green apple' and 'quince-like' smell, and is said to add to overall aroma intensity in wine. Two isomeric glucosidic precursors to damascenone, have been synthesised and their conversion to damascenone studied. Both are converted to damascenone rapidly and in high yield at wine pH and room temperature. The formation of damascenone from these glucosides is slower than from the corresponding aglycons, but nevertheless takes only a few days.

A glucosidic precursor of 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) has been synthesised, and hydrolytic studies on this compound and its corresponding aglycone are underway. Because the conversion of these precursors to TDN is relatively slow, the hydrolytic study is a long term one. The data will give us an insight into the importance of glycoconjugation to the formation of TDN during bottle storage. At higher concentration, TDN has a 'kerosene' like aroma and is mainly associated with some older Riesling wines. If glucosidic linkages influence the rate and scale of TDN formation, it is likely that the level of glycosidase activity in wine during and after fermentation could influence the degree to which 'kerosene' characters are observed in such wines.

Cork taint

The compound responsible for a 'fungal must' taint evident in industry assessments of wine corks has been identified as 2-methoxy-3,5-dimethylpyrazine (MDMP). The identification was made on the basis of gas chromatography/odour analyses, collection of material using micro-preparative techniques, determination of chemical properties of collected material and comparison by gas chromatography/mass spectrometry with an authentic sample, synthesised from 2-hydroxy-3,5-dimethylpyrazine. MDMP is an extremely potent compound with an unpleasant, musty, mouldy aroma and an aroma detection threshold in a white wine of 2.1 ng/L. The most common descriptors used by the sensory panellists for MDMP in the white wine were 'dirty', 'dusty', 'musty' and 'mouldy'. A few assessors also described the higher concentrations as 'chocolate' or 'coffee'. The aroma threshold of MDMP compares closely with that of 2,4,6-trichloroanisole (TCA), which has an aroma threshold in wine of 2-4 ng/L and is recognised as one of the most potent taint compounds affecting a wide range of foods and beverages.

MDMP was first identified 20 years ago when it was found to be responsible for an obnoxious odour, described as 'musty, foul drains, or sour dishcloths', present in certain machine cutting emulsions used in engineering workshops. An unidentified, aerobic, Gram-negative bacterium was isolated from these emulsions and gave MDMP as the only major component of the volatile fraction isolated from the culture broth. Since then, it has, to our knowledge, only been further reported once – as a component of coffee beans.

We have also prepared a deuterium-labelled analogue of MDMP and developed a stable isotope dilution assay for measuring MDMP in wines and cork extracts. Although only a small number of wine samples in which tainted corks have been soaked have so far been analysed, there are indications that, unlike TCA, MDMP can be efficiently extracted from cork by wine. Industry sensory assessments of cork closures have indicated that some cork batches contain a mixture of closures contaminated with TCA, closures contaminated with MDMP, and closures contaminated with both. We have since observed this co-occurrence of TCA and MDMP in a tainted commercial wine submitted for analysis. This wine contained TCA and MDMP in similar concentration. Whilst the contribution of MDMP to the frequency and intensity of cork taint in bottled wine has yet to be established, it has been assessed by some wine industry personnel as second only to TCA as a cause of cork taint in Australian wine. The discovery of MDMP

will allow cork suppliers and the wine industry to introduce a further component to their assessment of cork quality during manufacturing and use, to the further benefit of consumers.

Relationship between composition and sensory properties of wine

Following a detailed sensory study of 40 commercial wines (20 Riesling and 20 unwooded Chardonnay), a chemical analysis of these wines has been completed. The concentration of forty-nine compounds in these wines has been determined by stable isotope dilution analysis. The link between the sensory and compositional data has been explored using partial least squares (PLS) regression. PLS models were built using the composition data to predict the scoring of sensory attribute scores. Relating chemical composition to specific aromas in the wine has enabled the elucidation of particular volatile compounds that are most influential to the aroma of these two varieties. Future research will focus on validating the relationships found between aroma and volatile composition by means of sensory reconstitution experiments.

Following the preparation of the nature-identical isomer of *cis*-oak lactone and a determination of its detection threshold in a white wine (23 µg/L, and described in the previous annual report), we have also determined the aroma detection threshold of this compound in a bag-in-box red wine. The group aroma detection threshold was 46 µg/L, although there appeared to be a substantial variation in the sensitivity of individual panellists to this compound. A quarter of the panellists had individual thresholds for nature-identical *cis*-oak lactone at a concentration of 4 µg/L or below, while for several panellists, the individual thresholds were more than 280 µg/L. While these results need to be confirmed with a larger group, they indicate that there might be two or more population groups with completely different sensitivities to this compound. Since *cis*-oak lactone is probably the single most important oak-derived aroma/flavour compound found in barrel-aged wines or wines treated with oak chips, this observation could have important ramifications in determining optimum levels of *cis*-oak lactone in wines for consumers.

Identification of new grape and wine flavour components

Eighteen Shiraz grape berry sample homogenates were profiled for aroma and flavour attributes by a sensory panel in duplicate, as part of investigations into pepper flavour. The samples were sourced from South Australian and Victorian viticultural regions over two seasons. There were significant differences among the samples in a number of aroma attributes: 'grassy', 'green apple', 'compost', 'tobacco', 'red berry', 'raisin', 'pepper' and 'spicy', and flavour attributes: 'sweetness', 'grassy', 'green apple', 'compost', 'cooked fruit', 'chocolate', 'acidity' and 'pepper'. Two Mount Langi Ghiran berry samples from 2002 were rated the highest for the 'pepper' aroma and flavour attributes, and a further group of five samples were rated with intermediate 'pepper' scores. There was a weak inverse relationship between the level of 'pepper' character and the intensity of 'sweet' taste, 'grassy' and 'red berry' flavour, although this correlation was dominated by the two strongly peppery samples, which were rated as relatively low in 'sweetness', 'grassy' and 'red berry' flavour. This study provides further convincing evidence that a pepper aroma and flavour can be discerned in grape berries and has also provided valuable information for on-going investigations pursuing the chemical basis of the sensory differences among these Shiraz grape samples. The results indicate that the pepper flavour, being detectable by aroma as well as in-mouth, is not caused by non-volatile compounds and could be amenable to analysis by headspace analytical techniques.

Analysis of wine components and their precursors

The project is on track to achieve our aim of having rapid and accurate analytical methods for determining most known important flavour compounds in wine, as well as methods to determine at least several flavour precursors in grapes. So far, rapid and accurate analytical methods have been developed for more than 70 volatile wine components (including wine taint compounds) that are likely to be important to wine aroma and flavour. Of these compounds, the majority are derived directly or indirectly from the grape. Development of analytical methods for several other grape and wine flavour compounds and grape-derived precursors for important volatile wine phenols is in progress. As part of this program, we have now synthesised stable isotope-labelled analogues of several important thiols and their precursors. Methods have been developed to measure these compounds in model ferments, and these methods are currently being used by the AWRI's Molecular Biology team for their work on flavour release during fermentation.

A method for the quantitative analysis of 11 volatile sulfur aroma compounds in wine with a single gas chromatography analysis has been developed and validated. This method takes advantage of our newly acquired cool on-column injector for direct headspace analysis of labile wine volatiles. The specific and sensitive detection of sulfur compounds has been achieved with an atomic emission detector (GC-AED). The procedure provides us with rapid, accurate and precise measurement of compounds implicated in common wine off-flavours, is essential for studies aiming to optimise copper fining protocols and will be beneficial for the characterisation of 'reduced' characters in wines.

Applications of research and development to industry problems and opportunities: *Investigations into the relationship between* Dekkera/Brettanomyces *yeast and red wines in Australia*

Staff: Peter Godden, Jane McCarthy, Jenny Bellon, Dr Paul Henschke, Dr Miguel de Barros Lopes, Dr Leigh Francis, Kate Lattey, Dr Mark Sefton, Dimitra Capone, Dr Alan Pollnitz, Mark Gishen, Adrian Coulter, Geoff Cowey, Ella Robinson, Greg Ruediger, Narelle D'Costa, Chris Curtin

Approximately 120 isolates, confirmed to be *Dekkera/Brettanomyces* using Polymerase Chain Reaction (PCR) analysis, have now been obtained from barrel samples of Australian red wines by the Industry Services team. The distribution of genetically diverse strains within this species across Australia is becoming clearer as the number of isolates increases. Utilising amplified fragment length polymorphism (AFLP) analysis, seven strain groupings have been discerned that exhibited as little as 58% genetic similarity to one another. A majority of isolates (72.4%) (see Figure 1) were found to belong to strain grouping 'A', representatives of which were found across the country in 20 winemaking regions. Other strains were less common, although it is anticipated that when further isolates from some under-represented regions are obtained, more isolates from these other groups might be found.

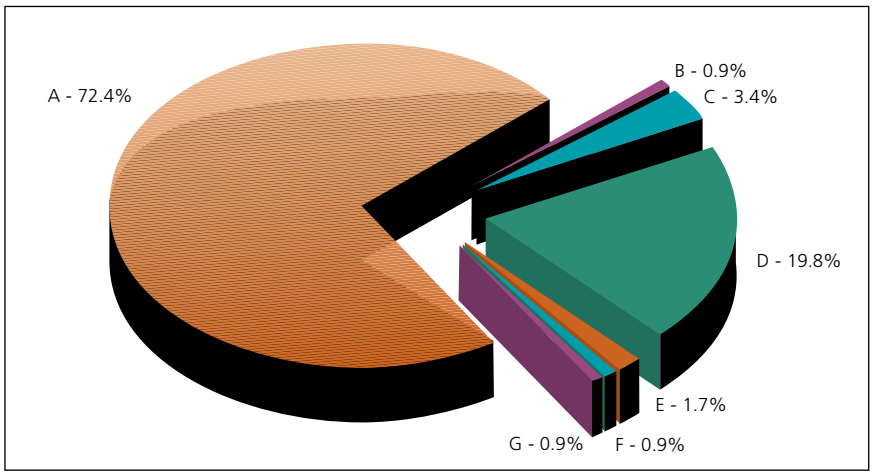


Figure 1. Relative abundance of *Dekkera/Brettanomyces bruxellensis* strains isolated in wine samples taken from 25 different winemaking regions in Australia, as determined by amplified fragment length polymorphism (AFLP) analysis

The next phase of this project will aim to link the genetic fingerprints of strains obtained by AFLP analysis, to physiological or metabolic capabilities relevant to cell growth and volatile phenol production. This phase began in earnest with the recruitment of Microbiologist Chris Curtin to work on this project, in January 2004. To facilitate rapid progress towards this objective, high-throughput growth assays conducted in microplate-wells have been developed. The approach being taken is to calculate kinetic parameters based on non-linear modeling of the growth data, which is a significant improvement over traditional physiological screening in terms of data quality. Technical difficulties that have been overcome during the reporting period include: extensive and non-uniform evaporation of growth medium from wells which is of particular importance given the slow growth rates of *Dekkera/Brettanomyces* isolates; atypical growth curves resulting from uneven sedimentation of yeast at the base of wells, and; unreliable curve fitting using standard Boltzmann and Gompertz sigmoidal growth models. Preliminary experiments comparing cultivation in microplates and shakeflasks found that *Dekkera/Brettanomyces* isolate BD13 (strain A) grew at the same rate in both systems, although the final yields of biomass and 4-ethylphenol differed. Ongoing work to characterise the nature of these differences will ensure the high-throughput microplate system generates valid and reproducible results. Experiments with representatives of the other strain groupings confirmed them all to be 4-ethylphenol producers in chemically defined medium, and the ability of these isolates to produce 4-ethylphenol in wine will be confirmed in the coming year.

The power of the high-throughput microplate approach was demonstrated in an experiment to investigate the effects of pH on growth of the major wine strain groups in chemically defined laboratory medium. With less than 30 minutes per day required to record growth using the AWRI's newly acquired microplate reader, (4-time points per day) over a period of seven days, six yeast strains were grown at five different pH intervals from 3.0 to 4.0, each in triplicate. One day of data analysis followed, but from this, the kinetic parameters could be calculated for each individual microfermentation and statistically compared. In contrast, a traditional experiment using viable-cell plating was conducted to investigate SO_2 tolerance of the major yeast strains in chemically defined medium. In this case, six yeast strains were exposed to four levels of SO_2 in duplicate and sampled four times over a 2-hour period. This approach necessitated as much as five hours of pouring plates and plating samples, followed by one and a half days of plate counting and a full day of data analysis. It is considered crucial that such high-throughput systems are adopted and properly validated, if the physiological characteristics of the large number of *Dekkera/Brettanomyces* isolates obtained from Australian wineries are to be fully elucidated.

Table 1. Tolerance of selected *Dekkera/Brettanomyces bruxellensis* wine-isolates and the CBS-74 type strain to 0.8 mg.l-1 mol. SO_2 in chemically defined medium (Yeast Nitrogen Base), determined by viable plating of cultures

Strain		Time to 90% kill
CBS-74	Type Strain	14 minutes
BD-51	AFLP-D	26 minutes
BD-31	AFLP-C	31 minutes
BD-6	AFLP-B	42 minutes
BD-13	AFLP-A	59 minutes

None of the wine strains exhibited sensitivity to pH levels in the range tested, which reflected typical wine conditions. This result confirmed anecdotal evidence that pH alone is not a significant factor for *Dekkera/Brettanomyces* growth, but is likely to be a factor in terms of SO_2 effectiveness. Interestingly, in the SO_2 tolerance experiment a representative of the strain-A group (the strain most frequently encountered) was the most tolerant of the wine strains, while the current *Dekkera bruxellensis* type-strain was the most sensitive (Figure 2). It took five times as long to achieve a 90% kill-rate for the strain-A representative than for the type-strain, and this might be one of the reasons why strain A is most frequently found across Australia. All wine strains were more tolerant to SO_2 than the type-strain, illustrating the importance of working with wine isolates rather than those available in culture collections, to ensure the results of this project are relevant to the Australian industry. However, as demonstrated in Table 1, marked differences in SO_2 sensitivity were identified between the wine strains. Preliminary experiments were carried out during the reporting period, to examine the possibility that enzymes released from lysed (dead) *Dekkera/Brettanomyces* cells could contribute to the production of 4EP. In chemically defined medium at a pH optimum for the phenol decarboxylase enzyme (the first step of the decarboxylation and reduction pathway to 4-ethylphenol production), a cell-free protein extract converted less than 1% of available coumaric acid to 4-ethylphenol, whereas whole-cells in the same conditions converted 100% to 4-ethylphenol. Nonetheless, it is considered that albeit inefficient conversion over sufficient time-periods could potentially lead to significant levels of 4-ethylphenol in wine, and it is unknown how stable the enzymes involved might be in wine. This will be an area further explored during the coming year.

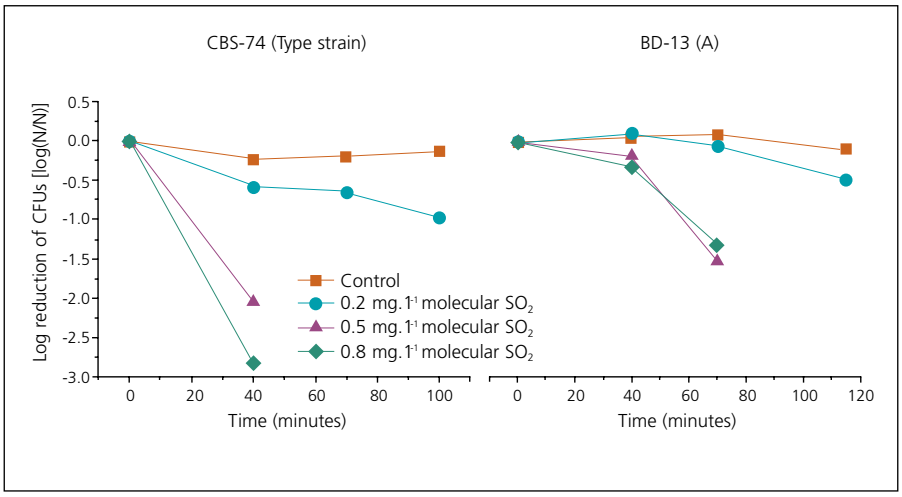


Figure 2. Comparison of tolerance to various levels of molecular SO_2 of the *Dekkera bruxellensis* type-strain and a representative of the major Australian strain grouping (strain 'A'). Log reduction in colony forming units (CFU) determined by viable plating of cultures exposed to SO_2 in chemically defined medium (Yeast Nitrogen Base)

Chris Curtin and Dr Paul Henschke (invited speaker) attended the American Society for Enology and Viticulture (ASEV) annual meeting in San Diego, a feature of which was a full-day session examining *Dekkera/Brettanomyces* on 2 July 2004. At this meeting, potential collaborations were discussed with researchers based in the USA and Portugal. Following the ASEV meeting, Chris Curtin travelled to the University of Bordeaux (II) and the Technical University of Denmark in Copenhagen, where he presented some of the AWRI's *Dekkera/Brettanomyces* project results, and discussed possible collaboration with researchers at both of these institutions. Other seminars attended by Chris Curtin during the year included the International Workshop on Anthocyanins held in Sydney, 26–29 January 2004, and the Yeast Products and Discovery (YPD) meeting held in Barossa Valley, 4–6 April 2004. At this meeting a

poster entitled *Genetic diversity of* Dekkera bruxellensis *isolates from Australian wine*, was presented.

While the future directions of the AWRI *Dekkera/Brettanomyces* project are currently being re-evaluated in light of other international research efforts, some key aspects will be undertaken in the next year.

An extensive microplate screen of wine isolates under a range of physiological conditions will be conducted, in order to differentiate the isolates, and a search for genetic polymorphisms elucidated by AFLP analysis will be conducted in an attempt to explain such differences. To facilitate this process, high-throughput non-linear growth curve modeling algorithms need to be implemented, and a high-throughput rapid method for 4-ethylphenol estimation developed.

The importance of oxygen in modulating 4-ethylphenol production by *Dekkera/Brettanomyces* is currently unknown, despite literature comprehensively demonstrating the link between oxygen and acetic acid production. Chemostat experiments will be conducted utilising a new bioreactor system recently acquired by the AWRI, in which it will be possible to accurately control the level of dissolved oxygen and monitor 4-ethylphenol production. Such experiments could not be conducted reliably using other systems, and will demonstrate the strategic importance of such instrumentation to the life-sciences teams at the AWRI.

An ongoing survey conducted by the Industry Services team remains an important element of the *Dekkera/Brettanomyces* project, and data collected in the current year provide further evidence that a reduction in mean 4-ethylphenol concentration occurred in Cabernet Sauvignon and Cabernet Sauvignon/Merlot wines produced in five regions during the 2001 and 2002 vintages, compared withthe previous five vintages (Table 2).
Wines from the Barossa Valley, Coonawarra, Hunter Valley, Margaret River and Yarra Valley regions have been surveyed in a representative manner. Two hundred and sixteen wines from the vintages 1996 to 2000, and one hundred and thirty nine wines from the 2001 and 2002 vintage have been analysed. There were no significant differences in the median or mean concentrations of 4-ethylphenol for the vintages 1996 to 2000. However, the mean concentration of 4-ethylphenol in wines from the 2001 and 2002 vintages, compared to the mean of the pre-2001 wines, fell by 50% (data not shown). Whilst these results are encouraging, care should be taken when interpreting these data, as it is possible that climatic or other factors during the growing season and winemaking might account for the apparent fall in the concentration of 4-ethylphenol in Cabernet Sauvignon and Cabernet Sauvignon/Merlot wines from these five regions in 2001 and 2002. It should also be noted that the sample of wines currently analysed from the 2002 vintage is probably skewed towards wines that have received shorter than average barrel maturation, and that the mean 4-ethylphenol for this vintage might increase as a more representative sample becomes available. Furthermore, the age of the wines at the time of analysis has generally decreased as the survey has progressed, although it is considered similar for wines from the 2000, 2001 and 2002 vintages (see note below Table 2). Valid comparisons are, therefore, dependent on there not having been development of 4-ethylphenol in bottle prior to analysis. Although, as discussed elsewhere in this report, some evidence of 4-ethylphenol production during bottle storage has been elucidated, multiple bottles of twelve wines included in the survey have now been analysed, approximately 14 months apart in each case. No variability in 4-ethylphenol concentrations has been identified in this sample of wines, beyond that which can be attributed to the uncertainty of the analytical method used.

Table 2. Mean 4-ethylphenol concentrations in Cabernet-Sauvignon-based wines from five Australian wine regions (Barossa Valley, Coonawarra, Margaret River, Hunter Valley and Yarra Valley) for the vintages 1996 - 2002

Vintage	4-ethylphenol (ug/L)	Number of samples
1996	1164	6
1997	1251	29
1998	979	44
1999	824	53
2000	849	84
2001	505	91
2002	385[a]	48

[a] Note: The average age of wines at the time of analysis has fallen as the survey has progressed. Analysis commenced in January 2002, and whilst wines from the 1996 to 1999 vintages were predominantly analysed during 2002, additional samples from these vintages are continually added to the survey as they become available. Analysis of wines from the 2000 vintage commenced when they became commercially available in early 2002, and likewise, analysis of 2001 wines commenced in early 2003, and 2002 wines in early 2004. Therefore, the average age of wines from the 2000, 2001 and 2002 vintages at the time of analysis is considered to be similar. However, it has also been noted that the date at which certain wines have become commercially available is up to four months later for the 2002 vintage wines, compared to the 2000 vintage wines. Furthermore, it should be noted that the sample of wines from the 2002 vintage analysed to date, might be skewed towards wines that have received shorter than average barrel maturation.

Wine and oxygen: towards an optimised management of wine manufacturing, maturation and storage

Staff and students: Dr Liz Waters, Dr George Skouroumounis, Mariola Kwiatkowski, Dr Leigh Francis, Kate Lattey and Dr Patrik Jones

Collaborators: Allen Hart, Ian Shephard (Southcorp Wines), Audrey Lim, Simon White (The Hardy Wine Company)

The negative effects of relatively large doses of oxygen on red and white wine aroma and colour are well documented and, for these reasons, winemaking processes are generally anaerobic. For red wines, some exposure to oxygen might be beneficial and this exposure is postulated to be part of the reason oak barrel maturation 'improves' wine quality. In recent years, equipment to simulate the slow permeation of oxygen through small oak barrels into wine ('micro-oxygenation, MOX') in large stainless steel tanks has been manufactured and is being used commercially by a significant proportion of Australian winemakers. The chemistry behind the process is not well understood.

For white wine production and storage, any exposure to oxygen, apart from oxidative juice handling (hyperoxidation), is considered negative. Oxidative spoilage of bottled wine is a significant problem for the Australian wine industry and shows itself in white wines as an obvious browning in a proportion of the bottled wine typically after six to 18 months of storage. The brown colour is accompanied by loss of SO_2 and ascorbic acid, and oxidised flavour. In 2002, the Grape and Wine Research and Development Corporation (GWRDC) estimated that the problem costs the industry at least $30 million per year in spoiled wine (http://www.gwrdc.com, accessed on 30/07/04 at 15:00). Prevention of browning of white wines is one of the challenges facing winemakers around the world for many decades.

Oxygen permeability

After fermentation, the main cause of accelerated browning in white wines is contact of the wine with oxygen. This can occur during normal transfer operations and barrel storage. One additional and more recently identified route for browning is that of oxygen permeation into bottled wines (Caloghiris et al. 1997, AWRI publication #534; Waters and Williams 1997, AWRI publication #580). Data on SO_2 and ascorbate levels in wines from the AWRI Closure Trial (Godden et al. 2001, AWRI publication #666) also suggests that closure types and/or the closure/glass interfaces vary in the amount of oxygen permeation they allow. This part of the project aims to determine the main parameters influencing permeability of oxygen through closures and/or the closure/bottle interface.

No satisfactory method to properly evaluate the permeation of oxygen into wine under natural conditions has yet been developed. The first step, therefore, was to develop an assay to quantify the relatively low levels of oxygen permeating through closures in wine bottles. The developed assay uses a water-soluble compound to trap singlet oxygen. An experiment with this compound in model wine was set up in February 2003 with one closure type. Data collected over the following four months indicated that the assay would allow us to estimate both oxygen permeation rates and the initial amount of oxygen in the headspace of a wine bottle within six to eight weeks. The experiment continued for a further five months with some of the closures 'sealed' with araldite, and other bottles inverted. The results confirmed that the changes in measured absorbance were due to reaction to the added compound with singlet oxygen and not, for example, due to non oxidative degradation. A method to recover the compound has also been developed to enable the reagent to be reused, saving considerable time in synthesis of the compound. The next experimental phase will be to assess a range of closure types and bottle and storage parameters likely to influence the permeation of oxygen in bottled wine.

Oxygen during bottling and bottle storage

Despite the body of work already undertaken by Godden et al. (2001, AWRI publication #666) and others, and the increasing choice in closures, there are many unanswered questions regarding what package (e.g. bottle/closure combination) is the most technically appropriate for particular wine styles and target storage times before consumption. The aim of this part of the project is to identify best practice bottling procedures for minimising oxidative spoilage during bottled storage of wine and our initial step has been to investigate ullage volumes under screw caps for bottling full-bodied red wines.

A commercial Cabernet Sauvignon wine was bottled with a roll-on-tamper-evident (ROTE) seal and headspaces of 4, 16 and 64 mL air, equivalent to 16, 53 and 104 mm of distance from the top of the bottle to the surface of the wine, respectively. A recent assessment of ullage space of 42 bottles of 21 different screw-capped white wines, indicated that the fill heights currently adopted by commercial producers ranges from 20 to 30 mm (Cowey and Godden 2004). As a comparison, the same wine was also bottled and sealed with natural cork (Reference 2) and a synthetic closure using vacuum insertion and ullages of 6 mL and 5.4 mL, respectively. Preliminary results indicated that differences in sulfur dioxide content could be observed among some of the treatments. The substantial initial drop in sulfur dioxide is most likely related to the amount of oxygen present in the headspace at bottling. HPLC analyses of key phenolic compounds or compound groups in these wines 12 and 18 months after bottling revealed that the concentration of 'pigmented polymers' and tannins were not different in wines sealed with different closures. However, ullage volume did have a slight impact on the polymeric phenol content and the concentration of malvidin-3-O-glucoside was concomitantly lower the greater the headspace. Sensory panel data obtained through duo-trio difference testing (by aroma only), performed six months after bottling revealed that there were no statistically significant differences between wines in bottles sealed under natural cork and any of the wines in bottles sealed with ROTE. However, nine months after bottling, significant differences could be found between the wine sealed under ROTE with 64 mL ullage and the wine sealed with natural cork and also with the ROTE with 16 mL of ullage.

Sensory descriptive analysis (13 aroma attributes, four palate attributes) was conducted after 12 months' post-bottling and there were no statistically significant differences found (at the 5% level) among the treatments for

any of the attributes rated except 'reduced' (struck flint/rubbery) and 'oxidised'. There was no significant difference for any attribute between the natural cork closed wine and the wine sealed with the ROTE with the intermediate headspace volume. The wine bottled with the ROTE closure with the smallest headspace volume was rated as highest in 'reduced' aroma, while the wine sealed with the ROTE closure with the largest headspace was rated as significantly higher in 'oxidised' aroma than the wine sealed with the other closure types. It should be noted that the average scores for both of these attributes were very low, especially in relation to the other attributes rated; for example overall fruit intensity was rated with a mean score of approximately 4.5 for each of the wines of each closure on the 0 to 9 intensity scale used. Thus, the results indicate that any struck flint/rubbery or oxidised aromas were relatively subtle features of the wine at 12 months' post-bottling.

After 18 months' storage, the wines were again subjected to sensory descriptive analysis (16 aroma attributes, eight palate attributes) but only the aroma attribute 'reduced' (struck flint/rubbery) was rated as significantly different across the treatments. The wine closed with ROTE with the smallest headspace was rated highest in this attribute, with the wine sealed with the natural bark and with the other two ROTE closures/caps rated as intermediate, and with the wine sealed with the synthetic closure rated as lowest in this attribute. All mean scores for this attribute were again low compared to the intensity scores given for the 'fruit' aroma attributes, indicating that 'reduced' aroma was not a large or dominating character in these wines. The scores for the aroma and palate term 'oxidised' were not significantly different across the treatments after 18 months.

The overall conclusion from the sensory and the phenolic chemical data obtained from this study to date is that this red wine sealed with the different treatments differed only slightly. Further data obtained over the next year of the wine's life will shed more light as to whether differences in oxygen level at bottling and closure type might substantially affect the flavour and aroma of the red wine used in this study. The ability to generalise from the results of this study to other red wines will also be established with further, extended investigations.

Reference:

Cowey, G.; Godden, P. (2004) Compositional data derived from AWRI *Advanced Wine Assessment Courses*: concentrations of *Dekkera/ Brettanomyces*-derived compounds and of isovaleric (3-methylbutyric) acid; the incidence of 2,4,6–trichloroanisole (TCA) taint in bottled wine, and; the ullage spaces in screw capped bottles, in a selection of wines from around the world. *Technical Review* (151) 8-18.

Oxygen during red wine maturation

Micro-oxygenation (MOX) is already commonly used within the Australian wine industry although a clear substantiated consensus view of the real effects and possible benefits is yet to emerge. Our limited discussions with winemakers practicing MOX have highlighted a need for methods to estimate which wines will benefit positively from MOX, how much oxygen to add, and when to stop treatment. Such methods are important in an effort to manage the outcome of the controlled addition of oxygen, without having to resort solely to sensory evaluation well in advance of commercial release. Several other issues that warrant consideration include the possible increased risks for microbial spoilage, interactions with oak wood additives, long-term effects and studies into actual economic gain.

Given the widespread use, it would appear that there are sensorial changes as a result of MOX treatment, although this is yet to be shown by a formal sensory evaluation. However, studies so far have not been able to detect differences in phenolic composition that explain reported sensory differences between treatments. This might reflect the current lack of knowledge and analytical techniques that can explain effectively the sensory impact of polymeric phenols in a complex and varying matrix. Alternatively, humans might be able to perceive very subtle differences, which our analytical machinery cannot yet detect. Whichever way we look at it, there is a need for further research on the topic. Preliminary studies into the effect of MOX will be initiated at the AWRI in collaboration with a large wine company. The aim of our study will be to confirm if MOX treatment has any measurable effect and, if so, use existing methods to describe what that effect might be. Guided by this initial trial, we will develop a plan for more comprehensive experimentation, in consultation with practitioners across the wine industry. Subject to the availability of resources, we aim to obtain an insight into how MOX treatments can be monitored so as to determine what wines to MOX, how much to MOX and when to stop.

Interactions of non-volatile and volatile compounds in wine: major influences on wine flavour perception

Staff and students: Dr Patrik Jones, Ken Pocock, Richard Muhlack, Jean Macintyre, Felicity Lloyd, Dr Miguel de Barros Lopes, Yoji Hayasaka, Gayle Baldock, Professor Peter Høj, Professor Sakkie Pretorius, Dr Liz Waters

Collaborators: Filomena Pettolino and Dr Tony Bacic (CRC for Bioproducts, University of Melbourne), Dr Chris Colby and Dr Brian O'Neill (School of Chemical Engineering, University of Adelaide), Audrey Lim (Hardy Wine Company), Dr Eileen Scott and Dr Belinda Stummer (CRC for Viticulture, School of Agriculture and Wine, The University of Adelaide)

There is a serious lack of knowledge as to what wine compounds are responsible for 'in-mouth' impressions. It has been observed that a synthetic wine made up of ethanol, acids and tannins is not close in character to a real wine. It is likely that other non-volatile components of wines, particularly proteins, and polysaccharides have an important role to play in the body or weight of wines, overall flavour impression and flavour persistence and as well as in their visual clarity.

Richard Muhlack

Ken Pocock

Studies on 'in-mouth' sensory properties of wine are notably those of Ann Noble and colleagues and more recently ourselves, undertaken as part of the CRC for Viticulture (Francis et al. 2002a, 2002b, AWRI publications #688 and 697; Vidal et al. 2002, AWRI publication #757; Vidal et al. 2003, AWRI publication #717; Vidal et al. 2004a, 2004b, 2004c, AWRI publications #759, 763 and 769). These studies have concentrated on the effect of pH, ethanol, and polysaccharides on astringency and bitterness of tannin and phenolic fractions.

The relationships and associations between aroma compounds and non-volatile/non-aroma active constituents in wines are also very likely to be of great importance to a wine's sensory properties, and have been little studied. Of the studies that have been investigated, the emphasis has been on measurement of volatiles in the headspace of a wine or model system, and looking at the effect of constituents such as mannoproteins, tannins or ethanol. These studies are very important to assess how non-volatiles might alter the concentration of aroma compounds, for example, by reducing volatility so much that the compound is below the sensory detection threshold and will not be perceived. However, it might be that a sensory effect will be observed which is solely as a result of the processing of signals from the several senses involved that reach the brain simultaneously when tasting wine, rather than physical interaction. A simple example of this phenomenon is that increasing the sugar concentration of fruit juices increases their fruitiness by mouth even through no differences can be perceived when smelling alone. Thus, not only the physicochemical interactions but, more importantly, the sensory consequences of mixtures of volatile fractions and non-volatile wine components such as proteins, polysaccharides and tannins need to be established.

One specific situation where an industry practice is believed to affect wine flavour is bentonite fining to prevent protein haze formation. Alternatives to, or improvements on traditional bentonite fining are sought because of its potential effect on wine quality but also because bentonite swells considerably and does not settle well, between 5% to 10% of the wine volume treated with bentonite might be lost as bentonite lees. Improved efficiency of bentonite use is being examined in collaboration with the School of Chemical Engineering, The University of Adelaide. An alternative to bentonite fining - the use of yeast mannoproteins with haze protective activity - was previously identified by us (reviewed in Waters et al. 2000, AWRI publication #631) and requires further examination to take this concept through to adoption by industry. Due to the mannoprotein nature of the active component, it is possible that the addition of the component and omission of bentonite fining will affect wine in-mouth sensory properties. This further work to take the concept of using haze protective mannoproteins in oenology through to industrial application is being undertaken under the auspices of this project. The sensory outcomes of such a practice will be rigorously evaluated and will fit into a framework of information about flavour interactions with polysaccharides. For this same reason, other alternatives to, or improvements on bentonite fining are also being undertaken in this project.

Haze protective factors

Previous work in this part of the project confirmed the identity of genes in the *Saccharomyces cerevisiae* genome, *HPF1, HPF1'* and *HPF2*, encoding the haze protective factors and investigated their biological function in *S. cerevisiae*. This intellectual property has been protected by patenting. Our focus now is on the sensory consequences of their use in general white wine and sparkling wine production and their potential viability as an alternative to bentonite fining.

A small scale trial in which either 6xhis-tagged Hpf2p or yeast invertase were added to a heat unstable wine and stored at 25°C indicated that the short to medium term stability, and thus continued haze protective activity, of these haze protective mannoproteins in wine was good, even at slightly elevated temperatures. Data from a sparkling wine trial suggested that improvement in heat stability of the wines following secondary fermentation might be due to additional release of haze protective factors by yeast during secondary fermentation. Whether the improved heat stability of these trial wines made them stable enough for commercial release is a decision that needs to be made after trials by individual companies and would depend on their specifications.

We are determining the feasibility of scale-up of a commercial process for manufacture of 6xhis–Hpf2p through our collaboration with the School of Chemical Engineering at The University of Adelaide. The PhD student on this project, Jean Macintyre, will spend up to six months at the Department of Applied Microbiology at Lund University, Sweden with Professor Bärbel Hahn-Hägerdal. This site is a specialised research and training centre for fermentation technologies used in the biotechnology industries and is equipped with dedicated state-of-the-art fermentation facilities not readily accessible in Australia. Training received and access to equipment at the site will enable identification of the best design and operational approach for a large-scale yeast or bacterial fermentation process for 6xhis–Hpf2p production.

Improving the efficiency of bentonite fining through chemical engineering

Through our collaboration with the School of Chemical Engineering, The University of Adelaide, and The Hardy Wine Company we are studying the adsorption of purified grape proteins by bentonite in various model wine solutions. The results of this study are being used to improve understanding of wine protein adsorption by bentonite. This project forms part of GWRDC-funded project from the School titled 'Better quality wine and lower production costs from new processing technologies for protein-haze removal'.

Richard Muhlack, the PhD student working on this part of the project, has developed a cation exchange chromatography method at pH 3 to isolate the thaumatin-like proteins and chitinases from grape juice without any initial sample preparation. This method has been scaled up and, to date, protein from 75 L of juice has been processed with almost one gram of thaumatin like protein (VvTL1) isolated and several other key protein fractions collected and awaiting final processing. An initial bentonite absorption experiment has been conducted with VvTL1 in a model wine system (12% ethanol, 1.5 g/L potassium hydrogen tartrate, pH 3.5). The collected data suggest that absorption of VvTL1 onto bentonite followed a 'classic' Langmuir type, as observed with other non-grape proteins.

A factorial experimental design approach to examine the influence of ethanol concentration, pH, temperature, potassium ion concentration, sugar concentration and concentration of simple monomeric phenolic compounds on absorption of VvTL1 onto bentonite has shown that only pH, temperature and potassium ion concentration were found to have a statistically significant effect on adsorption capacity.

The regression model indicated that bentonite had a maximum absorption capacity in this system of about 0.6 g protein / g bentonite and that the theoretical bentonite dose required to achieve 'heat stability' (protein concentration < 10 mg/L) for a range of initial protein concentrations would range from 50–550 mg/L. Commercially, however, a much higher bentonite dose (600–1000 mg/L) is typically required to achieve heat stability. The regression model was built from data from well mixed laboratory trials. This suggests there might be potential for enhanced adsorption, increased sustainability and reduced wine losses through the application of *alternative* contacting systems which are more efficient than the current batch method. Such a process improvement could potentially yield significant quality benefits and cost savings to the Australian wine industry.

Using grape proteins to confirm the varietal origins of finished wine

The ability of the electrospray mass spectrometry analysis of grape proteins to differentiate the variety of grapes was further extended this year to grapes infected with fungi. Work performed in collaboration with Eileen Scott and Belinda Stummer at the School of Agriculture and Wine, The University of Adelaide through the CRC for Viticulture, has shown that powdery mildew infection increased protein content and especially the levels of one of the thaumatin-like proteins in Chardonnay juice and wine. Despite the quantitative changes in protein levels in Chardonnay juice and wine due to powdery mildew infection of grapes, the characteristic qualitative protein profile was maintained. We, therefore, see no reason for powdery mildew infection of grapes to affect the ability of the electrospray mass spectrometric method to varietally differentiate grapes.

We also examined the impact of *Botrytis cinerea* infection on grape protein levels. Infection of Chardonnay or Semillon grapes by *Botrytis cinerea* in the vineyard resulted in decreased levels of all proteins in the free run juice and in a total protein extract from infected berries compared to that from uninfected grapes. Similar although less dramatic trends in reductions in protein levels were seen in laboratory experiments in which otherwise healthy berries or juice were inoculated with *B. cinerea*. These observations support the hypothesis that *B. cinerea* is able to degrade grape proteins, although this inference is tentative as the number of samples and their replication was small in this study. Despite the impact of *B. cinerea* infection on PR protein levels in the berry, juice and wine vinified from rotten grapes might still, in some cases, contain adequate levels of PR proteins or their fragments to enable varietal identification by mass spectrometry. This remains to be confirmed.

Wine flavour perception 'in-mouth'

It is well known that particular wine component groups and components (total acids and citric acid, for example) have a direct influence on sensory perception. However, due to interactions, the perception of a mixture of multiple components cannot always be predicted from the sum of perceptions of individual compounds. Such interactions might be either chemical, physiological (competition for receptors etc.) or cognitive.

In the present study, undertaken as part of the research program of the CRC for Viticulture, we are interested in assessing the relative importance of major components and component groups that influence the perception of wine sensory properties either directly or through interactions with other components. The chosen initial method involves separating wine component groups, such as phenolic compounds, proteins and polysaccharides, from wine and subjecting combinations of these purified component groups in aqueous solutions to quantitative sensory descriptive analysis.

The levels of an extensive range of known aroma compounds was quantified in a small selection of unoaked 2003 Chardonnay wines. All compounds at or above published aroma threshold levels were combined in simple model wine solutions and informal sensory evaluation indicated that the aroma was wine-like although less intense and less complex than the original wine.

To isolate sufficient quantities of polysaccharides, proteins and phenolics, several mild and gentle purification protocols were designed and tested and a method that resulted in samples with the greatest yield and purity was chosen. Informal tasting of the purified fractions indicated that the protein and polysaccharide fractions had no discernible aroma in water or model wine. A phenolic extract of a 2001 Shiraz wine was separated into multiple fractions by LH-20 chromatography. HPLC analysis of each fraction at equal dry weight concentration indicated compositional differences with regards to major anthocyanins, polymeric pigment and tannin. The purified phenolic fractions were reconstituted in basic model wine at original wine concentration. Informal sensory evaluation indicated that only the fractions containing polymeric phenolics were astringent at both the original wine concentration and at 500 mg/L. A formal sensory evaluation is planned for August 2004.

Identification and measurement of non-volatile phenolic compounds responsible for colour and mouth-feel of wine ('Tannin project')

Staff: A/Professor Markus Herderich, Dr Paul Smith, Dr Helen Holt (appointed as technical officer in October 2003), Maria Birse (nee de Sa), Mariola Kwiatkowski, Mango Parker, Holger Gockowiak, Dr Leigh Francis, Kate Lattey, Dr Liz Waters, Dr George Skouroumounis, Yoji Hayasaka, Gayle Baldock, Heather Donnell, Kevin Pardon.

Collaborators: School of Agriculture and Wine, The University of Adelaide: A/Professor Graham Jones, Dr Ewald Swinny, Dr Chris Ford, Mario Mazza, David Lee, Renata Ristic. School of Chemistry, Physics and Earth Sciences, Flinders University: Dr Michael Perkins, Eric Dennis, Caroline Sarneckis. Dr Patrick Iland.

The long-term objective of the 'Tannin project' is to understand how viticultural and winemaking practices affect colour, mouth-feel and flavour characteristics of red wine. Our research into occurrence, structure and function of non-volatile phenolic compounds involves three broad lines of research activities: the identification of wine compounds that contribute to colour and mouth-feel of red wine; studies into all transformations of grape phenolic compounds during winemaking and ageing; and finally the development of robust analytical techniques to efficiently measure non-volatile phenolic compounds in grape and wine samples.

Analysis of non-volatile phenolic compounds

The development and adaption of analytical methods to monitor the changes of phenolic compounds in grapes and during red wine fermentations remained a priority of the team. In addition, we continued to provide analytical support and training to staff members from an ever growing number of viticultural, winemaking and research projects at the AWRI, and facilitate analyses of phenolic compounds by other research providers. To assist identification of grape and wine phenolic compounds by routine HPLC analysis, we have developed an electronic library of UV/Vis spectra. Currently, more than 50 spectra of phenolic compounds and pigments commonly found in red wine have been added, including spectra of commercially available compounds, and reference standards that have been isolated or synthesised by the Tannin team. For example, malvidin-3-glucoside-6'-coumarate has been isolated and purified by a combination of MLCCC and column chromatography. This anthocyanin, which is not available from commercial sources, can serve as an exposure marker in Shiraz grapes and it has been speculated that it could represent a potential precursor of coumaric acid and might, thus, contribute to the formation of 'Brett' related vinylphenols. Also, malvidin-3-glucoside-6'-coumarate has been proposed by others to represent–together with malvidin-3-glucoside-6'-acetate–a suitable authenticity marker for Cabernet Sauvignon wines, a concept that has spurred some controversial discussion. Also, the anthocyanin-derived wine pigment Vitisin-B has been synthesised for the first time and has been included within our generic HPLC method. As already reported, we have isolated a number of anthocyanins and related pigments by MLCCC and the mass specrometric characterisation of the polar grape skin pigments has now been completed: ESI mass spectra have revealed that these hydrophilic pigments are anthocyanin oligomers and the presence of small amounts of such anthocyanin self-condensation products in grape skin and red wine has been demonstrated for the first time.

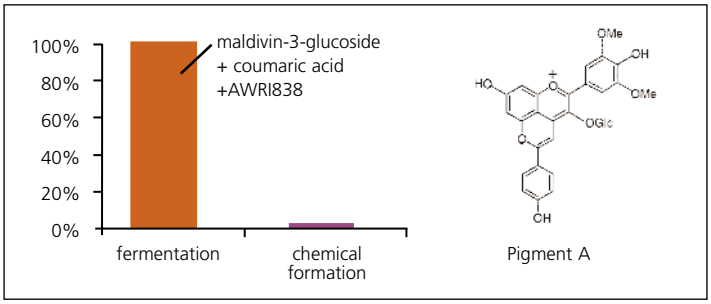


Figure 3. Formation of the pyranoanthocyanin, Pigment-A from malvidin-3-glucoside during fermentation in the presence of coumaric acid

Analysis of grape and wine tannins

A broad range of commercially available oenotannins, including grape-derived condensed tannins as well as hydrolysable tannins from oak and galls, has been sourced from Australian and overseas suppliers. The phenolic composition of these oenotannins has been profiled by HPLC analysis, the products are subject to ongoing characterisation and serve as reference material for HPLC and spectroscopic measurements. For example, we studied the composition of two commercially available grape-derived oenotannins by hydrolysis and HPLC analysis of the subunits after derivatisation. The data demonstrated significant differences between the products, with one oenotannin studied having approximately double the molecular mass as measured by the larger degree of polymerisation. This highlights that various products are not only differing with regard to the concentration of their 'active' ingredients, but might also exhibit different chemical properties. Consequently, the value of conducting individual trials on oenotannins within wineries, or in collaboration between wineries and research providers, cannot be underestimated.

To generate calibration functions for HPLC analysis of tannins, two commercially available grape-derived oenotannins have been purified further by column chromatography and these condensed tannins are presently used on a trial basis as reference materials. This is the first step towards a general reference standard for quantitative tannin analysis that will enable us to compare various methods for tannin measurement. Aided by such standards, we aim to monitor also the long-term performance of routine methods in our quest to generate quantitative data on 'total tannins' which are reflecting 'true' sample composition and are less likely to be affected by analytical protocols.

In collaboration with Dr Michael Perkin's natural products chemistry research group at Flinders University, Eric Dennis has initiated his Honours research with focus on the organic synthesis of condensed tannins. With this project, we are exploring various strategies to obtain structurally-defined reference compounds for tannin research in preparative amounts.

To complement our activities on HPLC analysis of tannins, various analytical approaches for the rapid and simple measurement of grape tannins have been investigated and the successful co-operation with the AWRI's Spectroscopy team was continued. Studies included research aiming to develop cost-effective and simple spectrophotometric measurements, which might enable wine industry personnel to quantify 'free' anthocyanins, coumaroylated anthocyanins, and tannins. As part of these research activities, we have investigated the precipitation of tannins with Ytterbium salts as an efficient technique to isolate tannins and to measure tannins by Yb-induced differences of the absorption spectra. The preliminary data looked promising for wine tannins as long as the ratio of Yb^{3+} to tannin was carefully controlled. The Yb-method, however, appeared not to be suitable for precipitation of grape tannins in 50% ethanol-containing grape extracts which are used for routine grape colour analysis. This was probably due to interferences of sugars or other grape compounds. Currently, polymer-based precipitation techniques in combination with spectrophotometric methods are studied by Caroline Sarneckis, a Honours student from the School of Chemistry, Physics and Earth Sciences at Flinders University and good progress has been made with the validation of a method for the measurement of total grape and wine tannins.

Characterisation of red wine pigments: studies on the formation of the pyranoanthocyanin Pigment-A

The concentration of malvidin-3-glucoside-6'-coumarate can be modulated in Shiraz grapes, and potentially other varieties, through viticultural practices such as sun exposure, and in wine through winemaking practices that affect extraction. We speculated that these coumaroylated anthocyanins could represent cofactors that would enhance the formation of pyranoanthocyanins and thus might contribute to the development of stable red wine colour. We also were interested to study the potential of yeast to utilise malvidin-3-glucoside-6'-coumarate as a direct precursor for the biochemical formation of Pigment-A, as opposed to the spontaneous chemical formation of pyranoanthocyanins that has been described in the literature.

Replicated mini-ferments (5 mL) using *Saccharomyces cerevisiae* AWRI838 in model grape juice medium with added malvidin-3-glucoside, malvidin-3-glucoside plus coumaric acid, or malvidin-3-glucoside-6'-coumarate have clearly demonstrated the substantial formation of the pyranoanthocyanin, Pigment-A, from malvidin-3-glucoside during fermentation in the presence of coumaric acid, while no formation of Pigment-A was observed from malvidin-3-glucoside-6'-coumarate (Figure 3). The formation of this anthocyanin-derived pigment was deemed responsible for the observed substantial colour changes following the fermentation with added coumaric acid and the presence of 4-vinyl-phenol (a precursor of the 'Brett'-associated compound, 4 ethylphenol) as a major metabolite from coumaric acid could be confirmed by HPLC analysis.

In conclusion, we demonstrated that yeast are able to biochemically decarboxylate coumaric acid and can modulate red wine colour through formation of pyranoanthocyanins such as Pigment-A. This reaction, however, appeared to rely on the presence of free coumaric acid, which potentially could be released from other common precursors such as coutaric acid during winemaking. Anthocyanins might have some potential to act as a 'sink' for yeast-derived vinyl-phenols. However, in view of the potential off-aromas caused by volatile phenols, this does not appear to represent an attractive strategy for colour stabilisation. As the formation of the vinyl-phenol derived pyranoanthocyanins, Pigment-A and Pinotin-A, in red wine relies most likely on a combination of biochemical and chemical reactions, these pigments appear to have little value as markers of red wine age in order to confirm label integrity.

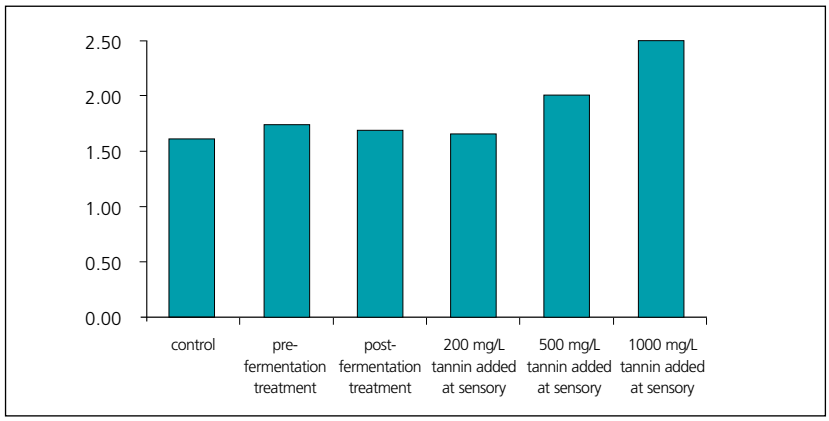


Figure 4. Panel score for 'overall astringency' between the control and the pre- and post-fermented treatments

Winemaking practices that affect tannin concentrations, and colour and sensory properties of red wine

In collaboration with AWRI's Wine Microbiology team, the influence of yeast strain on tannin and colour properties had been pursued with a winemaking trial in 2002 where we compared red wine made with *S. cerevisiae* AWRI838 or *S. bayanus* AWRI1375. We now have applied HPLC-UV/Vis, direct infusion ESI-MS/MS and HPLC-MS analysis to measure anthocyanin-derived pigments and pigmented polymers in these Cabernet Sauvignon wine samples. HPLC-MS analysis of red wine samples collected at day 8 and after 13 months demonstrated differences in formation and degradation of individual pyranoanthocyanins during winemaking and ageing, and confirmed that Vitisin-A and Vitisin-B were formed in substantial quantities during alcoholic fermentation. The acetaldehyde derivative, Vitisin-B, was found in significantly enhanced concentrations in the wines fermented with *S. bayanus* when compared to the *S. cerevisiae* wines. However, it declined rapidly during ageing and it is highly likely that the colour contribution of Vitisin-B in matured red wine is negligible. In contrast, the concentration of both 4-vinyl-phenol adducts, Pinotin-A and Pigment-A, was increased in the 13-month-old red wines, which is consistent with their proposed chemical formation during storage. Directly linked and ethyl-bridged anthocyanin-(epi)catechin dimers as model systems for pigmented polymer formation were also studied. The HPLC-MS/MS analysis established that multiple ethyl-bridged dimers were formed in substantial concentration during fermentation. The concentration of these conjugates decreased during ageing, while the dimers formed by direct condensation between malvidin-3-*O*-glucoside and (epi)catechin were observed only after fermentation. This observation is consistent with data from model experiments on the stability of ethyl-bridged dimers from research conducted by David Lee and Graham Jones at The University of Adelaide. With regard to the colour of the Cabernet Sauvignon wines studied, the results demonstrated that the colour properties varied significantly according to the yeast used for fermentation, while the anthocyanin concentration, when looked at in isolation, was not sufficient to explain the observed colour effects. It is conceivable that the enhanced orange characteristics of the young wines made with *S. bayanus* were mainly the consequence of the enhanced concentration of Vitisin-B and ethyl-bridged anthocyanin-(epi)catechin dimers, while the colour of the older red wines was significantly affected by the concentration of pigmented polymers, and, probably, other hitherto unknown pigments.

Following sensory discrimination testing to investigate differences among the fermentation replicates for each treatment, the two-year-old wines from this study were subjected to quantitative sensory descriptive analysis. The AWRI panel observed some aroma and palate differences among the treatments, and there were clear colour differences among the wines, with the *S. bayanus* wines rated as higher in a brown hue, with less purple hue. It is interesting to note that all wines made with *S. bayanus* had more pigmented polymers, and also more wine tannins compared to wines made with *S. cerevisiae*, despite being made from the same, homogenous batch of grapes. Whether fermenting with *S. bayanus* enhanced tannin extraction and/or resulted in enhanced formation of tannin-like polymers remains to be clarified. The observed differences in wine tannin concentration, however, did not cause measurable astringency effects at the time of sensory evaluation. This result indicates that the astringency properties of this sample set were reflecting grape composition rather than yeast strain used.

The effects on red wine of pre- and post-ferment additions of grape-derived tannin

Commercial grape-derived tannin preparations are becoming increasingly available for addition at various stages of winemaking. The extraction of such tannins during vinification influences mouth-feel of the resultant wine and the tannins are also thought to contribute to colour development in red wine. As already reported, a winemaking trial was performed in 2003 with pre- and post-fermentation additions of 200 mg/L of a commercial grape-derived tannin product. The wine was made using Shiraz fruit from the Riverland area of South Australia, was not oaked and was bottled under screw cap. The role of the additional grape-seed derived oenotannin on wine tannin concentration, pigmented polymer formation and colour parameters was investigated and no significant differences were found between any treated samples and the control samples for the analytical measurements of colour, tannin and pigmented polymers. A repeat small-scale experiment with additions of 200 and 1000 mg/L tannin (the same commercial grape-derived tannin product as for the first study used) also did not result in any difference in colour parameters or phenolics by HPLC. By sensory analysis of the one-year-old red wine, small but significant differences ($p<0.001$) were found between the control and the two treatments (Figure 4) for the 'overall astringency', 'adhesive', 'drying' and 'surface texture' sensory attributes, but not for 'fruit persistence', 'hotness', 'acidity' or 'bitterness'. However, no significant differences in sensory attributes were found between wines with pre-fermentation or post-fermentation additions of this particular oenotannin. Given the ample anecdotal evidence about sensory effects of tannin additions, it is highly likely that other tannin products of alternate composition or purity might cause larger or different effects and

Paul Smith

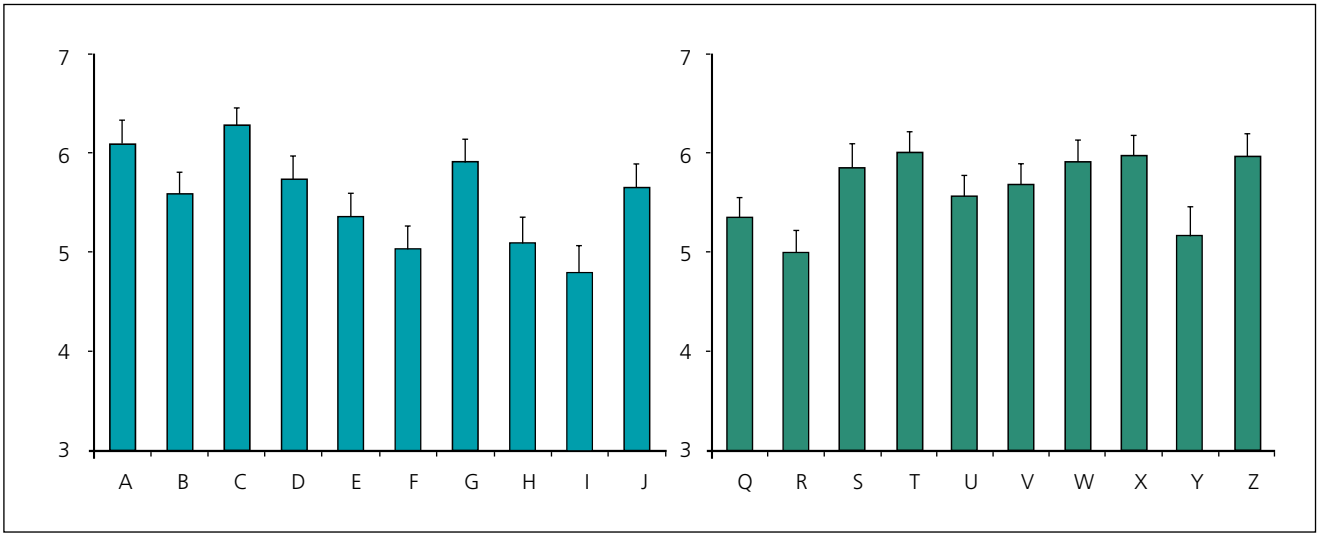
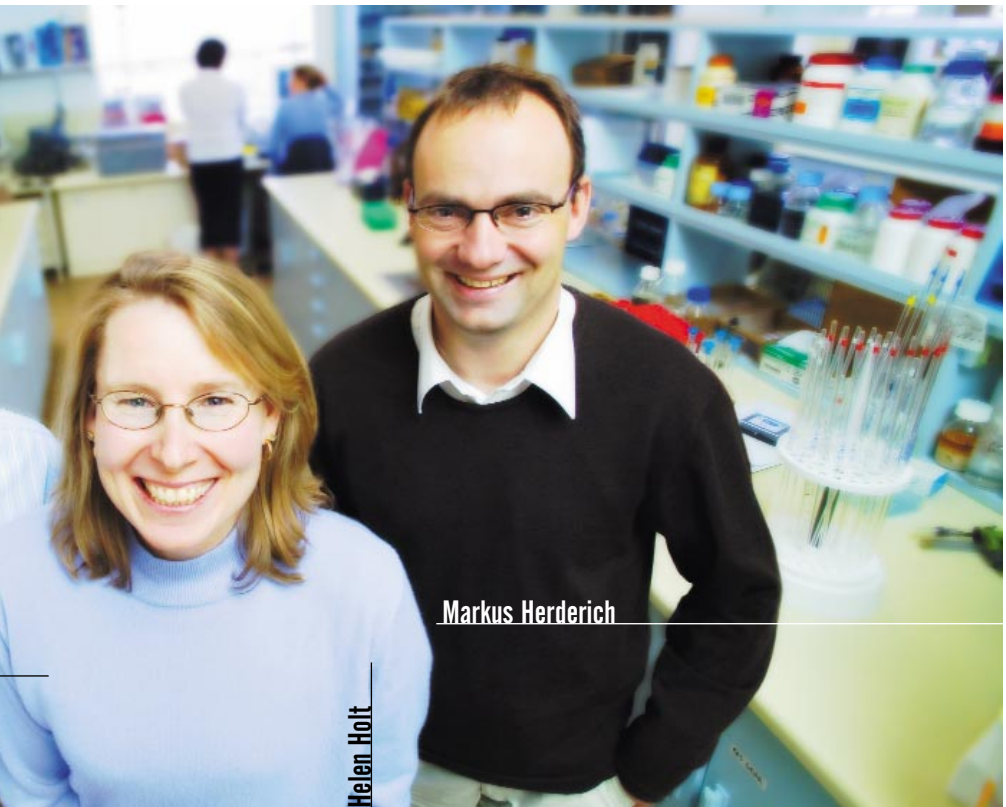


Figure 5. Mean liking scores for the Riesling (A to J) and Chardonnay (Q to Z) wines evaluated by 72 consumers.

that alternate winemaking styles or fruit might give alternate outcomes to those presented here. Consequently, we wish to emphasise the value of conducting individual tannin trials by wine industry personnel in a controlled and replicated fashion.

Effects of viticultural treatments on red wine colour and tannin levels

We continued investigating the effects of differences in berry size on grape seed and skin phenolics, anthocyanin/tannin ratios, and wine composition and sensory properties in Cabernet Sauvignon with the help of grape samples from a viticultural trial, which has been set-up by Hardy Wine Company/Leasingham Wines in the Clare Valley. This collaborative project, which forms part of our CRCV-funded research portfolio, has progressed considerably with the appointment of Dr Helen Holt as technical officer to the project. The wines made from the three different pruning treatments (spur, rod and machine) in 2003 were evaluated for a range of sensory attributes in November 2003 and the wines from the machine pruning treatment were rated as substantially more astringent, drying and coarse and with less finely grained mouth-feel texture than the wines produced from the two other treatments. Wine colour parameters have also been assessed. The stored 2003 berry samples have now been analysed providing data on berry weight, berry colour and HPLC profiles of anthocyanins, flavanols and tannins. These data are currently being compiled and will, with the wine colour and tannin data, be related to the sensory data. In 2004, the study was extended to include the collection of berries pre-harvest, at harvest and post-harvest to assess phenolic development and to monitor 'phenolic maturity'. The winemaking has been extended also to include replicated commercial (0.5 tonne lots), small-lot (50 kg) and mini-lot (1 kg) ferments to evaluate the suitability of each approach for assessing the viticultural treatments. The data from the 2004 vintage confirmed that berry size and concentration of some grape phenolics, including total berry tannins, could be modulated by the various viticultural treatments. The analysis of the 2004 berry samples is well advanced and the grape data will be compared to the wine phenolic profiles in the near future.



Wine quality and consumer preference: development of tools to understand market preferences and shifts

Staff: Dr Leigh Francis, Kate Lattey, Heather Smyth

Wine quality has traditionally been determined by wine industry personnel, with little use of the consumer testing methods that are commonly applied to other beverages and foods. Consumer preference data provide a direct indication of the degree of acceptability of a wine and allows the sensory attributes that most influence liking to be determined. A study was carried out to investigate consumer preference for a set of ten Riesling and, separately, ten unwooded Chardonnay wines, and to compare the relationships between consumer liking data with 'expert' wine judgements and quantitative sensory profiling data. Commercial wines of widely differing sensory characteristics–sourced from a range of Australian regions and with varied retail prices–were selected for the study. The Riesling wines were from the 1993 to 2003 vintages, while the Chardonnay wines were from the 2001, 2002 and 2003 vintages. The wines were all currently commercially available, and almost all had been previously analysed for composition of an array of volatile compounds. The sensory properties of the wines were characterised by a trained panel, and a group of highly experienced tasters scored the wines for overall quality in duplicate. Seventy-two consumers (37 males, 35 females) rated each wine for overall liking on a nine-point hedonic scale ('dislike extremely' to 'like extremely') over four sessions. The panellists were recruited based on the primary criteria of being regular drinkers of white wines, and demographic and wine use history data were obtained from each participant.

There were significant differences in mean liking scores (Figure 5) across the wines for both varieties, and preference mapping and cluster analysis methods allowed the identification of groups of consumers with similar patterns of liking. For the Riesling wines, for example, of the four consumer clusters identified, a group of 50% of the tested consumers most liked the wines with fruity, floral aromas, while a smaller group preferred wines with higher overall flavour intensity and persistence. There was no significant correlation between the expert panel quality scores and the consumer liking data for either of the varieties, assessing either the overall consumer mean data or the cluster means. This indicates that quality scores provide different information to consumer preference, and for this data set do not reflect consumer liking. This sensory study has generated data that allow an understanding of the diversity of consumer preference, and should assist producers in targeting wine styles to meet consumer expectations. This will be further assisted by identifying the compounds in wine that are responsible for some of the traits that are either liked or disliked (see report elsewhere, 'Relationship between composition and sensory properties of wine').

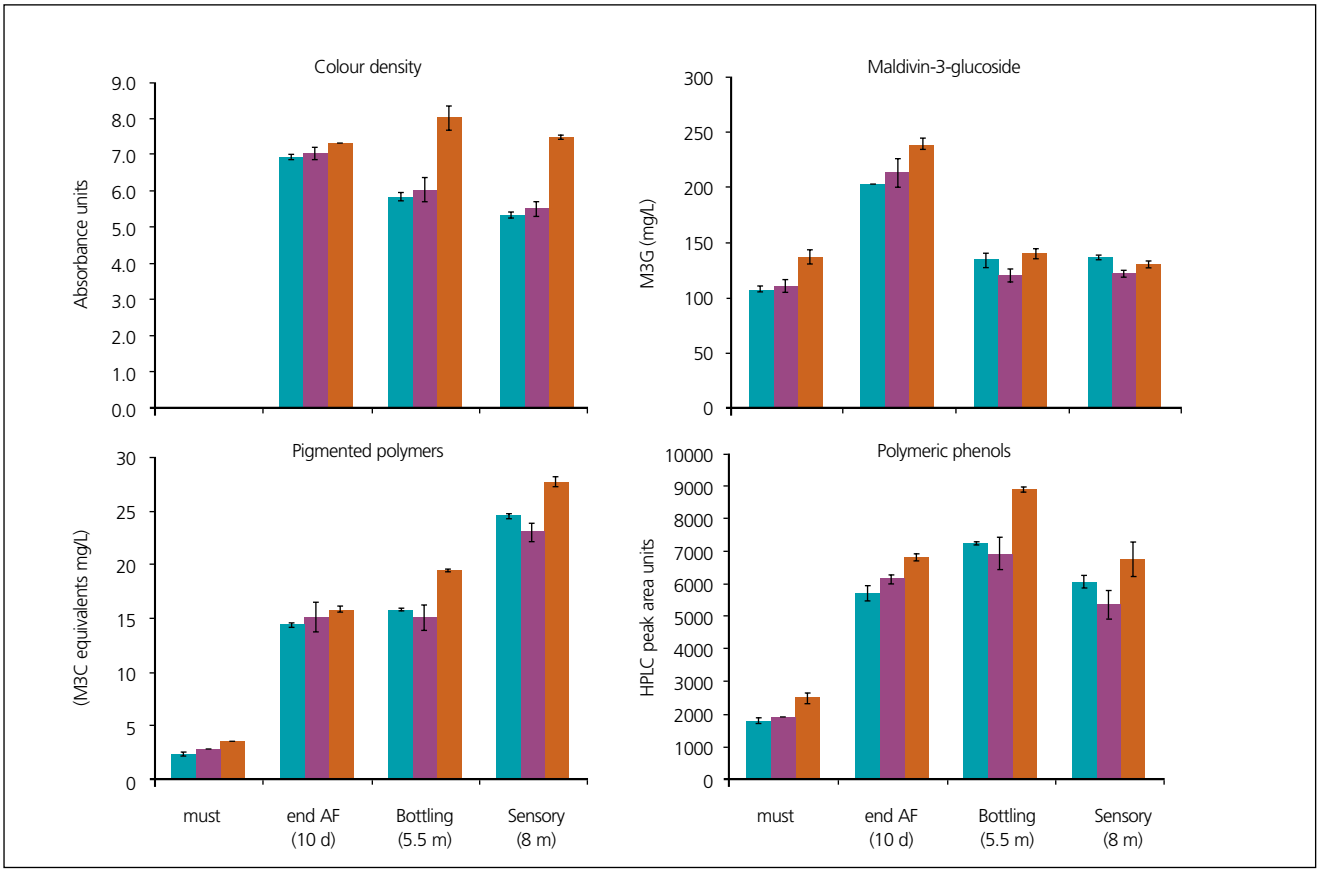


Figure 6. Colour and phenolic analysis of Shiraz wines determined up to eight months post alcoholic fermentation. The wines were made by fermentation with three *Saccharomyces cerevisiae* strains with different 'colour' properties as identified from previous micro-scale fermentation trials ('colour' yeast strain: 'low' - green, 'medium' - purple, 'high' - orange) (d - days; m - months).

Modulation of appearance, aroma, flavour and mouth-feel of wine with conventional and non-conventional yeast and malolactic bacteria

Staff: Dr Paul Henschke, Dr Eveline Bartowsky, Jeff Eglinton, Dr Diego Torrea, Dr Cristian Varela, Jane McCarthy, Dr Mark Sefton, Dr Alan Pollnitz, Tracey Siebert, Jason Siebert, Dimitra Capone, Kate Lattey, Briony Liebich, Dr Leigh Francis, Professor Sakkie Pretorius and Professor Peter Høj

The long term aim of this project is to more clearly define the roles that yeast and malolactic bacteria have in modulating wine sensory characteristics, in biochemical and physiological terms, so that winemakers can more effectively exploit the benefits of these microorganisms for commercial gain. During the past year we have continued our studies on using *Saccharomyces bayanus, S. cerevisiae* and *Oenococcus oeni* as research tools to understand the roles of yeast and malolactic bacteria in the development of red wine colour, flavour and mouth-feel. A strong focus has also been placed on determining the importance of the key yeast nutrient, ammonium, on the aroma profile of Chardonnay wine. The impact of various procedures for rescuing stuck fermentation on wine aroma profile has also been examined.

Dr Cristian Varela commenced as Microbiologist in May 2004 to undertake new project work on how yeast modulate the aroma profile of wine in response to different nutrient conditions. This work will be undertaken together with Dr Hentie Swiegers of the Molecular Biology team. Cristian is also involved in the ongoing project concerned with understanding how mixed culture fermentation can increase wine flavour complexity. He recently completed a PhD degree on modelling yeast metabolism during the wine fermentation at the Catholic University in Santiago, Chile. Maurizio Ugliano, a visiting PhD student from the University of Foggia, Italy, commenced studies on the role of yeast in enhancing wine varietal character in July 2004.

Dr Diego Torrea has recently completed his post-doctoral studies on defining better the role of nutrients in modifying the aroma profile of wine. His work, which focussed on the nutrients oxygen and nitrogen, described in quantitative terms the impact of these nutrients on wine aroma using the stable isotope dilution assay, recently developed by the AWRI Chemists working on wine 'volatiles' (led by Dr Mark Sefton). This work concludes that optimisation of nutrient addition is important for achieving the best sensory outcome. A preliminary report to industry has been published in the AWRI's *Technical Review* (150th issue June 2004, 59-63) and in a poster to be presented at the 12th Australian Wine Industry Technical Conference. Diego's research activities were supported by a post-doctoral fellowship from the Spanish Government. He has returned to Spain to take up a research position with a biotechnology company. Simon Dillon joined the Yalumba Wine Company in January 2004 after completing a three-year program of work within a collaborative project co-funded by Lallemand and the AWRI. This project led to the development of a micro-vinification methodology for studying the interaction of yeast with grape phenolics in order to evaluate and optimise the use of yeast for improving red wine colour. A preliminary report to industry has been submitted to *Australian Vignerons* for publication in the September/October issue in 2004, a poster will be presented at the 12th Australian Wine Industry Technical Conference, with papers now being finalised for scientific publication.

Modification of aroma and flavour of red wine with *Saccharomyces bayanus*

Staff: Jeff Eglinton, Kate Lattey, Dr Leigh Francis and Dr Paul Henschke

Saccharomyces bayanus has proven to be a very useful tool for exploring the role of yeast in modulating the sensory properties of white wine. This cryotolerant yeast, which can be managed in a similar manner to *S. cerevisiae*, has important metabolic differences which are proving of practical interest to several wine companies. These winemaking attributes have been summarised in an industry journal publication (Eglinton et al. AWRI publication #758). Compared to fermentations conducted with *S. cerevisiae*, higher amounts of glycerol and lower amounts of acetic acid (a small reduction in ethanol is also observed) together with increased succinic acid are typically formed at usual winemaking temperatures (AWRI publication #632, #647). *S. bayanus* is typically competitive and dominates fermentation (AWRI publication #707) and although it might exhibit a slower rate of fermentation towards the end of fermentation, the wines generally have low residual sugar. The prolonged fermentation times might be linked to improved palate structure and weight noted in a high proportion of an increasing number of industry trials.

Saccharomyces bayanus also appears to have red winemaking potential with respect to aroma, palate and colour. The limited laboratory and pilot scale trials undertaken so far suggest that young Cabernet Sauvignon wine made with *S. bayanus* AWRI 1375 was more red/red-orange in colour and showed more complex aromas and flavours than wine made with *S. cerevisiae* AWRI 838 which exhibited brighter purple colour and fresh fruit aromas and flavours. The chemical basis for these aroma differences is being investigated with stable isotope dilution GC/MS analysis as is being done for the *S. bayanus* white wines.

The perceived differences in red wine colour resulting from the use of *S. bayanus* have been supported by analytical measurements as reported in the previous annual report, presented at a recent international conference (Eglinton et al. 2003, ACS proceedings, in press) and recently published in an industry journal (Eglinton and Henschke, 2003, AWRI publication #729). This important attribute, which has its basis in wine phenolic composition, is the subject of a large research effort by the AWRI's Tannin and Wine Microbiology teams. Aspects of this work related to the role of yeast in determining wine colour are reported below.

Quantitative sensory descriptive analysis of the 2002 Cabernet Sauvignon wines that were made in collaboration with the Tannin Team has been completed in conjunction with Kate Lattey, Briony Liebich and Dr Leigh Francis. The wines had not been subjected to oak treatment, were aged in 750 mL bottles, and were evaluated at two years of age. A detailed report is included in the Tannin Team report. Briefly, a panel of experienced and trained judges assessed 17 aroma and nine palate attributes. The significant appearance and aroma attributes ($P < 0.05$) were 'colour intensity', 'brown hue', 'purple hue', 'chocolate' and 'sulfidic'. Wine made with *S. cerevisiae* AWRI 838 in rotary fermentors was rated the most 'estery' and least 'chocolate' with low 'fruit persistence', with the most intense purple hue. In comparison, wine made with *S. bayanus* AWRI 1375 under the same conditions was rated lower in estery and purple hue attributes with a higher 'cooked fruit' aroma, more 'fruit persistence' and more brown hue. Wine made with *S. bayanus* AWRI 1375 in a static (Potter) fermentor had the highest overall intensity rating for 'sweaty' aroma and the most perceived acidity. In general, wines made with *S. bayanus* were perceived as having greater 'fruit persistence' than that made with *S. cerevisiae*, although the difference was small.

To investigate any perceived differences in mouth-feel between wines made with *S. bayanus* AWRI 1375 or *S. cerevisiae* AWRI 838, the basic chemical composition of the wines was adjusted to render them as similar as possible. The concentration of the following compounds was adjusted: glucose, fructose, glycerol, lactic acid, succinic acid, tartaric acid and ethanol. After adjustment, wine made with *S. cerevisiae* was rated higher for perceived acidity and higher for 'fruit persistence', 'estery' and 'solvent' than the unadjusted wine. For *S. bayanus* wine, the perceived purple hue increased after adjustment but the acidity was perceived as no different to the unadjusted wine. All other attributes had similar sensory scores to the unadjusted wine. When compared to the adjusted *S. bayanus* wine, the ratings for the *S. cerevisiae* wine were higher for 'purple hue', 'solvent', 'acidity' and 'fruit persistence' with a much lower intensity rating for 'brown hue'.

In summary, based on the chemical and sensory analysis of red wines made on both laboratory and pilot scale in 2001 and 2002, together with anecdotal information gained from winemakers, the colour, aroma, flavour and mouth-feel properties of Cabernet Sauvignon wines made with *S. bayanus* can persist and can be differentiated from those made with *S. cerevisiae*. The sensory effects appear, at least in part, to be related to differences in non-volatile composition.

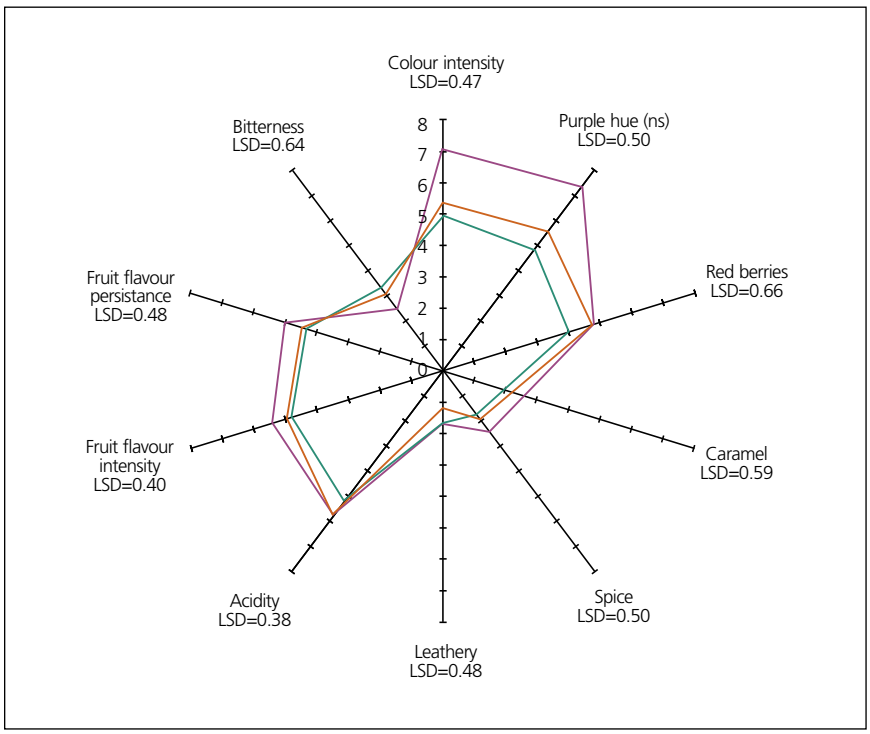


Figure 7. Radar plot of the sensory attributes of Shiraz wines determined at eight months post alcoholic fermentation. The wines were made by fermentation with three Saccharomyces cerevisiae strains with different 'colour' properties as identified from previous micro-scale fermentation trials ('colour' yeast strain; 'low' - green, 'medium' - orange, 'high' - purple).

Commercial *Saccharomyces cerevisiae* wine yeast can influence the colour and phenolics composition of red wine

Staff: Simon Dillon, Dr Eveline Bartowsky, Kate Lattey, Dr Leigh Francis, Professor Peter Høj and Dr Paul Henschke

The colour, flavour and mouth-feel properties of red wine depends on many factors during winemaking, including grape variety and maturity, and viticultural, fermentation and maturation conditions. The chemistry of grape and wine phenolics is very complex, and the understanding of how these compounds contribute to wine sensory properties is the subject of extensive studies by the Tannin and Sensory teams. In essence, monomeric anthocyanin grape pigments, and tannins, are extracted during fermentation, and undergo complex modifications that contribute to mouth-feel and the development of stable colour. Little is known, however, about the role of yeast in this process.

Yeast, through enhancement of maceration due to the evolution of ethanol, carbon dioxide and heat produced by fermentation, assist in the extraction of phenolics. A small proportion of phenolics becomes immobilised on the cell surface while yeast macromolecules might combine with various phenolics to modify their sensory properties. Enzymes possessing glycosidase activity can act negatively by destabilising anthocyanins. Fermentation metabolites, especially carbonyl compounds, such as acetaldehyde, are presently being studied to elucidate their role in anthocyanin modification and condensation with tannins to form new and more stable pigments that endure in wine as it ages. The latter mechanism is being studied by the Wine Microbiology and Tannin teams, as reported in previous Annual Reports; aspects of this work are being published (Eglinton et al. 2004, ACS proceedings, in press; Hayasaka et al. 2004, in preparation). An investigation into the interactions between commercial *S. cerevisiae* wine yeast and grape phenolics during red wine fermentations have been undertaken through a three-year collaborative project with Lallemand. The project has focussed on the development of micro-vinification methodology and the identification of yeast strains and conditions to maximise wine colour as has been highlighted in previous Annual Reports; the work is being prepared for publication (Bartowsky et al., *Australian Vignerons* September/October 2004, submitted). Present work has focussed on the impact of selected strains on the colour, flavour and mouth-feel of Shiraz wine made on a pilot winery scale.

From a survey of 17 Lallemand *S. cerevisiae* wine yeast for effect on wine colour density and phenolic concentration of young Shiraz wine, it was possible to subdivide the yeast strains into three statistically significant groups, that is, yeast that gave low, medium or high wine colour density (summarised in previous Annual Reports; Bartowsky et al., *Australian NZ Grapegrower and Winemaker* November 2004, submitted). We further confirmed these results with Shiraz fruit obtained from other viticultural regions, and with frozen versus fresh fruit. The generous provision of grapes by The Hardy Wine Company and Orlando Wyndham is acknowledged. These results suggested that there are intrinsic differences between some yeast strains, confirming the observations made by various winemakers and Lallemand.

To confirm the effect of yeast strain on the chemical, phenolic, sensory and ageing properties of Shiraz wine, we conducted a pilot-scale study in the Hickinbotham Roseworthy Wine Science Laboratory with the Tannin team. Shiraz grapes, sourced from the Banrock Station, Berri/Riverland, were generously provided by The Hardy Wine Company during the 2003 vintage. Three yeast strains, selected on the basis of previous trials, were used to produce the wines with replicated batches of must processed in 900 L capacity rotary fermentors. Alcoholic and malolactic fermentations proceeded efficiently with each of the three yeast strains. Phenolic composition and sensory analysis was determined on the wines at eight months' post fermentation.

Colour and phenolic analysis made on the wines immediately post alcoholic fermentation revealed that the low and high colour yeast strains produced wines that were significantly different in wine colour density and content of pigmented polymers (Figure 6), corroborating our earlier findings made by micro-vinification. These differences persisted and were still evident at eight months' post fermentation. The monomeric pigments (anthocyanins, particularly malvidin-3-glucoside) decreased over the eight month ageing period whereas the pigmented polymers increased. The wines with highest colour density also had the highest concentrations of pigmented polymers and polymeric phenols. The pigmented polymer and polymeric phenol composition of the wines suggest a chemical basis for their colour properties as a result of the yeast treatment.

At eight months' post fermentation, the wines were assessed by a sensory panel. Difference testing showed significant variation between the yeast treatments ($P<0.01$). Ten sensory attributes were rated significantly different amongst the wines (Figure 7). The wine prepared with the 'high' colour yeast showed relatively high visual colour intensity and purple hue, overall higher fruit intensity of the aroma attributes 'red berries', 'caramel', 'spice' and 'mint', exhibited more fruit flavour intensity and persistence and was reduced in bitterness. Differences in visual colour of the wines correlated well with the colour and phenolic data.

This study provides the first data to show that yeast strains characterised by micro-scale vinification can affect the colour properties of wines of, at least, up to eight months of age, in a similar way. Colour variations among the Shiraz wines prepared with the three different yeast strains was reflected by chemical and sensory analysis. The pigmented polymer and polymeric phenol composition of the wines suggest a chemical basis for their colour properties as a result of the yeast treatment. Further investigations are now required to elucidate the mechanism(s) by which yeast interact with wine phenolics to enhance and stabilise wine colour. A summary of this work will be presented as a poster at the 12th AWITC held in Melbourne in July 2004.

Ammonium supplementation of grape juice–effect on aroma profile of a Chardonnay wine

Staff: Dr Diego Torrea, Tracey Siebert, Briony Liebich, Dr Leigh Francis and Dr Paul Henschke

Collaborator: Professor Carmen Ancin, University of Navarra

Unless added by the winemaker, yeast fermentation is dependent on the nitrogen present in the grape juice or must at the time of inoculation. Yeast have an absolute requirement for assimilable nitrogen (N) needed for biomass formation and maintenance of fermentation activity (AWRI publication #424). Insufficient N can induce nitrogen limitation stress that leads to an inadequate rate of fermentation. Furthermore, under such stress some yeast strains produce a reductive off-flavour, due to, most notably, hydrogen sulfide and thiols (mercaptans) accumulation. The assimilable N content of musts and juices is highly variable (AWRI publications #412, 641). Ammonium salts, especially diammonium phosphate (DAP), are commonly used as N supplements when a deficiency is observed.

In order to establish a practical understanding of the impact of N supplementation on wine aroma, a low N Chardonnay juice (160 mg N/L), which was supplemented with ammonium to give juices with a moderate (320 mg N/L) and high (480 mg N/L) concentration of N, was fermented with AWRI 796 at 18°C. The wines were then profiled for 31 fermentation products using the stable isotope dilution assay procedure developed by the AWRI's Wine Volatiles team and subjected to sensory descriptive analysis by the AWRI's Sensory team.

As expected, yeast biomass yield increased and fermentation time decreased in response to increased initial juice N concentration (Figure 7A). Fermentation time was reduced from 14 days at low N to 9 days at moderate N to 6 days at high N. Juice N had no important impact on wine alcohol content (13.0-13.2 % v/v) and residual sugar (<0.1 g/L). Glycerol concentration, which ranged from 8.6 to 9.4 g/L, was lowest for the wine made from the moderate N juice, but differences noted are unlikely to be significant.

Ethyl acetate, the principle ester produced by yeast, increased from 40 to 100 mg/L in direct proportion to initial juice N (Figure 7B). Total esters, excluding ethyl acetate, also increased directly with N, except for the highest N juice, at which no further increase in ester content resulted. Total higher alcohols, by contrast to the esters, was inversely related to juice N, being only one-third the concentration for the high N juice compared with the low N juice. Acetic acid concentration increased from 0.01 to 0.21 g/L in direct proportion to the initial N content of the juice, whereas the total fatty acids, excluding acetic acid, peaked at moderate juice N. These observations are consistent with the published literature.

The sensory panel selected 16 terms to describe the aroma profile of the wines, and included 'fruit ester', 'floral', 'tropical', 'artificial grape', 'banana', 'musk', 'stewed fruit', 'bruised apple', 'citrus', 'honey', 'nail polish remover', 'acetic', 'cheesy', 'sweaty', 'stale beer' and 'wet cardboard'. The wine made from the low N juice was characterised by a low rating for most fruity and floral descriptors but was rated high in the unpleasant descriptors, especially 'stale beer', 'cheesy', 'sweaty' and 'wet cardboard'. The wine made from the moderate N juice was rated highest for most of the fruity and floral descriptors and lowest for the unpleasant descriptors. The wine derived from the high N juice was rated low for almost all descriptors except for 'acetic' and 'nail polish remover'.

A comparison of the fermentation products and aroma descriptors suggests a correspondence between several of them. The low N wine, which was generally rated low in the fruity and floral descriptors, had the lowest concentration of total esters and long chain fatty acids. In contrast, this wine had the highest concentration of the less preferred higher alcohols and short chain fatty acids, which might account, at least, for several of the least preferred aroma descriptors. The wine with the best aroma profile had moderate to high esters, moderate higher alcohols and moderate to high fatty acid concentrations. The high N wine was dominated by ethyl acetate, which would account for the highest VA rating awarded by the sensory panel.

Although these results were only obtained from a single Chardonnay juice fermented with one strain of *S. cerevisiae*, they suggest that the initial concentration of N in the juice not only has a considerable effect on the concentration of volatile fermentation aroma compounds but also impacts, surprisingly highly, on the sensory profile of the wine. The fruity and floral esters tended to increase in response to the increased initial juice N concentration whereas the higher alcohols decreased in response to increasing juice N concentration. The fatty acids showed a more complex pattern, with the short chain acids being present at low concentrations for moderate to high initial juice N concentrations. Although the response to N of yeast fermentation products has been well documented previously, the sensory significance of these data is wholly unknown.

Overall, the juice that had a moderate N concentration gave rise to a wine with the best balance of pleasant to unpleasant aroma descriptors. These data suggest that there might be an optimum N concentration, or range, to give the most preferred wine aroma profile, at least for a young Chardonnay made with *S. cerevisiae* AWRI 796. In this case, the moderate juice N concentration was 320 mg N/L, but the experimental design does not allow us to deduce whether 320 mg N/L is really the optimum value and over what range of values a good aroma profile can be achieved. However, when the juice N is too low, there is a risk of augmenting the unpleasant yeast-derived aromas, which might include H2S, whereas when the juice N is too high there is a different risk, that is, introducing a 'volatile' aroma profile, due to excessive ethyl acetate formation; although the concentration of acetic acid had also increased, it probably was not sensorially important. In red wine fermentations, high N can also impair (Pinot Noir) wine colour. Further work is needed to show whether different yeast strain and juice combinations give different aroma descriptors for a given N concentration, and the variability of the optimum N, and its range for these combinations.

Given that the N content of juices prepared from some grape varieties is typically low, and in some years, juices from other varieties can also be low (i.e. <200 mg N/L; AWRI publications # 412, #424, #641), N supplementation might benefit the aroma profile of wines made from such juices. Clearly, more work needs to done to confirm the general nature of these preliminary observations, however, this work highlights the need for routine N determination of juice and must.

A summary of this work has been published in the Institute's *Technical Review* (150th issue June 2004) and is to be presented as a poster presented at the 12th Australian Wine Industry Technical Conference held in Melbourne in July 2004.

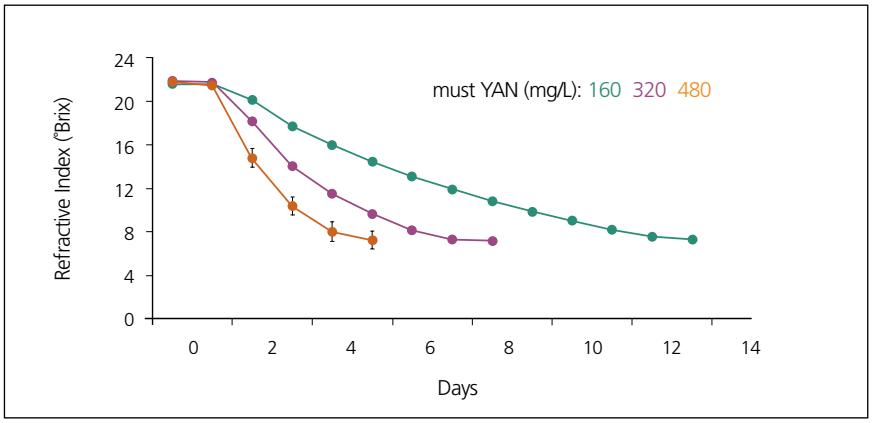


Figure 7A. Fermentation kinetics of *Saccharomyces cerevisiae* AWRI 796 in a Chardonnay juice with different initial concentrations of yeast assimilable nitrogen (YAN).

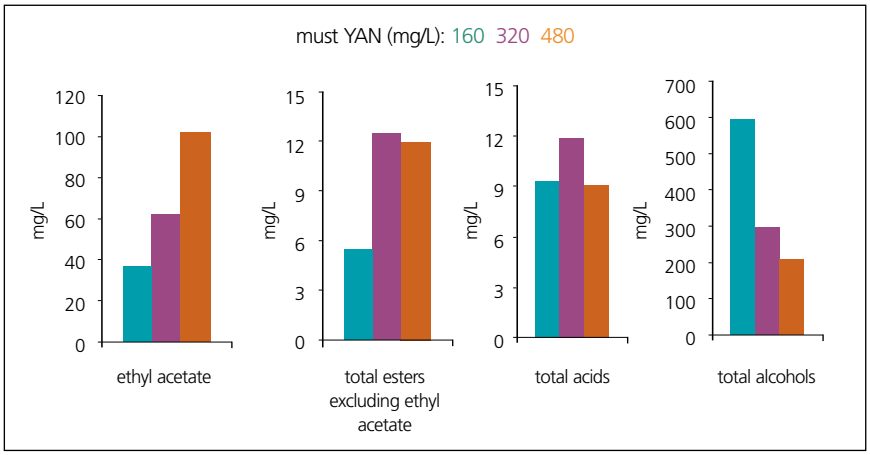


Figure 7B. Accumulation of fermentation products produced by *Saccharomyces cerevisiae* AWRI 796 in a Chardonnay juice with different initial concentrations of yeast assimilable nitrogen (YAN).

Strategies for ensuring complete fermentation using a non-conventional wine yeast–effect on aroma compounds

Staff: Jeff Eglinton and Dr Paul Henschke

We have used a model system in which a non-conventional yeast, *S. bayanus* AWRI 1375, was used to ferment a 2002 vintage Chardonnay grape juice under very difficult vinification conditions (e.g. pH 2.9, TA>9 g/L, sterile processed and cellar bright) in order to establish the efficacy of several intervention strategies for overcoming sluggish fermentation. The strategies were (1) no intervention (leading to a very sluggish fermentation), (2) sequential inoculation with *S. cerevisiae* AWRI 838 at approximately the half-way point of fermentation, (3) aerobic handling (pump over and 'splash') when fermentation slowed, or (4) sequential inoculation with AWRI 1375 when fermentation had stopped. We have reported previously that use of any of the intervention strategies resulted in complete fermentation of the juice, thereby providing reliable options for complete fermentation to winemakers. The suitability of these strategies for commercial winemaking is reliant, however, on the commercial quality of the finished wine. There have been no studies published, to date, on the effect of these strategies on the aroma and/or flavour impact of the strategies, particularly those that are applied at the end of fermentation when the wine can be in a state that is relatively vulnerable to spoilage. We have conducted quantitative sensory descriptive analysis and GC-MS analysis of important aroma compounds in the Chardonnay wines.

There was no statistically significant difference between the wines made using any of the intervention strategies, as judged by the mean aroma intensity score for the 12 most important aroma descriptors determined by a panel of experienced wine assessors. The attributes scored in the wines were 'estery', 'floral', 'pineapple', 'melon', 'citrus', 'cooked apricot', 'ethyl acetate', 'acetic', 'sweaty', 'sulfidic' and 'yeasty/caramel'.

The lack of an effect on aroma was consistent with results of GC-MS analysis of wine volatiles using the stable isotope dilution assay that has been developed by the AWRI's chemists. Of the 11 volatile compounds that were present with an odour activity value (ratio of the concentration in the sample to the published sensory threshold in the same type of matrix) in excess of one (that is, the concentration was above the published threshold), the concentration of only five compounds was statistically significantly ($P < 0.05$) different between any of the treatments. The results indicated that, in these ferments, any of the strategies could be used without having a detrimental effect on the aroma of the wine. The validity of these results requires confirmation using a number of grape juices and other, ideally commercial, wine yeasts.

A summary of this work is to be presented as a poster at the 12th Australian Wine Industry Technical Conference held in Melbourne in July 2004.

Winery trials with AWRI *Saccharomyces bayanus* yeast

Staff: Jeff Eglinton and Dr Paul Henschke

Winery trials are the 'acid test' of a yeast selection program. In order to assist winemakers in successful deployment of experimental non-conventional yeasts, such as the AWRI's *S. bayanus* strains, technical notes and industry publications (AWRI publications #632, #647, #693, #707, #729, #758) have been produced and AWRI roadshows and workshops (11th and 12th AWITC) have been undertaken, with some 90 winemakers registered to attend three workshops to be held at the 12AWITC in Melbourne July 2004. Following on from promising barrel and tank trials conducted over recent vintages by several medium and large wine companies, the scale of trials was expanded for the 2004 vintage to include 20 kl tank trials. Additional winemakers have initiated trials in 2004 for the first time. One Barossa Valley winery has recently launched a premium quality Semillon wine made with *S. bayanus* AWRI 1375. Encouraging progress has been made on the development of a commercial quantity of dry yeast, which should facilitate trials during the 2005 vintage, as reported below.

Evaluation of *Saccharomyces bayanus* AWRI 1375 active dry wine yeast (ADWY)

Staff: Jeff Eglinton, Jane McCarthy and Dr Paul Henschke

The success of any program of yeast strain selection or improvement is ultimately dependent on the availability of the yeast for commercial production. In Australia, success will be greatly aided by the availability of ADWY. We are collaborating with a commercial yeast manufacturing partner to provide winemakers with *S. bayanus* AWRI 1375 ADWY for future vintages, and with a winery to test the ADWY prior to commercial release. Microbiological and activity assessment of the *S. bayanus* AWRI 1375 ADWY preparation using methodology previously developed for monitoring the quality of commercial yeast (AWRI publications #367, #406, #542) showed that this preparation essentially met the minimum specifications set by the OIV. During the 2004 vintage, *S. bayanus* AWRI 1375 ADWY was compared to a liquid culture of the same yeast in barrel ferments of Barossa Valley Chardonnay pressings (228 L French oak barriques). Results to date are encouraging, with the initial rate of fermentation being comparable for the ADWY and liquid culture as prepared by usual industry practice. Rapid onset of fermentation is an important property of any yeast inoculum. Despite high initial rates, ferments that were inoculated with ADWY took longer than those made with the liquid culture, however, and further work is required to assess the full properties of the ADWY. We will be working in the laboratory and closely with industry to determine if the *S. bayanus* AWRI 1375 ADWY is suitable for commercial wine production.

Malolactic fermentation and wine flavour–evidence for palate modification not related to acidity

Staff: Dr Eveline Bartowsky, Jane McCarthy, Kate Lattey, Tracey Siebert, Dr Leigh Francis, Dr Paul Henschke

Malolactic fermentation (MLF) is essentially a 'fermentation' of the dicarboxylic acid, L-malic acid, to the monocarboxylic acid, L-lactic acid, with the release of carbon dioxide. Citric acid is also degraded with the formation of acetic acid and pyruvic acid; the latter acid is further metabolised to other products, with diacetyl being the most important from a sensory perspective. The implications and practical management of bacterial citric acid-diacetyl metabolism were described in the Annual Report for 2003.

These bio-deacidification steps, which affect wine acidity by increasing pH and decreasing titratable acidity (TA), therefore, have a notable effect on the palate of wine. Acidic wines can benefit from MLF by acquiring a 'softer' palate, and consequently, the wines are ready for consumption at an earlier stage. These wines also often show differences in their aroma and flavour profile. The chemical basis for these changes has been the subject of recent research. A better understanding

of these chemical changes can then be linked to biochemical and/or chemical processes, which could lead to the development of strategies to emphasise the desirable benefits of MLF and to achieve them more reliably.

Our recent investigations into the aroma and flavour changes associated with MLF have been focussed on red wine and involve a combination of chemical and sensory techniques. Three MLF treatments were studied in Cabernet Sauvignon wines contained in 18 L stainless steel vessels. The treatments were 'spontaneous', with the indigenous bacteria, and induced, with MLB1 and MLB2, using commercial starter cultures. The Cabernet Sauvignon wines used in these studies had been prepared from Padthaway fruit provided by Orlando Winery in 2002. These wines were made on a pilot winery scale (approx. 700 kg) at the Hickinbotham Roseworthy Wine Science Laboratory by collaboration between the Wine Microbiology and Tannin teams, with Stephen Clarke (The University of Adelaide) as winemaker. The results of this trial, which compared the effect of *S. bayanus* and *S. cerevisiae* on the chemical and sensory composition of Cabernet Sauvignon wine, have been reported in the 2002 and 2003 Annual Reports.

Changes to the composition of yeast-derived volatile aroma compounds (esters, alcohols and fatty acids) have been quantified by the AWRI's suite of 31 stable isotope dilution assays. Overall, the impact of MLF seems to be associated with the esters, with little or no impact on the volatile fatty acids or higher alcohols. In general, the concentration of most fatty acid ethyl esters increased whereas the acetate esters decreased; the changes are, however, relatively small when compared with those during primary fermentation. Ethyl lactate, commonly associated with MLF, increased, whereas MLF had no effect on the main yeast-derived ester of wine, ethyl acetate.

In addition to the aroma changes associated with MLF, winemakers commonly observe flavour or palate differences. These are typically described as 'softer', and increased 'mouth-feel' and 'palate weight'. The approach adopted to gain evidence for these palate changes in wine, that are not related to pH and TA changes, involved quantifying the acids in the wine before and after MLF, and restoring them to pre-MLF concentrations. The wines were then subjected to quantitative descriptive analysis with techniques recently developed by the Sensory Team led by Dr Leigh Francis.

A total of 27 attributes, appearance, aroma and palate, were rated by the sensory panel. Statistical analysis revealed that 15 attributes were significantly different either for ML strain, acid adjustment, or both treatments. The MLF-treated wines could be distinguished from the control non-MLF-treated wines, and the acid-restored MLF-treated wines could be distinguished from the MLF-treated wines, which had not been subjected to acid restoration, for the attribute 'acidity'. As would be predicted, if the analysis and restoration was performed accurately, the acid-restored MLF-treated wines could not be distinguished from the non-MLF wines (i.e. the control wines) for the attribute 'acidity', however, these acid-restored wines did exhibit other differences in aroma and palate attributes. Depending on the ML bacterial culture used, statistically important sensory differences were found in the aroma for the attributes 'plum', 'floral' and 'herbaceous', and for the palate attributes 'fruit flavour intensity', 'coarse texture after expectoration' and 'bitterness' (Figure 8).

These investigations formally confirm that MLF can modify the sensory properties of red wine other than by diacetyl and deacidification and provide some of the first objective evidence that MLF can modify palate or mouth-feel independent of the pH and titratable acidity effects. A preliminary report of this work will be presented in poster format at the 12th AWITC in Melbourne.

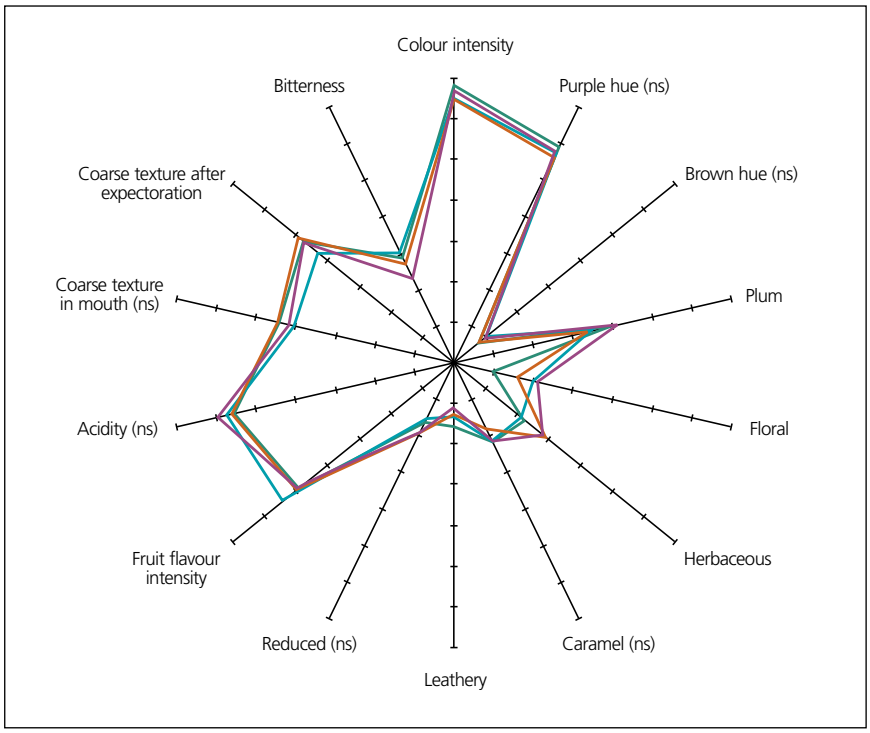


Figure 8. Descriptive sensory analysis of MLF-treated wines for which pH and titratable acidity have been restored to pre-MLF concentrations (pre-MLF - green, MLB1 - blue, MLB2 - purple, 'spontaneous' MLF - orange). NS, non-sigificant

Selection and improvement of wine yeast using molecular biology

Staff: Jenny Bellon, Dr Miguel de Barros Lopes, Jeff Eglinton, Lorelie Flood, Jaromir Guzinski, Anthony Heinrich, Alana Hill-Ling, Kate Howell, Mathias Klein, Professor Sakkie Pretorius, Dr Hentie Swiegers, Tina Tran, Emmie van Veeren, Robyn Willmott

In collaboration with Dr Eveline Bartowsky, Dr Alan Pollnitz, Dr Gordon Elsey, Dr Mark Sefton, Tracey Seibert, Dimitra Capone, Dr Daniel Cozzolino, A/Professor Markus Herderich, Yoji Hayasaka, Professor Peter Høj (amongst others at the AWRI) Dr Paul Chambers (Victorian University), Grant Stanley, Professor Graham Fleet (University of New South Wales), Louisa Rose and Simon Dillon (Yalumba), Dr Vladimir Jiranek and Carolyn Leach (The University of Adelaide)

The Molecular Biology team is focusing on providing improved wine yeast strains, with an emphasis on generating yeast that produce wines with enhanced sensory qualities. During the past year, the research has focused on understanding the mechanisms of volatile thiol release. This is a project that was started by a PhD student, Kate Howell, and is being continued by Hentie Swiegers and two Honours students, Robyn Willmott and Alana Hill-Ling. Studies by Anthony Heinrich, a PhD student in the laboratory, has also provided interesting results in identifying genes that are likely to be important for yeast to tolerate the stressful conditions encountered during fermentation, and work by Jenny Bellon is providing a more detailed characterisation of novel hybrid wine yeast.

This year, the Molecular Biology team has benefited greatly from visitors from both national and international universities. Dr Paul Chambers, from Victoria University, completed one year of sabbatical research in the laboratory. Paul's extensive experience in yeast fermentation and molecular biology has been invaluable to the laboratory. This has led to the attainment of a Victoria University scholarship for Tina Tran to perform her PhD studies; dividing her time between the AWRI and Victoria University. Also, two visiting students, Mathias Klein and Emmie van Veeren, from Archen University of Technology and Delft University, respectively, have greatly

assisted in the team's research. The Molecular Biology team has recently undergone personnel changes. Dr Miguel de Barros Lopes has left the AWRI to take a position at The University of South Australia as Senior Lecturer. A collaboration between Miguel and the Molecular Biology team will continue, providing benefits to both organisations. Dr Hentie Swiegers, a Research Molecular Biologist has recently joined the team. Hentie received his PhD from the Institute for Wine Biotechnology, at the University of Stellenbosh in South Africa, and brings with him extensive experience in yeast molecular genetics.

Formation of volatile thiols by yeast during fermentation

Fermentation of grape must by *Saccharomyces cerevisiae* results in the biosynthesis of aroma compounds by the yeast, as well as the liberation of aroma compounds from the grape. A class of compounds that has been shown to have an important influence on wine aroma is the sulfur-containing volatile thiols, in particular, 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA). These compounds have been shown to contribute strongly to the varietal aroma of Sauvignon Blanc wines and are found in many other varieties including Cabernet sauvignon, Merlot and Riesling. These compounds can have blackcurrent, grapefruit and passionfruit characteristics depending on their concentration. Most of these thiols exist as non-volatile cysteine conjugates in the grape must. Although the extraction of the cysteine conjugated precursors into the juice appears to be correlated to the final concentrations of the volatile thiols present in wine, only a small and varying proportion of the precursor is converted to the active aroma compound during fermentation, indicating a huge remaining potential for thiol release. We, and other researchers, have shown that this conversion is yeast strain dependent.

The volatile thiols are present in very low concentrations in wine and therefore measurement requires specialised analytical methods. In addition, the volatile thiols and their cysteine precursors have to be chemically synthesised as they are not available commercially. Together with Dr Gordon Elsey, Dimitra Capone, Tracey Siebert and Dr Mark Sefton, the thiols and their precursors were synthesised and sensitive methods using the GC/AED (Gas Chromatography/ Atomic Emission Detector) and GC/MS (Gas Chromatography/Mass Spectrometry) have been developed to accurately measure the concentrations of 4MMP, 3MH and 3MHA in model ferments. With these methods, it has been shown by Kate Howell and Dr Hentie Swiegers that commercial wine yeast strains differ greatly in their ability to release 4MMP (up to 20-fold) and that the release is temperature dependent. In order to elucidate the molecular mechanisms the yeast cell uses to cleave the cysteine precursors, Kate Howell, together with Mathias Klein, a visiting student from Aarchen, produced yeast deletion mutants in a commercial wine yeast. Fermentations with strains where specific genes involved in sulfur metabolism were deleted resulted in a reduced release of 4MMP from the precursors (Figure 9). This information can be used in order to select wine yeast strains with optimal capabilities for releasing thiols.

Dr Hentie Swiegers, together with Robyn Willmott and Alana Hill-Ling, are performing similar experiments with the volatile thiol 3MHA. These studies are highlighting the importance of wine yeast selection, and they have identified the genetic factors that regulate the synthesis of this important aroma compound.

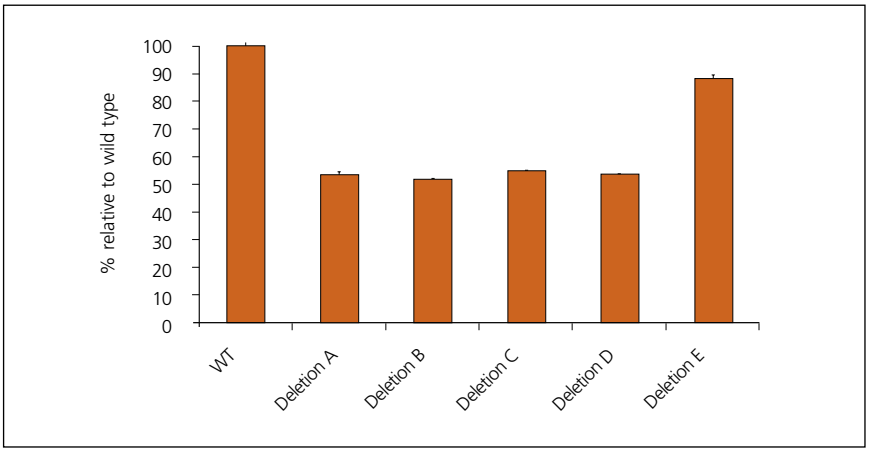


Figure 9. Yeast deletion mutants release less 4MMP than wild-type yeast (WT)

Hybrid wine yeasts

It is generally believed that there are limitations to changes in wine flavour that can be generated by using the current selection of *Saccharomyces cerevisiae* commercial wine strains. For this reason, Jenny Bellon has been exploring the potential of using novel yeast that are interspecific hybrids between commercial wine strains and other related *Saccharomyces* species (de Barros Lopes et al., 2002). Previous research has shown that these hybrid yeast ferment grape juice efficiently in both small-scale winemaking and commercial trials, and preliminary chemical and sensorial studies produce wines with diverse characteristics.

The continuing development of stable isotope dilution assays by Dr Mark Sefton's team for accurately quantifying yeast volatile compounds has allowed us to further analyse wines made with hybrid yeast in commercial trials. The results demonstrate that the concentration of a number of important wine aroma compounds are modified in the hybrid-made wines. For example, the concentrations of acetic acid and ethyl acetate, which produce 'vinegar' and 'nail-polish' characters respectively, are substantially decreased in the hybrid-made wines in comparison to the commercial wines. Conversely, concentrations of the fruity compound ethyl propanoate are increased in the hybrid-made wines.

Presentation of the hybird-made wines to winemakers gave rise to mixed reactions but it is generally viewed that the wines are sensorially different to the wine made with commercial yeast. In particular, a wine yeast X *S. kudriavzevii* was viewed as having potentially useful winemaking properties.

The generation of hybrids between a commercial wine yeast and *S. bayanus* strain, AWRI 1176, a yeast isolated and characterised by Dr Paul Henschke's team, has been important in allowing us to compare the winemaking properties of the hybrid yeast with both its parents. Recent tastings and preliminary chemical analysis indicate that this hybrid produces wines with characteristics more similar to the commercial wine strain, than the *S. bayanus* parent. Chemical analysis of ferments in defined medium indicate a similar trend with some of the other hybrids. Molecular studies by Jaromir Guzinski and Jenny Bellon have shown that some of the hybrids have lost a chromosome from one of the parents. This hybrid appears to ferment more efficiently than the parental hybrid strain. These results are important as they might offer a means to alter the hybrid genome so that the novel aromas and flavours of the non-*S. cerevisiae* parent can be enhanced.

Natural interspecific hybrids, such as S6U, also exist (de Barros Lopes et al., 2002). Recently, a *S. cerevisiae X Saccharomyces kudriavzevii* hybrid commercial wine yeast has been identified (Heinrich et al., submitted).

Identifying genes for wine yeast improvement

The wine habitat is a demanding environment and wine yeast need to be able to withstand a number of stresses, including the high concentrations of sugar and ethanol at the beginning and end of fermentation, respectively. Several studies have used microarray analyses to identify genes that are induced when cells undergo an ethanol shock (a sudden response to high ethanol). In these experiments more than 100 genes were induced and repressed shortly after ethanol treatment. In a wine fermentation, yeast cells do not respond to a sudden

ethanol shock; the ethanol builds up progressively. Anthony Heinrich, together with Emmie van Veeren, studied gene expression during ethanol stress under conditions that were more similar to a wine fermentation. In this environment, only a few genes were shown to be up regulated. Together with Jeff Eglinton, Anthony has been using bioinformatics to gain insight into the role of these genes and their mechanism of regulation. Genetic studies are continuing to establish the importance of these genes in winemaking.

Construction of a wine strain producing less ethanol

A strategy using genetic modification has been used to produce a yeast that makes less ethanol. Attempts are being made to isolate a yeast with similar characteristics, but without using genetic manipulation. To advance this project a partnership has been established with Victoria University. A collaborative PhD scholarship offered by Victoria University has been awarded to Tina Tran. Tina will be co-supervised by Dr Paul Chambers and Dr Grant Stanley at Victoria University, who both have extensive experience in ethanol research in commercial strains of *Saccharomyces cerevisiae*.

Waite Campus Mass Spectrometry Facility

Staff: Yoji Hayasaka and Gayle Baldock

Purpose

The three important roles of the Waite Campus Mass Spectrometry Facility are to act; (i) as a leader in the application of mass spectrometry to grape and wine research; (ii) as an investigator to solve the problems facing the wine industry and individual winemakers, using mass spectrometric techniques; and (iii) as a collaborator with The University of Adelaide and CSIRO in the research activities involving mass spectrometry.

Mass spectrometry facility usage trends

The application of the electrospray technique has continued to increase in the reporting period. In particular, usage of the facility for analytical service related activities dramatically increased and accounted for 66% of the total instrument running time for AWRI. With respect to research activities, the instrument was used for a wide range of projects. These included: the 'Tannin project' (11%); 'Interaction of volatile and non-volatile compounds in wine flavour perception' (10%); 'Molecular tools for the evaluation and improvement of wine fermentations' (6%); and 'Defining, measuring and controlling volatile flavour compounds' (2%).

Table 3. Usage of the TSQ GC-MS/MS and API LC-MS/MS for the period 1 July 2003 to 30 June 2004

	TSQ GC-MS/MS	API LC-MS/MS
AWRI	79%	86%
The University of Adelaide	21%	14%

The mass spectrometer (ThermoFinnigan LCQ *Deca XP Plus*) purchased by Provisor was evaluated for the applicability to our research. The new instrument has versatile capabilities in LC-MS and MS/MS functions, which were found to be very useful for the characterisation and quantification of juice and wine components. Access to this new instrument has provided us with opportunities to investigate new methods and adapt current methods of analysis as well as the ability to overcome the current and increasing problem of limited mass spectrometric resources. The capabilities of this new instrument underlines the necessity to upgrade advanced instrumentation at regular intervals.

The TSQ GC-MS/MS and API LC-MS/MS are used for various purposes and appropriate financial arrangements for all users are in place to recover the running cost of the facility. The usage of the TSQ GC-MS/MS and API LC-MS/MS for the period between 1 July 2003 and 30 June 2004 is detailed in Table 3.

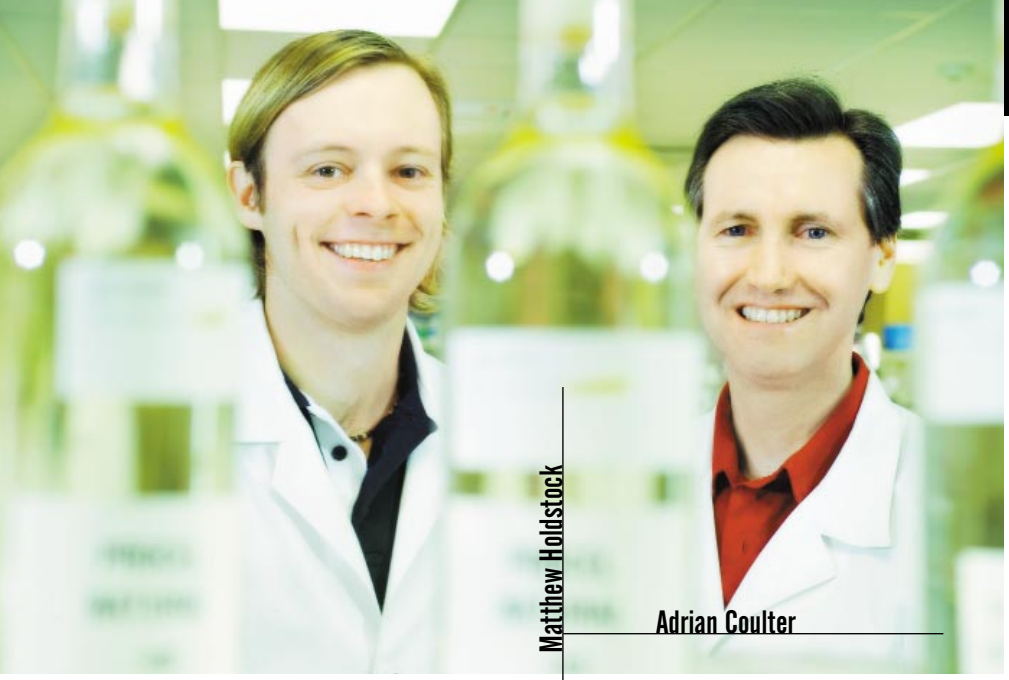
Collaboration with the AWRI Industry Services team and AWRI's Analytical Services

It is necessary for juice and wine to be contamination and taint free in order to meet specified quality criteria. Hence, the availability to monitor contaminants in juice or wine is essential to ensure and maintain the quality of our products at a high standard.

The MS Facility conducted mass spectrometric analyses on 272 samples for the investigation of taint or contamination problems, in collaboration with staff of the Industry Services team and Analytical Services. The analyses conducted during the reporting period are detailed in Table 4. Staff members from Analytical Services were also trained in analysis of brine contamination on the API 300 MS/MS.

Table 4. Investigation conducted in the period from July 2003 to June 2004.

Type of investigation	Number of samples analysed
Brine contamination	217
Aromatic hydrocarbon taint	11
Flavouring	6
Protein analysis for varietal differeniation	5
Hydraulic oil contamination	5
Faulty 'Stelvin'	3
Sanitiser contamination	2
Chlorophenols	1
Others	4
Total Samples	254



Collaboration with research groups

The contribution of the MS facility to the research projects is detailed in the respective research team reports.

Collaboration with Provisor

The new ThermoFinnigan LCQ *Deca XP Plus* with *Surveyor* HPLC Pump, PDA detector and autosampler was purchased by Provisor and was installed at the MS Facility on 13 November, 2003. Staff of the MS Facility carried out familiarisation with the instruments' operation and performance and maintenance of the instrument. CSIRO, the AWRI's Tannin team and the AWRI Industry Services team benefited from this familiarisation and subjected samples to analyses on this new mass spectrometer.

Industry development and support—technical problem solving and extension and information transfer

Staff: Peter Godden, Adrian Coulter, Mark Gishen, Geoff Cowey, Ella Robinson, Narelle D'Costa, Matthew Holdstock, Professor Peter Høj, Yoji Hayasaka, Gayle Baldock and Greg Ruediger

The Industry Services team provides technical development and support services to the Australian wine industry, primarily in the form of an advisory service that disseminates a wide range of technical information, and a problem solving and analysis service, which collectively represent a significant proportion of the team's workload. The team coordinates an AWRI-wide project that is investigating the relationships between the potential spoilage yeast *Dekkera/Brettanomyces* and wine in Australia, and this project became the team's major research focus during the year. Additionally, the development of a web-based technical reference manual for the Australian wine industry has continued, which complements the team's problem solving and analysis service, and workshops that are presented in conjunction with AWRI Roadshow seminars in 26 winemaking regions around Australia.

In addition to the team Manager's membership of the Conference Planning Committee and the Program Sub-Committee, the team played a major role in planning the workshop program for the 12th Australian Wine Industry Technical Conference, which was held in Melbourne in July 2004. In conjunction with other members of the Industry Services team, particularly Ms Narelle D'Costa, the team Manager took primary responsibility for coordinating the staging of 67 workshops at the conference. The input of staff of the Cooperative Research Centre for Viticulture, particularly Gerard Hogan, in contributing a portion of the workshop program is also appreciatively acknowledged. During the reporting period the team Manager also devoted a significant amount of time to the formulation of a bid for a new Cooperative Research Centre for Wine.

Industry Services team members are also regular contributors to the AWRI's *Technical Review*, periodically provide presentations for external seminars and conferences, and also provided eleven hours of lectures to Oenology students at The University of Adelaide (see Appendix 2).

The Industry Services Laboratory analysed in excess of 1,200 samples during the year (Table 5), using a wide range of routine or novel analytical techniques. An increased reliance on advanced analytical methods provided by the Waite Campus Mass Spectrometry Facility and the AWRI's commercial Analytical Service is acknowledged. The majority of samples analysed are wine; the analysis of which is supplemented by detailed sensory evaluation by a panel of experienced tasters. The remaining samples predominantly consist of wine additives, closures, or compounds that are suspected to have caused taints and or deposits in wine. The capability of the Industry Services team was enhanced during the year by the recruitment of Narelle D'Costa in the position of Administrator–Industry Services, and Matthew Holdstock in the position of Chemist–Industry Services. Matthew holds a BSc in Chemistry, and had worked for the AWRI's Analytical Service for eight years before joining the Industry Services team. Matthew undertook vintage experience in California in 2003, after completing the Graduate in the Diploma in Oenology at The University of Adelaide, and his recruitment has strengthened the team's already broad scientific, tertiary science, winemaking, and industry experience.

Industry Services staff members increasingly regard their role as educational, seeking to disseminate information in a variety of ways in order to foster industry wide understanding of the causes of many common winemaking problems, in order to prevent their frequent recurrence. The continued enhancement and increased frequency of delivery of workshops on AWRI Roadshows, and the further development of the AWRI *Practical Solutions* website, are part of a coordinated strategy that seeks to provide wine producers with targeted information to enable them to avoid wine quality loss during processing and packaging.

With appropriate cross-charges in place, the Industry Services team also provides technical support to the AWRI's Analytical Service, particularly in the maintenance and auditing of the quality management system, and the interpretation of analytical results. The Analytical Service also supplies chemical analysis on problem solving and research samples to the Industry Services team, on a contractual basis.

The AWRI's investigative and advisory services are provided according to strict Terms and Conditions, and client confidentiality is an important aspect of the provision of the services. This facilitates a frank exchange of information between the AWRI and its clients, which in turn allows the maximisation of the knowledge gained from the provision of these services.

A summary of the number and type of investigations conducted by the Industry Services team over the past three financial years is presented in Table 5. The number of investigations conducted decreased by 32% from the previous year, and the total number of samples analysed as part of these investigations decreased by 43%.

Taking into account that the previous year's high sample numbers were, in part, due to a large number of grape and wine samples analysed for taints derived from exposure of grapes to bushfire smoke, a general downward trend in the number of investigations has now been evident for the last six quarters. Nevertheless, the number of investigations related to hazes and deposits and microbiological instabilities has remained stubbornly high despite the emphasis placed on addressing such issues in AWRI Roadshow workshops and the *Practical Solutions* website. It is apparent, though, that an increased awareness of these problems generated by Roadshows tends to lead to a spike in the number of samples received from particular regions immediately following the staging of workshops. Ten such workshops were staged during the current year.

Most of the investigations recorded in Table 5 result in a comprehensive written report being prepared for the client. These reports contain a large amount of technical information relating to the problem being investigated and are written in a way which seeks to explain the underlying causes of the problems encountered, and provide advice on how to prevent their re-occurrence. The reports are often accompanied by a number of technical references relating to the area of investigation.

The types of investigations recorded in Table 5 as 'other investigative analyses' are extremely varied, and some particularly interesting and unusual cases have been investigated during the year.

> The AWRI was contacted by a levy-paying winery that produces a 'fruit-flavoured' alcoholic beverage that is classed as a wine product. A particular batch of the beverage was reported to exhibit a higher than usual level of fruit flavour during sensory assessment. Analysis was requested in order to investigate if (i) there were higher concentrations of fruit flavour compounds present in the 'affected' batch of the beverage compared to 'control' samples from a different batch of the beverage, and, if so, (ii) had this situation arisen due to the wine company adding an excess of a 'fruit flavoured concentrate' to the batch of beverage in question, or due to the concentration of certain flavour compounds differing between batches of the 'fruit flavoured concentrate'. 'Affected' and 'control' samples of the beverage were submitted, as were samples from two different batches of the fruit flavoured concentrate.

  The two most abundant flavour compounds in the flavouring concentrate were determined. Internal standards of both compounds were available at the AWRI, as both compounds have been found to occur naturally in wine. Yoji Hayasaka and Gayle Baldock determined the relative concentrations of these two compounds in the 'fruit flavoured concentrate' and in the 'affected' and 'control' samples of the beverage, using solid phase micro extraction (SPME), followed by gas chromatography-mass spectrometry (GC-MS).

  The 'affected' beverage samples were found to contain higher concentrations of the two most abundant flavour compounds than the 'control' beverage samples. It was also established that the batch of 'fruit flavoured concentrate' that had been used for the 'affected' beverage samples, contained higher concentrations of the two most abundant flavour compounds than did another batch of 'fruit flavoured concentrate' analysed. The ratio of the concentrations of the most abundant flavour compound in the 'affected' and 'control' beverage samples, and between the two batches of concentrate analysed, was found to be similar. No such relationship could be established for the second most abundant flavour compound, possibly because it could not be established if the 'control' concentrate sample was from the same batch of 'fruit flavoured concentrate' that had been used in the batch of beverage from which the 'control' beverage samples had been taken. It was nonetheless concluded that varying concentrations of flavour compounds between batches of the 'fruit flavoured concentrate', was the most likely cause of the confirmed varying concentrations of fruit flavour compounds present in different batches of the beverage. It appears, therefore, that careful assessment of individual batches of 'fruit-flavoured concentrate' should be made prior to their use.

> An investigation was conducted into a drum of 'double strength' brandy, which had been returned to a winery from a customer because the brandy had developed a green/blue colour. Samples of the 'affected' brandy and 'control' samples of the same product that did not exhibit the green/blue colour, were submitted for investigation.

  Both the 'affected' and 'control' samples of the brandy were analysed for the concentrations of a range of metals using inductively coupled plasma atomic emission spectrometry (ICPAES), as a metal instability was suspected to be the cause of the problem. The assistance of The University of Adelaide ICPAES unit is acknowledged. The only notable difference in the metal concentrations between the affected and 'control' samples was the concentration of iron. The affected sample was found to contain 1.4 mg/L of iron whilst only a trace of iron was detected in the 'control' sample (<0.08 mg/L). A Fe(II) salt was incrementally added to a portion of the 'control' sample until a similar green/blue colour was induced, suggesting that the colour associated with the affected brandy sample was indeed related to the concentration of iron. Further, a commercially bottled sample of a different batch of the brandy product was purchased, and again, a similar colour was induced in the sample by adding a small amount of iron.

  It was suspected that the integrity of the drum liner in which the brandy had been stored might have been compromised, such that iron was able to be leached from the drum and combine with oak derived tannins in the brandy, to form a green/blue complex.

> The AWRI was contacted by a winemaker who was concerned that the pH of a Pinot Noir wine had increased dramatically after the completion of malolactic fermentation (MLF), from 3.58 prior to inoculation of the wine with malolactic bacteria, to 4.92 after the completion of MLF. The winemaker indicated that the vineyard from which the grapes were sourced was 'organic' and that a powdered milk spray had been used in the vineyard to control powdery mildew. The winemaker queried whether the use of milk powder in the vineyard might have been related to the problem associated with the Pinot Noir wine. The winemaker asked this question because it was the first time he had sourced grapes from this particular vineyard and it was also the first time he had observed such a large increase in pH after MLF during his ten years of winemaking.

  Chemical analysis confirmed that the wine exhibited a very high pH value and that it contained an unusually high concentration of lactate, a high concentration of

Table 5. Summary of the number and type of problem solving investigations conducted, and numbers of samples analysed by Industry Services during the past three years

	Investigations conducted and samples analysed		
	2001/02	2002/03	2003/04
Identification of hazes and deposits	85	112	87
Microbiological investigations	89	95	93
Sensory assessments	98	89	60
Taint problems	43	72	46
Other investigative analyses	209	113	36
Closure-related investigations	22	20	19
Total number of investigations	546	501	341
Total number of samples analysed	1233	2231	1262

succinate, an elevated concentration of acetate, and no detectable citrate, tartrate or malate. Approximately half of the lactate detected in the wine was found to be D-lactic acid, which is not usually found at high concentrations in wine. The lactic acid present in most wines tends to be L-lactic acid, which is produced during the malolactic fermentation of L-malic acid. This suggested that lactic fermentation of some wine constituents, other than malic acid, might have occurred with the wine in question. The fact that no tartaric acid was detected in the wine suggests that the microorganism responsible for the lactic fermentation might have been a species of *Lactobacillus*, possibly *Lactobacillus plantarum* and/or *Lactobacillus brevis*. These species were suspected because it has been reported that of the numerous species and strains of lactic acid bacteria, only *Lactobacillus plantarum* and *Lactobacillus brevis* are able to utilise L-tartaric acid. In particular, *Lactobacillus plantarum* can produce lactic acid from tartaric acid (Fugelsang 1997). It, therefore, appears that *Lactobacillus plantarum* might have utilised the tartaric acid in the wine, consequently increasing the pH due to the effect of replacing a stronger acid (tartaric acid) with a weaker acid (lactic acid).

It is not known whether the use of milk powder in the vineyard might have been related to the problem associated with the Pinot Noir wine. However, it is possible that the milk powder contained *Lactobacillus* sp. bacteria, or that the milk powder might have provided a substrate for growth of other bacteria that might have already been present on the grapes.

> An investigation was conducted into a shipment of oak barrels imported from France because the importer had observed a "strange chemical smell" when opening the container on arrival in Australia. Shavings were taken from one of the barrels, and these shavings were subsequently delivered to the AWRI.

It was noted by the AWRI's Oenologist that the plastic bag containing the oak shavings gave off a 'chlorine-like' odour. Based on similar investigations previously conducted at the AWRI, oak shavings that smell 'chlorine-like' have subsequently been found contaminated with chlorophenol compounds. It was, therefore, suspected that the oak shavings submitted might have been contaminated with chlorophenols.

The oak shavings were subsequently analysed for the presence of chlorophenol compounds using a solid phase microextraction (SPME) technique, followed by analysis using gas chromatography–mass spectrometry (GC-MS).

Contamination of the oak shavings with chlorophenol compounds was confirmed by GC-MS analysis, which indicated the oak shavings were contaminated with 2,4-dichlorophenol, 2,6-dichlorophenol and possibly, to a lesser extent, with 2-chlorophenol, 4-chlorophenol and 2,4,6-trichlorophenol.

It was suspected that the wooden floor of the shipping container might have been sprayed with a wood preservative containing chlorophenols, or that a chlorine-based cleaning agent might have been used in the container. Circumstances such as these could have led to aerial contamination of the oak barrels with chlorophenol compounds, or with chlorine that could have subsequently reacted with oak-derived phenolics to form chlorophenol compounds.

> An investigation was conducted into a Riesling fermentation which the winemaker indicated was 'sluggish' and 'going brown', despite there having been 23 mg/L of free SO_2 measured in the must prior to fermentation. The winemaker also indicated that other Riesling fermentations, with fruit from nearby vineyards, were all proceeding normally.

The fermentation sample submitted to the AWRI was initially analysed for laccase activity, although the winemaker indicated that the grapes showed no obvious sign of *Botrytis* infection. No laccase activity was detected in the sample, suggesting that any oxidation of the wine was likely to have been chemical rather than enzymatic.

The sample was then analysed for the concentration of copper, as it is known that copper is a catalyst for oxidation in wine (Danilewicz 2003, Ribéreau-Gayon et al. 2000, Singleton 1987). The concentration of copper in the sample was determined to be 1.2 mg/L, which is considerably higher than typically observed in white wines previously analysed at the AWRI. It should be noted that the winemaker had indicated that the fermentation sample submitted would have been more than two thirds of the way through the fermentation by the time the sample arrived at the AWRI, due to transport delays. The copper concentration in the must might, therefore, have been considerably higher before fermentation commenced, as yeast cells are known to effectively adsorb copper ions (Tromp and de Klerk 1988). It has often been observed at the AWRI that the concentration of copper can decrease by as much as 90% or more during fermentation of musts that initially contain high concentrations of copper.

In order to determine if the copper concentration in the must might have been higher than that observed in the fermentation sample, the winemaker (first producer) contacted a second producer who sourced grapes from the same vineyard, and requested that a juice sample of the grapes from the vineyard be forwarded to the AWRI for copper analysis. The second producer had harvested their fruit a few days after the first producer had contacted the AWRI. The concentration of copper in the juice sample from the second producer was determined to be greater than 10 mg/L. It was, therefore, likely that the first producer's Riesling juice also contained a concentration of copper greater than 10 mg/L before fermentation, which might explain the browning observed. It should also be noted that the sluggish fermentation might also be explained by the presence of a high concentration of copper, as Tromp and de Klerk (1988) found that a copper concentration of 10 mg/L could inhibit fermentation. A possible cause of a high concentration of copper in juice and must is the application of copper-containing sprays in the vineyard and non-adherence to correct withholding periods before harvesting.

> Two separate investigations were conducted into variable post-bottling *Dekkera/Brettanomyces* yeast growth. In the first case, the winemaker suspected that there was variation in the degree of 'Brett character' between bottles of a particular wine about ten months after bottling, and had submitted a sample of the wine to the AWRI's Analytical Service for analysis of 4-ethylphenol, a compound known to be produced in wine by *Dekkera/Brettanomyces* yeast. The concentration of 4-ethylphenol in the wine was determined to be 430 µg/L, a concentration that might negatively affect the sensory properties of many wines. Five months later, the winemaker submitted a second sample of the wine to the AWRI's Analytical Service for analysis, and the 4-ethylphenol in this sample was found to be 2380 µg/L. The winemaker contacted the AWRI's Industry Services team regarding the problem, and subsequently submitted 12 samples of the wine and a copy of the bottling documents that showed that the wine was bottled in one run on one day.

Chemical analysis of the 12 samples showed that one particular sample contained a concentration of 4-ethylphenol (1400 µg/L) three times higher than the mean of the remaining 11 samples. The mean 4-ethylphenol concentration (465 µg/L) for these 11 samples was similar to the concentration of 4-ethylphenol determined in the first sample submitted to the Analytical Service. The sample that contained the high concentration of 4-ethylphenol also contained a significantly lower concentration of *glucose plus fructose* (0.6 g/L) than the remaining 11 samples (0.9 g/L), suggesting that *Dekkera/Brettanomyces* yeast might have metabolised some of the residual sugar. During sensory assessment of the 12 samples by three of the AWRI's tasters who are known to be most sensitive to the effects of 4-ethylphenol in wine, the sample that contained the relatively high concentration of 4-ethylphenol also obtained a significantly higher mean score for the attribute *bandaid/medicinal aroma*, which is commonly associated with elevated concentrations of 4-ethylphenol in wine.

The second case of variable post-bottling *Dekkera/Brettanomyces* yeast growth that was investigated, was similar to the first case discussed above in that the winemaker had noticed that some bottles of wine appeared to be affected by 4-ethyphenol some months after bottling. Chemical analysis of 11 samples subsequently submitted to the AWRI showed that the concentration of 4-ethylphenol ranged from 295 µg/L to 565 µg/L. These results considered in isolation were not compelling enough to prove that there was definitely variation in the concentration of 4-ethylphenol between the samples. However, there was a highly statistically significant relationship between the concentration of 4-ethylphenol in the samples and the mean score for the attribute *bandaid/medicinal aroma* obtained during the sensory assessment of the samples by the AWRI's three tasters most sensitive to the effects of 4-ethylphenol in wine. Once again, correspondence has been received from the winery concerned indicating that the wine was bottled in one bottling run on the same day.

> In the *2003 AWRI Annual Report*, the development of a GC-MS analysis for the compound rhodamine, which is used as a dye in several commercially available refrigerant brines, was reported. The development of this method by the AWRI's Mass Spectrometry facility manager, Yoji Hayasaka and technical officer Ms Gayle Baldock, has greatly aided the Industry Services team. During the current reporting period the sensitivity of the rhodamine method has been greatly improved, allowing definitive detection of brine contamination at much lower concentrations than was previously possible. With red wines, typically 10 mL of brine per 1000 L of wine can now be detected, and greater sensitivity is possible with some white wines. Whilst Industry Services staff members are always disappointed to learn of new cases of brine contamination, they are now able to provide wine producers who suspect contamination, with an analysis that provides much greater certainty with regard to the integrity of wine products.

References

Danilewicz, J.C. Review of reaction mechanisms of oxygen and proposed intermediate reaction products in wine: central role of iron and copper. Am. J. Enol. Vitic. 54(2): 73-85; 2003

Fugelsang, K.C. Wine Microbiology. New York: Chapman & Hall: 1997; 35

Ribéreau-Gayon, P.; Glories, Y.; Maujean, A; Dubourdieu, D. Handbook of Enology Volume 2: The Chemistry of Wine Stabilisation and Treatments. Chichester: John Wiley & Sons Ltd; 2000; 92

Singleton, V.L. Oxygen with phenols and related reactions in musts, wines, and model systems: observations and practical implications. Am. J. Enol. Vitic. 38(1): 69-77; 1987

Tromp, A.; de Klerk, C.A. Effect of copper oxychloride on the fermentation of must and wine quality. S. Afr. J. Enol. Vitic. 9(1): 31-36; 1988

Winemaking and other technical consultation

The Industry Services team provides a winemaking consultancy service principally through the Manager, Peter Godden, a qualified and experienced winemaker; Adrian Coulter, a Graduate in the Diploma in Oenology from The University of Adelaide; and Mark Gishen, a qualified chemical engineer with a masters qualification in mechanical engineering science. Mark had gained six years wine industry experience before joining the AWRI in 1994. Greg Ruediger, a Graduate in the Diploma in Oenology from The University of Adelaide, is the AWRI Analytical Service's Good Laboratory Practice Supervisor, and worked with the Industry Services team on a 0.25 basis until March 2004. Of the other members of the team, Geoff Cowey (BSc) had gained five years of wine industry experience before joining the AWRI in 2001, and is currently undertaking undergraduate studies in winemaking at Charles Sturt University. Matthew Holdstock (BSc), who was recruited to the team during the current reporting period, is also a Graduate of the Diploma in Oenology from The University of Adelaide, has overseas winemaking experience, and previously held the position of Laboratory Supervisor with the AWRI Analytical Service.

Table 6. Enquiries received by advisory staff during the past three years

	2001/02	2002/03	2003/04
Wineries	1008	1184	1220
Government organisations	101	97	57
Other	469	368	431
Students	31	27	40
Total	1609	1676	1748

Most queries received are technical in nature and arise predominantly from Australian winemakers. However, many queries are also received from wine industry suppliers and Government bodies, as well as a relatively small number from the general public and secondary and tertiary students. The majority of queries are answered either by telephone, increasingly by e-mail, or by facsimile. Thus, Industry Services staff supplies approximately five hundred technical papers or other pieces of relevant literature to callers each year, via the John Fornachon Memorial Library. Increasingly, Industry Services staff members are also able to direct callers to web-based information, both on the AWRI's own, and other web sites, and the support facilities provided by other AWRI research and library staff members are important in supplying relevant information to callers. Furthermore, the analytical capacity of the Industry Services Laboratory plays an important role in responding to many of these enquiries.

A summary of the enquiries received by Peter Godden, Adrian Coulter, Mark Gishen, Ella Robinson, Geoff Cowey, Matthew Holdstock and Greg Ruediger during over the last three years is presented in Table 6.

The current year figure shows a 4.3% increase in the number of enquiries received, compared to the previous year. The total of 1748 calls during the current reporting period is a new record figure, surpassing that recorded for the 1999/2000 year (1700). The proportion of calls received from wineries has stabilised at a historically high 70%, indicating that a large number of personnel in the Australian wine industry regard the AWRI as an important or primary source of technical information. Additionally, it is clear from consistent feedback received on AWRI Roadshows, that there is a high degree of awareness and use of the *Practical Solutions* section of the AWRI website, which might negate the need for many people to contact advisory staff directly.

The Investigative and Advisory Services are supported by Roadshow seminar and workshop tours, which are made on a rotating basis to 26 wine growing regions. During the year, workshops were held in two regions that had not been previously visited on Roadshows (Bunbury and Manjimup, WA), reflecting the geographical divergence of new grape growing and winemaking enterprises in that state.

Roadshows are generally organised in conjunction with local vignerons' associations, and are held in the second half of the calendar year, although the Bunbury and Manjimup events were organised solely by AWRI Industry Services staff, and the three workshops staged in Manjimup were held in January. Whilst Industry Services staff members are responsible for the organisation of Roadshow seminars, they rely heavily on input from all of the AWRI's research teams, with six senior Institute staff members making twelve presentations in each full-day seminar. During the current year, Roadshow seminars were presented in four regions, and ten *Trouble-free winemaking* -

Identification and management of common wine instabilities workshops were held in three states.

Regional winemakers' and growers' associations are asked to select the presentations to be made at each Roadshow seminar from a list of approximately forty areas of current AWRI activity, in order that seminars are closely tailored to the interests and needs of the audience. In addition to the formal presentations, Roadshows are also considered an important vehicle for the delivery of informal advice, and it is considered that if this contact were formally recorded then it would account for a substantial increase in the number of enquiries recorded in Table 6. The most common areas of informal discussion on recent Roadshows have continued to be *Dekkera/Brettanomyces* and other microbiological stability issues, red wine phenolics, salinity both in terms of soil and vineyard effects and effect on wine, closure issues, and the use of non-conventional yeasts. However, except for one region visited during the current year, stuck fermentation seems to have declined markedly as a topic of concern. The number of calls received by advisory staff on this subject during the past two vintages apparently mirrors this decline.

The seventeenth Advanced Wine Assessment Course was held in July 2003, giving another thirty participants the opportunity to develop and test their sensory evaluation performance. This was the fifth course presented under a four-day format, which includes over 40 hours of activities over the four days, and 14 leading wine show judges, journalists and winemakers assisted in the presentation of the course. The course content is continually being enhanced, and the seventeenth course included a number of new tasting and discussion exercises that were intended to simulate the dynamics associated with judging as part of a wine show panel. As in the past, Associate Judges for the 2003 Adelaide Wine Show were selected from the most successful recent participants in the Course, and in a continuation of a trend discussed in the previous Annual Reports, during the current year other shows have accessed the course results for use in the selection of new judges. Similar approaches from other wine shows are encouraged.

The team Manager participated in five other seminars during the reporting period, which were organised by regional winemakers' associations or other state-based winemakers groups, or large wine companies. Additionally, the Oenologist, Adrian Coulter, presented a paper developed by the Industry Services team on the management of *Dekkera/ Brettranomyces* yeast during winemaking, at the *ASVO Impacts on Wine Flavour* seminar (AWRI publication # 756), which was held in the Barossa Valley in July 2003 (see Appendix 1).

Beginning on 1 July 2003, the previous *Targeted training of wine industry personnel: compilation of a technical reference manual and delivery of complementary workshops* project, was incorporated into the newly created *Industry development and support technical problem solving* project. The primary aim of this continuing project is to produce flexible and updateable information packages on selected technical subjects, to be delivered to wine grape levy payers via the internet, and Roadshow workshops which will be held in all major grape growing areas of Australia on a rotating basis.

The AWRI has a vast amount of collective knowledge pertaining to grape and wine production, much of it generated over the years by research and Industry Services' projects, that have been supported by industry research-levy funding. Although this research has produced many technical publications, there is also a great deal of information generated and recorded in a more informal manner by the staff concerned. In addition, a great deal of data pertaining to the composition of Australian wine is stored in various databases at the AWRI, some of which have been in existence for several decades. Collectively, this information is a resource of great potential value to the Australian wine industry. This project, therefore, seeks to make a record of this information, so that it can be delivered to the industry in a manner in which it is both readily useable, and relevant to those involved in day-to-day wine production. The development of these resources will be ongoing, and many areas of interest to winery technical personnel will be addressed in due course.

New material is periodically added to the website, and the existing information is enhanced. During the current year, a new section dealing with microbiological instabilities has been added to the website. Australian wine industry personnel can access the information at http://www.awri.com.au/practical_solutions/ or, alternatively, via the AWRI Home Page by selecting 'Practical Solutions', followed by 'Hazes and Deposits' and 'Microbiological Instabilities'. This section of the site is password protected (the password has previously been supplied to Australian wineries). Australian grapegrowers and winemakers who require the password, and who can confirm that they pay the *Wine Grapes* or *Grape Research Levy,* can obtain it from the AWRI's Librarian, Catherine Daniel Catherine.Daniel@awri.com.au. However, other sections of the AWRI's website also contain a great deal of other technical information, which is readily accessible by interested parties.

As discussed elsewhere in this report, the combined and related areas of hazes and deposits and microbiological instabilities continue to represent a significant proportion of the problem wine investigations conducted by the Industry Services team, and accounted for more than 50% of such investigations conducted during the current reporting period. In many cases it is apparent that the problems could have been avoided if winemakers were more aware of the causes of many types of instability, and were able to access practical and relevant tools with which to trouble-shoot the problems before significant wine spoilage had occurred. The web site and workshops address these issues, providing practical trouble-shooting information and simple diagnostic tests with which to isolate and identify a wide range of hazes and deposits and microorganisms in wine.

In addition to the development of the website and staging of Roadshow workshops, the 'mining' of AWRI databases for information that can be of use to Australian wine producers has been a major focus of this project. The *2003 AWRI Annual Report* presented some such data obtained from the AWRI Analytical Service's database of analytical results, relating to trends of alcohol content in Australian red wine. This trend was discussed in relation to the manner in which it might be contributing to the continued high number of wines that are presented to the Industry Services team with some form of microbiological instability, or haze or deposit. During the current year, data from the same database relating to trends in SO_2 and volatile acidity (VA) concentrations have also been collated and analysed by Mark Gishen, and are presented below (Figures 10-12). Whilst it is considered that this database contains results from a broad cross section of Australian wines, it should be noted that it is a self-selected sample of clients of the Analytical Service, and is probably skewed towards relatively higher rather than lower value wines.

The data presented here might indicate that, at least in part, key strategies for the avoidance of common winemaking problems advocated in the workshops developed under this project, are being widely adopted by Australian wine producers. The key strategies discussed in workshops include those for the control of stuck fermentation, the control of *Dekkera/Brettanomyces* yeast, and other microbiological spoilage, the maximisation of the effectiveness of SO_2 additions, the minimisation of oxidation during the winemaking process, and the maintenance of low turbidity during winemaking.

Data relating to the concentration of SO_2 in red wines analysed by the Analytical Service are presented in Figure 10. The data demonstrate that for red wines the mean concentration of Free SO_2 has increased steadily over the last 20 years, while the mean concentration of Total SO_2 had increased up to 2001, but has decreased since then. The decrease in Total SO_2 since 2001 is coincident with the concentration of Bound SO_2 in red wines having fallen notably since 2001. Consequently, the ratio of Free to Total SO_2 has increased strongly over this period.

The importance of optimising the effectiveness of SO_2 has been stressed in relation to the avoidance of many common winemaking problems, in the Roadshow workshops developed by the Industry Services team in 1999, and from the first presentations made in 2000. In particular, the team has emphasised the importance of monitoring the ratio of Free to Total SO_2 concentrations as an extremely useful winemaking tool. Microbiological activity, or chemical instability, can both lead to the formation of hazes in wine that are able to directly or indirectly lead to the binding of SO_2 and hence a decline in Free SO_2. Thus, a change in the ratio of Free to Total SO_2 can be a simple and early indicator of problems that might lead to wine spoilage, such as microbiological activity and oxidation. While the monitoring of both Free and Total SO_2 concentrations, and calculation of the concentration of molecular SO_2 are advocated in most winemaking texts and are considered useful tools for quality control in their own right, the simple calculation of the ratio of Free to Total SO_2 seems to have been ignored until now.

The *2003 AWRI Annual Report* provided data from two wineries that had been particularly successful in producing red wines with lower concentrations of the *Dekkera/Brettanomyces* yeast-derived compound 4-ethylphenol, than was present in wines produced by them in previous vintages. It became apparent to the wineries concerned and to the Industry Services team as early as 2000, that these lower 4-ethylphenol concentrations in the more recent vintages had been achieved with the use of less Total SO_2 than was present in wines produced by these wineries in previous vintages, and where substantially higher concentrations of 4-ethylphenol were observed. Thus, it was considered that the manner in which SO_2 is used during winemaking and packaging, is perhaps more important than the final total concentration of SO_2 that is present in wine. Fostering an understanding of the manner in which the SO_2 concentrations currently being used by Australian winemakers could be optimised, therefore, became a key focus of this project, and standard wording was developed to encapsulate the AWRI's position with regard to the use of SO_2, which is reproduced below:

The AWRI does not advocate that Australian winemakers should address microbiological and chemical stability issues simply by increasing their use of SO_2–although this would be recommended for many of the wines that are submitted to its problem solving service.

Rather, the AWRI seeks to promote an understanding of the interactions between SO_2 and other wine components, in order to maximise the effectiveness of the concentrations of SO_2 currently being used in many wineries.

An article discussing the optimal use of SO_2 was published in the August 2003 issue of *Technical Review* (Robinson and Godden 2003, issue 145). The Industry Services team has advocated that winemakers should achieve as high a ratio of Free to Total SO_2 as possible and should aim for the range of 0.35–0.40 in the first instance. It is encouraging to note that the mean ratio in red wines analysed by the Analytical Service has now surpassed 0.40 (Figure 10). It seems probable that an improvement in the effectiveness of SO_2 additions facilitated by implementing the strategies discussed by Robinson and Godden (2003), has contributed to the apparent reduction in the number of red wines exhibiting *Dekkera/Brettanomyces*-related problems discussed elsewhere in this report. Data in Figure 11 also demonstrate that there has been an apparent positive effect on the control of microorganisms responsible for the production of VA in red wines.

Data in Figure 11 also demonstrate that there has been a similar, if less dramatic fall in the concentration of VA, in white wines analysed by the AWRI's Analytical Service since 2000. It is considered that a possible explanation of this trend are two strategies discussed during Roadshow seminars and workshops, namely: the avoidance and management of stuck or sluggish fermentations, and advice that advocates addition of SO_2 to grapes at harvesting and/or crushing, in order to eliminate microorganisms such as *Gluconobacter* sp. which are capable of forming acetic acid during the early stages of fermentation. During Roadshow seminars staged over the last two years, each audience has been asked to indicate by a show of hands, if they consider "stuck fermentation to be a greater or lesser problem than it was five years ago". The overwhelming majority, perhaps as strong as three to one in most regions, is that it is now considered to be less of a problem. This is a particularly encouraging sign given the trend towards higher and higher alcohol concentrations in Australian wines (see 2003 Annual Report).

The compilation of data relating to volatile acidity was refined during the reporting period, to consider only wines made from Pinot Noir, and the team Manager presented these data at the *Victorian Pinot Noir Workshop* in November 2003 (see Appendix 1). The same downward trend in volatile acidity concentrations is evident with Pinot Noir as with all red wines. At this workshop many wines were presented, with compositional data available for most of them. It was apparent that among the approximately 55 winemakers present, approximately ten were able to consistently identify VA as a negative attribute in multiple samples, at lower concentrations than the remainder of the group. The proportion of people identifying VA as a negative attribute increased with increasing VA concentration, and all attendees considered concentrations of slightly above 0.7 g/L, to be negative. The data presented in Figure 12 demonstrate that the mean concentration of VA in the Australian Pinot Noir wines analysed by the Analytical Service was, until recently, above this albeit anecdotally determined threshold, which was based on data produced by a number of different laboratories.

It is apparent that data collated under this project collectively represent powerful tools with which wine producers are able to benchmark their own wines for a range of compositional variables, and it might be expected that as a result of the widespread dissemination of this type of information to personnel employed in the Australian wine industry, the apparent current trends in the composition of Australian wines discussed above, might continue.

Reference:

Robinson, E.M.C, and Godden, P.W. (2003) Revisiting sulfur dioxide use. The Australian Wine Research Institute *Technical Review*, issue 145

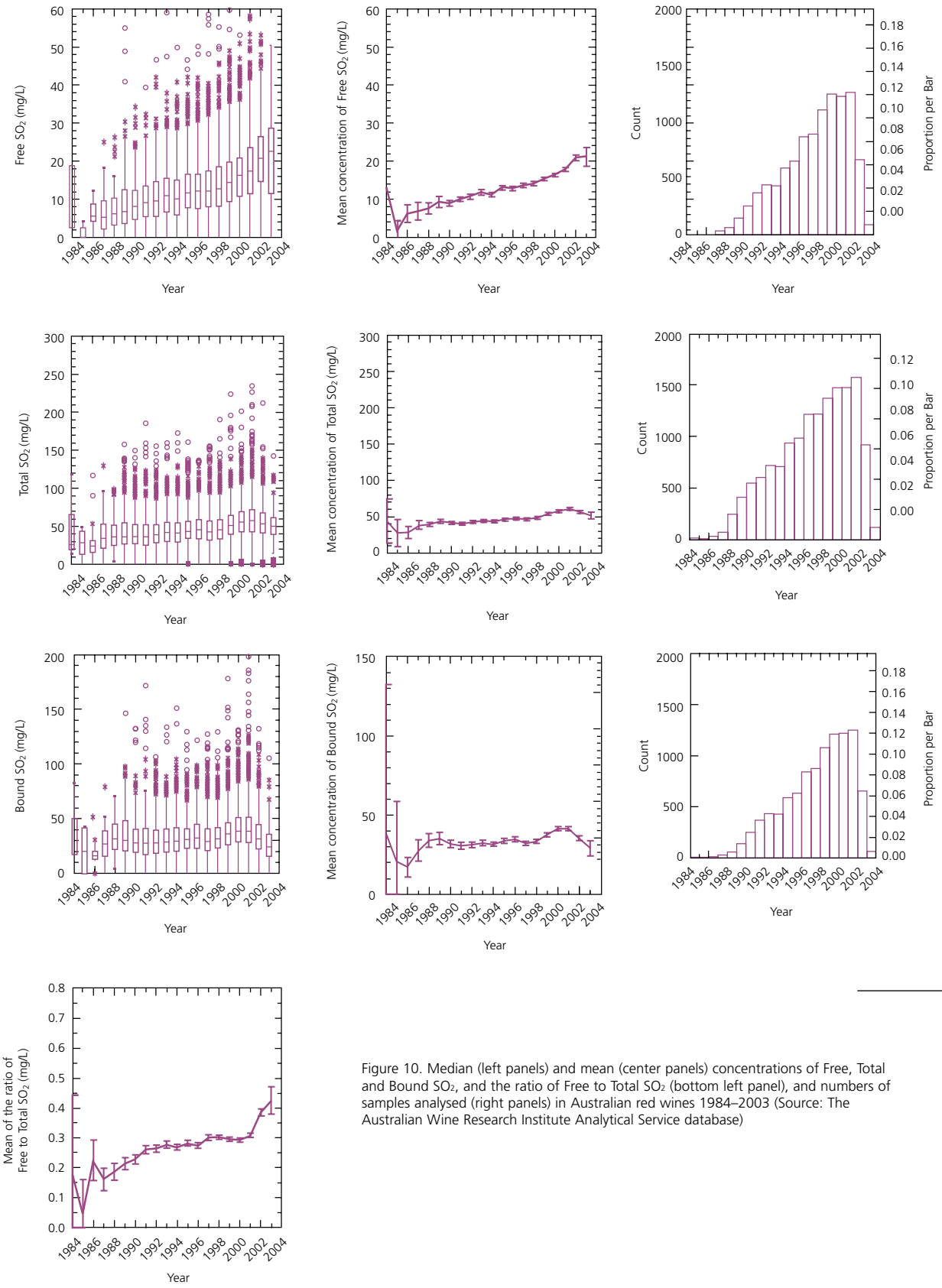
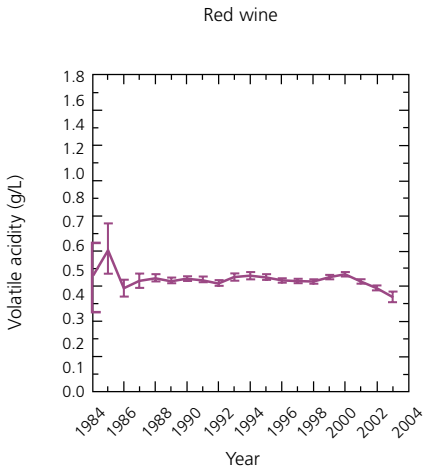


Figure 10. Median (left panels) and mean (center panels) concentrations of Free, Total and Bound SO_2, and the ratio of Free to Total SO_2 (bottom left panel), and numbers of samples analysed (right panels) in Australian red wines 1984–2003 (Source: The Australian Wine Research Institute Analytical Service database)



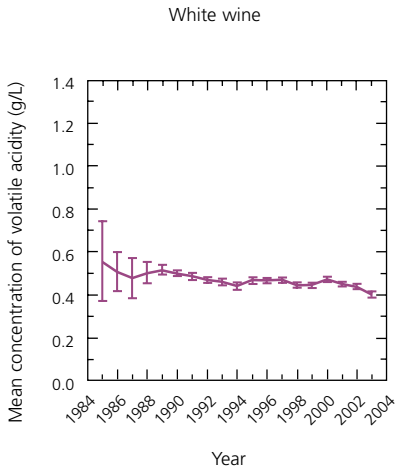


Figure 11. Mean concentration of volatile acidity (g/L) in Australian wine 1984–2003 (Source: The Australian Wine Research Institute Analytical Service database)

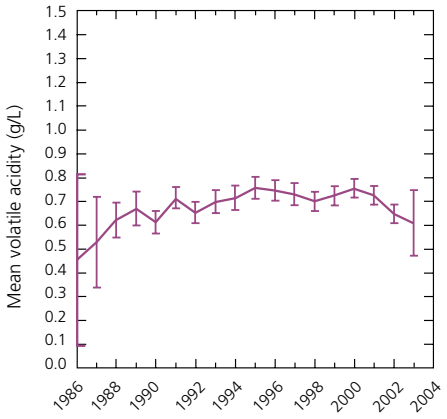


Figure 12. Mean concentration of volatile acidity (g/L) in Pinot Noir wine 1984–2003 (Source: The Australian Wine Research Institute Analytical Service database)

Viticultural practices: ensuring best practice in relation to the use of agrochemicals and other viticultural production; information and determination of the effect of selected viticultural practices on grape and wine composition

Staff: Dr Sally-Jean Bell, Kevin Pardon

Objectives:

Assess and disseminate information from a variety of sources where viticulture interacts with oenology.
Participate in viticultural research on winegrapes in relation to wine quality.

Progress

During 2003/2004 the Viticulturist responded to 430 enquiries. Eleven thousand copies of the AWRI's annual publication, *Agrochemicals registered for use in Australian viticulture 2003/2004* were produced and the information duplicated on the AWRI's website. The booklet was distributed with the *Australian Grapegrower and Winemaker, Technical Review* and in the *Research to Practice*™ IPM and Spray application manuals. The tables within the booklet were featured in *Australian Viticulture* and *The Grapevine Management Guide 2003/2004* (Somers et al., 2003). The Viticulturist and Jelka Software have developed an agrochemical database. This database is similar to the currently existing MRL database except that it contains all the information related to and presented in the publication, *Agrochemicals registered for use in Australian viticulture 2003/2004*. The ultimate aim is to provide a searchable database to replace the static retrieval of agrochemical information currently available from the AWRI's agrochemical website. A common spray diary format was developed in conjunction with industry for the 2004/2005 season and was placed on the AWRI website. Along with this format are explanations of the spray diary terminology and other relevant information. The 2003/2004 MRLs for Australian export markets were updated for the AWRI's website.

The Viticulturist participated in the AWRI Roadshow visit to Canberra, ACT and delivered presentations entitled 'The link between bentonite requirements and vineyard and winemaking practices' and 'Managing Botrytis in your vineyard' (refer Appendix 1). Lastly, abstracts of relevant articles for the six issues of *Technical Review* were prepared.

The Viticulturist participated in the following *Research to Practice*™ workshops: Winegrape Quality Management (Langhorne Creek and McLaren Vale) (Appendix 1). Two lectures on 'Herbicides in viticulture' and 'Agrochemical issues - selling quality wine' were presented to viticulture and oenology students at The University of Adelaide (Appendix 2). Two invited presentations on 'Spray diaries and new agrochemicals' and 'Agrochemical issues for grapegrowers and winemakers' were made at an Adelaide Hills Wine Region seminar day and the Barossa Valley Grapegrower Technical Group meeting, respectively (Appendix 1). The Viticulturist attended the 7th International Symposium on Grapevine Physiology and Biotechnology in Davis, California (21-25 June, 2004) and gave a presentation entitled 'A tale of two seasons - impact of viticultural practice on red grape phenolics' (Appendix 1).

Vineyard aspects of the research project 'The effect of plant water status and canopy management on the phenolic composition of Shiraz grapes and the resulting impact on red wine quality' has come to a close. Collection of 2003/2004 viticultural data was completed at the end of March. The 2003 wines underwent sensory analysis in June. In addition to data presented at the *7th International Symposium on Grapevine Physiology and Biotechnology* in June, a poster and a presentation of data from this trial were prepared for the 12AWITC poster session and 'Red Wine Phenolics' workshops respectively. The vastly differing climatic data over the past vintages necessitated the collection of an additional season's data for this trial. The vast amount of viticultural, grape compositional and wine sensory data are now being analysed in preparation for publication of several papers.

Quality Liaison Manager

Mark Gishen remains heavily involved in the collaborative research project evaluating the use of spectroscopy for the rapid determination of a number of compositional parameters in grapes, must, wine and grape spirit, and continues to take primary responsibility for the project as team leader. The details of this project are reported below. New collaborations have been established with the Department of Chemical Engineering at The University of Adelaide through co-supervision of PhD and Honours students' projects including: alternative tartrate stabilisation, optimisation of winery scheduling, and monitoring of fermentation.

The major output of the Institute's activities in the provision of advice on quality management techniques to industry remains the *From grapes to glass* program, which was published in August 1997 and enhanced with a simple HACCP module in 1999. Industry interest remains greatest in the HACCP (hazard analysis and critical control point) module - a simple program delivered in a one-day course that incorporates an HACCP-type food safety plan. This module was designed to satisfy the requirements of the national food hygiene regulations, and meet the needs of the smaller scale businesses in the wine industry. Only one course for the HACCP module was conducted throughout the year, adding another six to the growing list of companies having attended (now totalling 100). *The From grapes to glass* program provides a simple and relatively cheap program that uses a staged approach in the attainment of internationally recognised standards, starting from the Codex HACCP principles and leading to the full ISO 9000 quality management standard.

Mark Gishen takes primary responsibility for the internal quality management systems of the Analytical Service, overseeing management reviews, documentation, auditing, and corrective actions, and is the Authorised Representative in respect of its NATA accreditation. Validation reports prepared by the laboratory for several new methods were reviewed or approved and these included: the determination by HPLC of boscalid and pyraclostrobin, shikimic acid, salicylic acid, and resveratrol; determination by GC-MS of dimethomorph; the use of the FOSS WineScan for the determination of density, pH, TA, VA, and the concentrations of alcohol, glucose+fructose, acetic acid, malic acid and citric acid. The Analytical Service continues to participate and excel in both national and international proficiency testing programs for routine wine analysis and for agrochemical residue testing. The quality system of the Analytical Service has been reviewed and significantly upgraded in preparation for an audit of the system for compliance with the OECD Principles of Good Laboratory Practice that is scheduled in September 2004.

Mark Gishen is the Institute's representative on the Winemakers' Federation of Australia (WFA) working group known as the Legal Metrology Group. This group was formed in response to a proposal put by the National Standards Commission (now National Measurement Institute) and aims to develop a metrological control system for measurement instruments used by wineries for the receival of and payment for winegrapes. The group has prioritised the various areas to be addressed and has commenced a work program to progress its aims. Mark Gishen is also liaising with another of WFA's committees, the Packaging Committee, as part of the process of reviewing and updating the industry's *Code of Good Manufacturing Practice* in collaboration with Creina Stockley.

Rapid instrumental techniques (CRCV 1.4)

Staff: Dr Daniel Cozzolino, Wies Cynkar, Dr Bob Dambergs, Narelle D'Costa, Mark Gishen, Peter Godden, Les Janik, Elizabeth Waters

Research into rapid instrumental methods has continued to concentrate on pursuing the promising technique of near infrared (NIR) spectroscopy but with some expansion of the activities to include visible and infrared spectroscopies. This analytical approach has been shown from earlier work to be capable of providing very fast, low cost analyses of a range of parameters important to commercial wine production. Spectroscopic techniques offer the potential to simplify and reduce analytical times for a range of grape and wine analytes. It is this aspect, together with the ability to simultaneously measure several analytes, which was the impetus for developing NIR methods.

In response to the wine industry's need for rapid analytical methods for the determination of objective indicators of grape quality, a decision was made to concentrate efforts on development of an NIR method for determining colour, total soluble solids (TSS) and pH in red grapes, that would be suitable for practical industry use. Several reports and roadshow presentations by the project team have introduced the potential use of NIR for red grape quality assessment and a detailed article was published in the 2003 *Australian and New Zealand Grapegrower and Winemaker* Technical Issue (AWRI publication #730). Several large wine companies have now put this technology into practice.

As reported previously, it has been proposed that transfer of the technology to the industry might best be achieved through direct commercialisation activities, and this is being carried out under the responsibility of the Cooperative Research Centre for Viticulture's dedicated commercialisation company, CRCV Technologies Ltd, which continues to negotiate with several instrument manufacturers to this end. A considerable effort was made this year to clarify the commercial impacts arising from the existence of several patents related to this technology. Whilst there might be issues to address with respect to the current path of commercialisation, none of these are considered to be insurmountable.

The consolidation of calibrations developed for the analysis of grape berry colour, total soluble solids and pH by NIR scanning has continued, involving study of as wide a range of samples as possible. The continued cooperation of industry partners has meant that more than 3000 berry samples from the 1999, 2000, 2001, 2002, 2003 and 2004 seasons, and from a wide range of growing regions and red varieties (but predominantly Shiraz and Cabernet Sauvignon), have been analysed by the conventional laboratory method and scanned with a research grade NIR instrument located at the Institute. The berry samples were scanned with no preparation other than homogenisation using a high-speed laboratory homogeniser.

Sample storage and processing are important issues that require consideration when performing analysis on grape samples. Recently the effects of overnight freezing, time in frozen storage and method of homogenisation of red grapes on the determination of total anthocyanins, TSS and pH was determined (Table 7). Three different commercial homogenisers were used for sample preparation. Neither the homogeniser type, nor the sample state (fresh or overnight freezing) had significant effects on the determination of total anthocyanins and TSS by the laboratory methods. However, we observed an effect of overnight freezing on total anthocyanins in samples of high concentration. The strongest effects of both homogeniser type and the state of the sample were observed with pH measurement, however, these statistically significant differences might not be very important from the practical point of view. Phenolic extraction was also affected by the type of homogeniser used, but not by overnight freezing. Frozen storage for more than three months affected all the measured quality parameters.

This study brings to light evidence for the need and benefits of a strict, detailed laboratory protocol outlining the conditions of sample processing and storage for grape compositional analysis. As a result, the team has prepared a technical note outlining the laboratory procedure for determination of the concentration of total anthocyanins in red grapes that is now available to industry and will hopefully become the industry's 'standard' method through endorsement by the Winemakers' Federation of Australia's legal metrology group. Furthermore, an interlaboratory proficiency test for the measurement of red grape colour conducted this year between four laboratories performing this assay and showed good agreement.

Mark Gishen

Audrey Lim, Hardy Wine Company

Table 7. A summary of the effects of homogeniser type, freezing and storage time on grape total anthocyanins, TSS, pH and total phenolics (* = p<0.05, ** = p<0.01, NS= not significant)

	Total anthocyanins	TSS	pH	Total phenolics
Homogeniser	NS	NS	**	**
Sample state	NS	NS	**	NS
Homogeniser x Sample state	NS	NS	**	NS
Storage : 1 month	NS	NS	**	NS
3 months	NS	NS	**	NS
6 months	*	*	*	*
12 months	*	*	*	*

Although samples can be analysed either fresh or after storage in a frozen state, the effect of freezing and frozen storage on the VIS-NIR spectra has not been reported before. Calibration equations for colour were developed using either fresh or frozen samples alone, respectively. Preliminary results show that the standard error of prediction (SEP) for total anthocyanins in all samples was slightly increased by freezing and storage when compared with calibrations developed on fresh or frozen samples alone. These results suggest that it might be possible to use NIR calibrations developed on fresh or frozen samples alone to measure the concentration of total anthocyanins in either fresh or frozen samples after appropriate slope and bias correction. Furthermore, in a collaboration with a PhD student from James Cook University, these data are being further investigated using alternative multivariate analysis techniques to evaluate the effect of freezing and frozen storage on the NIR spectra.

The project has continued to investigate the effect of sample presentation in NIR analysis, comparing homogenised with whole red grapes. Initial work by the project team on the prediction of quality parameters in red grapes using NIR began with the scanning of homogenized grape samples using a research grade laboratory NIR spectrometer, the FOSS *NIRSystems 6500*. However, the recent availability of faster scanning Vis/NIR spectrometers with alternative optical configurations has provided the possibility of presenting the grape samples to the instrument without the need for homogenisation. Simplifying the sample presentation for NIR prediction of colour, TSS and pH could dramatically increase sample throughput. Furthermore, this mode of presentation might even offer the potential of scanning whole, intact, single berries. Preliminary investigations conducted in collaboration with a major winery for whole grape berry presentation were promising, indicating that NIR might have potential for use in the streaming of fruit on receival at the weighbridge or for in-field analysis. These studies have been performed using several compact, fast, diode-array spectrometers including the Zeiss C*orona*, and has included investigation of the feasibility of scanning samples directly in plastic jars that are commonly used when collecting grape samples.

In a similar approach to the investigation of sample presentation, trials have commenced in the use of NIR for the non-destructive analysis of bottled wine *in situ* using several instruments available to the project team. As well as being able to measure, for example, the relative degree of wine oxidation, as previously demonstrated by the AWRI Oxygen and Wine team, it might be possible to perform some more routine analyses, such as alcohol content for on-line monitoring of packaging for quality control purposes.

Since the 2001 vintage, the NIR team has been collaborating with the Tannin Project team in their investigations of process scale fermentations. The main objective was to examine the potential of NIR spectroscopy to predict the concentration, and monitor the extraction and evolution, of phenolic compounds during red wine fermentation. If successful, NIR spectroscopy techniques might offer potential as a rapid, low cost and non-invasive tool for monitoring the fermentation process. The results from the 2001, 2002 and 2003 winemaking trials conducted at the Hickinbotham Roseworthy Wine Science Laboratory showed that NIR spectroscopy could predict the concentration of malvidin-3-glucoside (the major anthocyanin in grapes), pigmented polymers and tannins in Cabernet Sauvignon and Shiraz wines during fermentation. However, the specificity of the calibrations developed must be confirmed, as there are many simultaneous changes occurring during fermentation. A more robust application of this technology that might be more immediately applicable could be to qualitatively monitor fermentations directly using NIR spectroscopy without the need for any compositional analysis. These findings were presented to an international conference in analytical chemistry in wine held in Aveiro, Portugal in July 2003 (*InVino Analytica Scientia*) and subsequently published in a peer reviewed journal (AWRI publication #769).

The authentication or identification of food is emerging as one of the most important areas in order to comply with food industry regulations and standards and to address consumers' concerns. Spectroscopic techniques such as NIR and Fourier transform infrared (FT-IR) have proved to be a suitable tool for authentication with demonstrated applications including discrimination among coffee varieties, honey adulteration, and beef contamination and adulteration. As reported in 2003, the project team has demonstrated the use of NIR spectroscopy to successfully discriminate between commercial samples of two white wine varieties (unwooded Chardonnay and Riesling), sourced from a PhD project on identifying white wine flavour compounds, conducted by Heather Smyth. This finding was published in an international peer reviewed journal during the reporting year (AWRI publication #750). Further work is continuing in order to understand the influence of the wine matrix on the classification specificity and it is proposed that red wines will also be investigated using this technique.

The project team continues to collaborate with several research teams in investigating further applications of NIR spectroscopy. These include the discrimination and identification of yeast strains (AWRI Molecular Biology), the detection of moulds in grapes (The University of Adelaide), the potential to monitor compositional changes during wine fermentation (AWRI Tannin and Microbiology) and oxidation in white wines (AWRI Oxygen and Wine).

The project team has continued with investigations on the application of the MIR (mid-infrared) technique, using a rapid and simple sample presentation method called attenuated total reflectance (ATR). When using transmission cells, extreme absorption of the signal can result from strong MIR signals, while short path-length cells are susceptible to blockages from suspended particles (i.e. grape homogenates). With the ATR method, the sample grape homogenate or white juice is simply spread onto an ATR crystal surface and scanned. Prediction calibrations developed for total anthocyanins (colour), pH, total soluble solids (TSS) and electric conductivity (EC) in red grape homogenates, and ammonia, a-amino and yeast assimilable nitrogen (YAN) in white grape juice, were compared with those using a FOSS *NIRSystems 6500* spectrometer. Better calibrations were obtained with ATR for pH, TSS and EC than for NIR, but not for total anthocyanins. The prediction of ammonia, a-amino and YAN nitrogen in white juice was slightly better than that using NIR. It was concluded that MIR-ATR is worthy of further investigation as a method for grape and juice analysis. It can be used in the same manner for both white juice and red grape homogenates, as well as for other grape-derived products, with very little sample preparation thus providing several advantages as a simple cost-effective technique.

In late 2002, the AWRI Analytical Service commenced evaluation of an FTIR-based spectrometer (FOSS *WineScan*) for assessment of its capability in the rapid routine analysis of wines. Wine samples, and corresponding analytical data, were used for re-calibration of the instrument, and the resulting calibrations used to enhance the 'global' calibrations provided by FOSS with the instrument. Although the FOSS calibrations allow analysis of up to 18 wine properties, the current study is assessing only nine of these *viz.* alcohol, pH, density, glucose/fructose, total acidity, volatile acids, acetic acid, citric acid and malic acid. Preliminary results have been very encouraging and were presented in Perth at the April 2004 FOSS Directions conference. A full operations manual for the *WineScan*, setting out the instrument maintenance, methodology and calibrations has been written and will be updated once all the required validations have been conducted. This is an essential pre-requisite for obtaining NATA accreditation for use of the *WineScan* for routine operation in the AWRI Analytical Service laboratory.

The team also maintains a strong commitment to the training and teaching of the principles and use of multivariate analysis techniques (chemometrics) to other team members as well as for industrial and external research partners. In-house workshops and training commenced in March 2004 and is being extended to industry through workshops to be conducted as part of the 12th Australian Wine Industry Technical Conference to be held in Melbourne in July 2004. One example of an external collaboration is that with a GWRDC-funded RITA project that is being conducted in Tasmania on benchmarking Pinot Noir production, where team members have been providing advice and assistance in the use of multivariate techniques to analyse large amounts of complex data from the analysis of grapevine sap, vineyard soil and harvested grapes. Another example of an internal collaboration is the team's contribution in the use of chemometrics to relate sensory assessment of wine to visible wavelength spectral data of samples from the AWRI Oxygen and Wine project, which revealed that the critical wavelengths differ from those traditionally used to compare relative oxidation in white wines (Table 8). This illustrates the value of the multivariate data analysis approach to complex problems.

It has been proposed that an 'IR spectroscopy users group' be established within the industry during the latter half of 2004 bringing together the AWRI team, and other researchers and practitioners involved in the development and application of IR spectroscopy in the research and industrial communities. This will be an important step in further strengthening the links between the AWRI and industry in the application of the IR technology in the Australian wine industry.

Technical, human and environmental health and management issues impacting on the Australian wine industry–research, repository, interpretation and communication of information

Staff: Creina Stockley, Professor Peter Høj

Ms Creina Stockley, a clinical pharmacologist, has assumed the position of Health and Regulatory Information Manager since 1991. One of the activities of the AWRI has been to provide health, regulatory and technical advice and assistance to the Australian wine industry, through the Health and Regulatory Information Manager and the Industry Services team; from 1 July 2003, environmental information was added to the position description. From 1 July 2003 until 30 June 2004, 272 independent information requests were received by the Health and Regulatory Information Manager from industry (149), the general public (111) and government (12).

Industry committee membership

During the year, support to the industry has been derived from the Director's membership on the Australian Wine and Brandy Corporation's (AWBC) Compliance Committee, the AWBC/WFA Wine Industry Technical Advisory Committee, the Management Committee for Viticultural Publishing (ASVO) and the Royal Adelaide Horticultural Society's Wine Show Committee. One of the important aspects of the AWRI's support of the Australian wine industry is its pivotal role in facilitating the triennial Australian Wine Industry Technical Conference (AWITC), in conjunction with the Australian Society of Viticulture and Oenology. Professor Peter Høj is the Chair of the 12th AWITC.

During the year, the Health and Regulatory Information Manager was a member of the following industry committees: AWBC/WFA Wine Industry Technical Advisory Committee (as Technical Liaison Officer); the AWBC Legislation Review Committee; the Wine Industry National Environment Committee; the Eco-efficiency Working Group (Sub-committee of SAWIA's Environment Committee); and was the DAFF nominated Australian delegate for the Organisation International de la Vigne et du Vin (OIV) Nutrition and Health Subcommission. She is also a member of the National Drug and Alcohol Research Centre's Young People and Alcohol Project Advisory Group on behalf of WFA.

Table 8. Prediction of 'visual brown' score with spectral data: a comparison of Partial least squares (PLS) regression using absorbance over 400–800 nm and classical linear regression using absorbance at 420 nm or 500 nm. PLS regression with full spectral data and linear regression using absorbance at 500 nm both outperformed absorbance at 420 nm in predicting the 'visual brown' rating.

Variety	Regression Method	Wavelength(nm)	R^2	Standard error*
Chardonnay	partial least squares	400-800	0.81	0.61
Chardonnay	linear	500	0.82	0.60
Chardonnay	linear	420	0.48	1.03
Riesling	partial least squares	400-800	0.76	0.43
Riesling	linear	500	0.75	0.44
Riesling	linear	420	0.42	0.68

* units refer to browning scale values used in visual assessments (0 to 9)

Technical and regulatory issues

During 2003/2004, technical and regulatory information and/or issues that have been reviewed, and material prepared includes: a review of the 20th Australian Total Diet Survey that included 21 Australian white wines; dossiers on the 18 additives and processing aids used in Australian winemaking, which are not approved for use in Japan and a dossier on Japanese maximum residue limits (MRLs) that are inconsistent with Australian MRLs; responses to Food Standards Australia New Zealand applications A482-plant proteins as wine processing aids, A503-collagen: processing aid for winemaking and A463–copper citrate: processing aid for winemaking, A474-Winemaking-Draft Assessment, P283 Winemaking-Draft Assessment, and A480-Mandatory declaration of the presence of allergenic substances in food at the behest, and on behalf, of WITAC. Amongst several reports, a report on the *Analysis of Australian wine for bromide and chloride* was prepared for the AWBC, and a report on *The content of minerals in Australian soils, water, grapes and wines* was also prepared for the Department of Agriculture, Fisheries and Forestry (DAFF) in order to facilitate the export of Australian wine into the European Union.

This technical and regulatory support to the Australian wine industry is ongoing as issues are regularly raised by industry or government, both in Australia and internationally, and often span several years. The Health and Regulatory Information Manager also coordinated Wine Industry Technical Advisory Committee working groups on wine component separation and blending and the AWBC Label Integrity Program, and on gene technology, participating in the redrafting of the Australian wine industry's position statement on gene technology.

In addition, a guide *Winemaking provisions for wine in Australia and its export markets*, has been made available on the AWRI's website; this complements the Institute's other guide *Analytical specifications for export of Australian wine*, which was published in 1995 and 2001.

The Director and Health and Regulatory Information Manager also coordinate Course 3005WT *Grape industry practice, policy and communication* for the School of Agriculture and Wine at The University of Adelaide. In its tenth year, 37 students enrolled in the Course, which exposes students to organisational, commercial, environmental, political, societal and technical issues relating to the wine industry's operating environment.

Health and nutrition issues

A database of research on the beneficial and detrimental health effects of alcohol and in particular, wine, has been established on the internal database of the John Fornachon Memorial Library. This is facilitated by the subscription to relevant medical and scientific journals, and by the formal and informal exchange of information between complementary organisations, both national and international. The journals have been regularly scanned, the database of research on the health effects of wine has been added to and articles have been prepared for inclusion in the AWRI's publication, *Technical Review* and for other Australian wine industry and international alcohol industry newsletters. Articles and other material have also been prepared for the electronic and print media. For example, three articles have been prepared for the bimonthly international publication, *AIM-Alcohol in moderation*, and two articles for the monthly *Australian and New Zealand Grapegrower and Winemaker*, in addition to one article for their annual technical issue (refer Appendix 4).

The Health and Regulatory Information Manager has also co-authored with Dr Michael Fenech of CSIRO Health Sciences and Nutrition two papers entitled '*Wine - derived phenolic compounds are protective against radiation - induced DNA damage-results of a comparative in vivo intervention with alcohol, dealcoholised red and whole red wine*' and '*Reduction of damage to LDL from oxidative free radicals by the regular and moderate consumption of wine*' for refereed journals; these papers ensue from the GWRDC-funded project *CSN97/2 Reduction of damage to LDL and DNA from oxidative free radicals by the regular and moderate consumption of wine*, which the Health and Regulatory Information Manager coordinated. Another paper entitled '*Recommendations on alcohol in moderation: an international comparison*', was also co-authored with Dr Elisabeth Holmgren (formerly of the US Wine Institute) (refer Appendix 4). The latter was also an oral presentation at the *Fifteenth international conference on the reduction of drug related harm* in Melbourne on 23 April 2004. Two oral presentations were also made at the *Organisation International de la Vigne et du Vin (OIV) 2004 Congress and General Assembly* in Vienna on 5 and 7 July 2004 (refer Appendix 1).

Submissions prepared on behalf of the Australian wine industry include draft resolutions *OENO/ASP/99/129/Step 5 - Health warning, OENO/SCVNS/00/150/Step 5 OIV/WHO relations* and *OENO/ASP/01/190/Step 3 - Wine consumption and young adults* of the Nutrition and Health Sub-commission. The Health and Regulatory Information Manager was re-elected as Vice President of the OIV Nutrition and Wine Expert Group in March 2004.

Recently reviewed/updated are three external course booklets–Topic 3: The toxicological aspects of winemaking and wine, Topic 4: The pharmacological and physiological effects of alcohol and wine, and Topic 5: The beneficial effects and issues of alcohol and wine in moderation–for the Courses 2001WT and 7030WT *Wine and society* in the Bachelor of Wine Marketing at The University of Adelaide, to complement the two lectures and tutorial presented on 15 March 2004 (refer Appendix 2).

Environmental issues

Complementary to the AWRI's databases on health effects of alcohol, a database on environmental issues impacting on the wine industry has been instigated by the Health and Regulatory Information Manager at the behest of the Wine Industry National Environment Committee (WINEC). At present, the JFML Librarian has sourced the availability and cost of certain environmental journals and magazines recommended by WINEC for inclusion on the database and for abstracting in *Technical Review*, and organised a keyword specific alert service. Bibliographic details of journal articles already included in *Technical Review* have been entered onto a database available via the AWRI website. Bibliographic details of additional journal articles sourced from the existing password protected database are also being loaded onto the database. This environmental database is accessible to the general public and is accessible without a username and password. Since its launch in May 2004, the database has received 123 unique research requests, highlighting the relevance of the service.

Project coordination

Through Creina Stockley, the AWRI has played a coordinating and a participating role in a GWRDC–funded research project on a medical and technical aspect of wine consumption entitled (ADF02/01) *The identification and measurement of potential allergens in wine.*

The identification and measurement of potential allergens in wine

A collaborative project between the Department of Asthma, Immunology and Respiratory Medicine, The Alfred Hospital/ Monash University and The Australian Wine Research Institute commenced on 1 July 2002. The project is funded by the GWRDC through The Alfred Hospital and Creina Stockley is the project supervisor and Professor Robyn O'Hehir is the principal investigator. The project was developed in response to labelling requirements for potential allergens introduced on 14 December 2002 by Food Standards Australia New Zealand, where use of the proteinaceous processing aids casein, egg white, isinglass, milk and milk products in winemaking requires declaration of use on the label of each wine and wine product.

The project comprises two sections. The first section is the determination of detectable allergenic proteins in wine by establishing an *in vitro* functional assay to determine if wines fined with the proteinacous processing aids are capable of activating blood basophils from subjects with known sensitivity to milk, egg, fish, or nuts, and by establishing a panel of sensitive and specific antigen-capture ELISA to detect and measure the processing aids casein and potassium caseinate, egg white, isinglass and milk in final bottled wine. The second section is a double-blind

placebo-controlled clinical study of fined wines in subjects with confirmed sensitivity to eggs, fish, milk or nuts.

A panel of 100 commercially available Australian wines was collected, representing 20 wines for each of the five proteinaceous processing aids. An ovalbumin enzyme-linked immunosorbant assay (ELISA) using commercial monoclonal and polyclonal antibodies has already been adapted and optimised to detect ovalbumin in egg white-fined wine, where the lower limit of detection is 1 ng/mL. Monoclonal and polyclonal antibodies are not commercially available for the other processing aid proteins. A panel of relevant monoclonal and polyclonal antibodies for ovomucoid, casein, ß-lactoglobulin, and isinglass is, therefore, currently being generated.

Further investigation of potentially detectable allergenic proteins in the wines has been obtained from *in vitro* basophil activation tests (BAT) using flow cytometry. BAT have been developed and optimised for use with wine and the processing aids. Challenge subjects with sensitivity to the relevant food allergens were recruited to provide a blood sample, such that analysis of the 100 wines using BAT occurred in tandem with the clinical challenges of subjects.

Thirty-seven challenge subjects were recruited, clinically characterised, and compared to a group of 11 healthy subjects without a history of food or wine reactions and in the absence of specific IgE to any of the study allergens. As milk allergy is rare in adults, only one milk allergic subject could be identified for this study. The subjects were challenged with the relevant fined wine and with a control unfined wine on separate occasions and monitored clinically for a period of two hours after challenge. A diary card was completed over the six days following wine consumption. Data are currently being analysed and will also be correlated with the data obtained from the *in vitro* BAT studies conducted, with a target release date by end 2004.

Provision of technical information: facilitating innovation through customised information delivery to industry practitioners and researchers

Staff: Rae Blair, Catherine Daniel, Ingrid Oats, Melissa Francis

The John Fornachon Memorial Library holds the largest collection of wine technical literature in Australia. The Library's principal responsibility is to provide technical information to the Australian wine industry and to the researchers of the AWRI.
The Library is also used extensively by other groups such as students, institutional researchers, government bodies and private companies (see Table 9 for requests for information serviced during the year).

Information and document delivery services

The Library has excellent access to international databases, particularly in the fields of science, technology and medicine. If requested, the Librarian, Catherine Daniel, will carry out online searches on commercial databases on any appropriate topic (on a fee-for-service basis). Alternatively, Library staff can provide, free of charge, a report of relevant articles indexed on the Library's in-house databases.

The Library's traditional focus on being a centralised 'one-on-one' service provider has shifted to meet the demands for 24 hour, 7 day per week access to industry-specific trade and research literature that is of interest and relevance to the Library's key clientele. The Library, whilst still providing the essential personal service, has focussed on expanding and making more valuable its web-accessible database. While the Library continues to enjoy strong support for its traditional suite of services, the Library's electronic resources, particularly the web-accessible database, are being used increasingly by industry as indexes to wine and grape literature already available in personal and company collections. An increasing proportion of the Library's resources are, therefore, devoted to the maintenance and development of such systems (number of records shown in Table 10). Table 9 provides information on the number of enquiries serviced by Library staff during the year. Whilst the number of information requests received by the Library has maintained a steady level comparative with last year, the number of articles and AWRI publications forwarded showed a slight decrease. We believe that this trend is indicative of a larger number of traditional Library customers using the web-accessible database for this purpose. We have been able to monitor the usage of our web-accessible database for the last two months of 2003/2004, and intend to track usage against the level of personal service requested over the next year.

Document delivery

The Library can supply either books or photocopies from its collection or obtain such items for wine industry clients through the interlibrary system. Patents or standards can also be ordered. Electronic ordering and delivery services mean that most interlibrary requests are fulfilled within five days. Charges apply for the supply of some items.

Specialised information services

The Library staff continue to be actively involved in the production of specialised information products for the benefit of the wine industry, such as the annual and web-based editions of the *Agrochemicals registered for use in Australian viticulture*, the bimonthly print and electronic editions of T*echnical Review*, and several in-house technical information databases.

Library collection

A total of 101 monographs and 31 conference proceedings and over 4,700 new records were added to the library databases during the year.

The Library subscribes to 54 journals and receives approximately 70 annual reports, journals and newsletters through exchange and donation. The Library also maintains a collection of over 23,000 reprints.

Table 9. Summary of information requests during 2003/2004

	Wine industry		Staff		Other[6]		Total		% change
	2004	2003	2004	2003	2004	2003	2004	2003	
Information requests	1151	1081	1035	943	1602	1584	3788	3608	5%
Interlibrary loans:									
* requests sent[1]	53	29	514	505			567	534	6%
* requests received[2]							83	53	57%
Technical Review requests[3]							189	193	(2%)
Technical Review articles forwarded[4]							915	970	(6%)
Articles forwarded[5]							869	1424	(39%)
Number of AWRI publications forwarded							627	725	(14%)
Articles photocopied in JFML							3899	3874	0.6%

1 Staff at the JFML sent a request to another library for an article.
2 Requests received by the JFML from other libraries for articles from our collection.
3 Number of requests received for articles published in the *Technical Review.*
4 Number of articles forwarded (usually more than one article is requested).
5 Number of articles forwarded, excluding staff publications.
6 90% of 'other' requests come from students and Government sources.

Library databases

A single search screen provides access to the Library's collection of over 45,000 books, conference proceedings, scientific, technical and medical reprint articles which are indexed on the Library's database catalogue; the bibliographic details of the Library's collection of the European Union wine legislation and details of the library's journal holdings are maintained on separate in-house databases.

The Librarian provides reports, either on particular subjects or authors, listing the records retrieved from any of the Library's in-house databases. A summary of the size of the Library's catalogue and information databases is given in Table 10.

The Library provides access to its databases via the internet to Australian winemakers and grapegrowers paying the *Wine Grapes Levy* or the *Grape Research Levy*. The restriction in access is enforced to comply with copyright approvals obtained from the various publishers whose journals are the source of the abstracts that are accessible via the database. Library staff members continue to edit database records to post onto the Library's web database, on an ongoing basis.

Agrochemicals Grid

As reported elsewhere in this Annual Report, Dr Sally Bell and Catherine Daniel prepared the fourteenth edition of the Agrochemicals registered for use in Australian viticulture. All levy payers receive a printed copy of the revised edition automatically, and the web-based edition (http://awri.com.au/agrochemicals/) is updated on a regular basis.

Technical Review

Technical Review is received by all *Wine Grapes* and *Grape Research Levy* paying organisations in Australia and, through subscription, by government and other organisations and individuals, both in Australia and overseas. *Technical Review* provides progress reports to the industry on the AWRI's research as well as updates on relevant conferences, regulatory amendments and medical issues. *Technical Review's* 'Current Literature' section provides citation details and abstracts of recently published technical and scientific articles. Recipients of *Technical Review* may order articles featured in the 'Current Literature' section via a request form available within each issue. Restricted password access to *Technical Review* is also available on the AWRI's website (http://awri.com.au/technical_review/latest_issue/). Dr Barbara Hardy AO and her sons continue to support the publication of *Technical Review* through regular generous financial contribution to the Thomas Walter Hardy Memorial Trust, and their ongoing support is gratefully acknowledged.

The collection of the 2003/2004 issues of *Technical Review* has been made available via a CD ROM and distributed free of charge to *Wine Grapes* and *Grape Research* Levy paying organisations. A simple search mechanism within the CD ROM facilitates fast access to technical notes, current literature abstracts and other matters of interest published throughout the year within the six issues.

Table 10. Number of records on the Library's catalogue, information and web-accessible databases

Library catalogue databases	
AWRI_Database: Books, conference proceedings, Theses, scientific and medical papers	45,833
JOURNALS: journals, newsletters, statistics and annual reports	391
Library information databases	
REGS: European Community wine legislation	393
ISYS – full text retrieval database covering	
United States of America *Federal Register*	877
Web accessible database (with searchable abstracts)	29,509

Email service

The Email Advice and Information on Technical Issues Bulletin service continues to be a fast and cost-efficient way of disseminating important technical information to interested members of the Australian wine industry. There are 908 email addresses recorded to receive the email bulletins, and interested members of the Australian wine industry should submit their email address (to Rae.Blair@awri.com.au) should they wish to receive the email bulletins. Eighteen email bulletins were issued during the year and are shown in Table 11.

The John Fornachon Memorial Library Endowment Fund

The AWRI acts as the Trustee of this fund, which was established in 1969 by donations from winemakers and friends of the late John Fornachon, the first Director of Research of the Institute. The Library is funded by an annual grant from the Grape and Wine Research and Development Corporation, together with the income generated from investment of the Endowment Fund.

Table 11. Email bulletins sent during 2003/2004

Date	Bulletin topic
3/7/03	AWRI Roadshow regions for 2003
9/9/03	AWRI Roadshow program for NSW
4/11/03	Benomyl registration
6/11/03	Cabrio registered
17/11/03	AWRI Library closure over Christmas/Information services at AWRI
20/11/03	Insecticide Tokuthion is no longer registered for use on winegrapes
21/11/03	*Technical Review* issue 147 (December 2003) available on website, plus copies of 2002-2003 issues of *Technical Review* available on CD
27/11/03	AWRI Roadshows in WA 'Trouble free winemaking: *the identification, management and avoidance of wine instabilities*' Bunbury (Dec 2003), Manjimup (Jan 2004)
28/11/03	Industry aroma and flavour workshop, 10 December 2003
5/1/04	'Wine instabilities' workshop at Manjimup WA (places still available)
20/1/04	Maintaining label integrity - options for varietal testing of juices
3/2/04	*Technical Review* issue 148 (February 2004) available on website.
5/3/04	GWRDC support for students and regional representatives to attend the 12AWITC
23/3/04	Early registration for the 12th AWITC closing 24/3
26/3/04	April 2004 *Technical Review* available on line
26/3/04	Four 12AWITC workshops sold out
7/5/04	Agrochemical update on Benomyl registration for 2004
24/5/04	150th issue of *Technical Review* available on line

Acknowledgements

The AWRI wishes to thank all individuals and companies who contribute to the collection through donations or exchange agreements. The support of the following persons and organisations that have donated books or journals is acknowledged:

Australian Bureau of Agriculture and Research Economics, Australian Dried Fruits Corporation, Australian Wine and Brandy Corporation, Australian Wine and Brandy Producers' Association, Commonwealth Scientific and Industrial Research Organisation, K.F. Pocock, Dr B.C. Rankine, Viticultural Publishing Inc., Winemakers' Federation of Australia Incorporated, Petaluma Australia.

Communication and Publicity Manager

The Communication and Publicity Manager's role is a part-time position for Rae Blair, who is also the Personal Assistant to the Director and the Conference Manager of the Australian Wine Industry Technical Conference (details of her other activities can be found under the report for *Provision of Technical Information*).

As Communication and Publicity Manager, she is responsible for ensuring that industry and stakeholder groups receive a clear understanding of the positioning (value) of the AWRI. This positioning is developed in line with the AWRI's mission statement and business plan objectives. Part of her role is to coordinate the AWRI's printed material and other non-technical communications, and to act as a conduit for media. The Communication and Publicity Manager is also responsible for the performance and output of the John Fornachon Memorial Library. The report of the activities of the Library can be found elsewhere in this Report.

AWRI staff members handled 59 approaches from international and national media during the year. Increasingly, the AWRI is called upon to be the technical mouthpiece for the Australian wine industry. Details of these approaches can be found in Appendix 5.

Details of some of the activities undertaken during the year are shown below. Due to the demands of planning the Twelfth Australian Wine Industry Technical Conference to be held in Melbourne 24-29 July 2004, the great majority of Rae Blair's time, during 2004, was directed to meeting her commitments as the Conference Manager and Treasurer of the AWITC Inc.

> Assisted the coordination of the Southcorp Winemakers' Seminar held at the AWRI and The University of Adelaide's Sensory laboratory (July 2003) - ~ 70 winemakers over three days.

> Coordinated the AWRI's sponsorship of the Royal Adelaide Wine Show (*Best Riesling of Show Trophy*, by the Analytical Service; and *Sponsorship of two Associate Judges who have participated in the Advanced Wine Assessment Course* – sponsored by the Advanced Wine Assessment Course).

> Liaising with our legal advisors, and then writing, producing and distributing the AWRI's *Privacy Policy.* The AWRI has appointed an anonymous Privacy Officer, and this staff member's email address has been given an alias of 'Privacy@awri.com.au'. The policy has also been placed onto the AWRI's website.

> Editorial assistance with the AWRI's regular 'column' in the *Australian and New Zealand Wine Industry Journal*.

> High proportion of time working with the Director of Research in developing the Master Plan for the physical infrastructure of the AWRI, and also participating on the Project Control Group for the Provisor building.

> Assisted the coordination of the Orlando Wyndham R&D seminar at the AWRI (14-16 October 2003) (15 viticulturists over three days).

> The Director and the Communication and Publicity Manager provided comments on a press release issued from the CSIRO regarding the *Wine Industry Survey: grape and wine aroma and flavour, long term plan*. The results of the survey and subsequent workshop will inform research to be undertaken collaboratively between the AWRI, CSIRO, The University of Adelaide and Provisor – funded by the GWRDC, in the areas of wine and grape aroma and flavour.

The survey was distributed in the *Australian and New Zealand Wine Industry Journal* and *Australian Viticulture* (7,500 surveys), and an email was distributed to about 1,400 addresses with a link to the electronic copy of the survey. Industry associations including regional grapegrowing associations were also be emailed.

> Attended a Waite Campus Communication Manager's meeting on 16 December 2003. The purpose of the meeting is for the Communication Managers of the co-located partners to recommend to the Waite Campus Executive Committee a plan for the promotion and communication of benefits of the Waite Campus. In response to an action item from the meeting, the Communication and Publicity Manager drafted up a creative brief for the development of: a) a Waite Precinct logo; and b) a folder and similarly designed insert sheets. This brief is currently with two other co-located partners for comment, before it can be provided to two creative agencies as a basis for them to provide a competitive quote for the work.

> Working with our graphic design agency, finessed the AWRI logo.

> Assisted the Manager – Analytical Service with developing the copy for their new suite of magazine advertisements.

> A new corporate leaflet was developed and printed, and will be distributed at the Twelfth Australian Wine Industry Technical Conference.

> Coordinated an exhibition booth at the Twelfth Australian Wine Industry Technical Conference on behalf of the John Fornachon Memorial Library and the Australian Wine and Brandy Corporation.

> Coordinated the production of the 2003/2004 annual *Technical Review* CD.

> With the Library staff, authored three posters for display at the Twelfth Australian Wine Industry Technical Conference (Melbourne July 2004).

> Managing enquiries from the AWRI website: with the AWRI's website being more widely accessed, the Communication and Publicity Manager receives regular requests for information (as her email address is used as the AWRI's 'general enquiries' email address). Many requests for information are simply forwarded to the relevant AWRI staff member, however, there are many enquiries that are handled directly.

The AWRI Analytical Service

Foreground: Peter Eichinger
Back row, left to right: Matthew Cream, David Boehm, Slavko Bekavac, Daniel Tynan, Anthony Marafioti
Front row: Carol Sigston, Jelena Jovanovic, Danielle Leedham, Maria Mills, Heather Brooks, Sandra Lloyd-Davies, Stella Kassara, Randell Taylor

Analytical Service

Staff: Dr Peter Eichinger, Greg Ruediger, Matthew Cream, Randell Taylor, Sandra Lloyd-Davies, David Boehm, Danielle Leedham, Maria Mills, Heather Brooks, Belinda Bramley, Stella Kassara (part time), Carol Sigston, Slavko Bakavac, Daniel Tynan, Steve Smith (external contract), Anthony Marafioti (external contract) and Jelena Jovanovic (part time)

This has been an extremely busy year, due to a steady high volume of work being processed by the Analytical Service. Unlike previous years, we have not had to deal with any major unexpected problems, resulting from either adverse seasonal rains or fires causing damage to the vintage. However, increasingly, we have been servicing clients who are keen to ensure the integrity of their products and the absence of unintended contaminants.

This year's challenges have arisen through the continued high growth in the number and complexity of analyses being undertaken, the loss of a number of experienced staff members, equipment failures and resourcing new and improved services. In spite of these difficulties we have, considering the complexity of the tasks at hand, managed to maintain short turnround times. Overall, the Analytical Service conducted approximately 56,200 individual tests on wine and grapes in 2003-2004. While the number of tests has grown satisfactorily (*ca* 4.2%), the number of data points has grown by 14%, demonstrating the increasing complexity of the analyses being performed.

To keep pace with the expanding demands, we have carefully targeted our efforts to achieve measurable improvements. Many of the problems solved have been quite mundane, such as the lack of storage brought about by the growth of AWRI activities and the physical constraints of our working environment.

In this report, the focus will be on summarising the rationale behind many of the operational changes and the benefits that have accrued from the strategies we have implemented.

Staff

There have been several staff changes in the past 12 months. Due to the timing of the departure of some staff it has been difficult to find suitable people to cover and train them for the range of services offered by the laboratory. Nevertheless, the current staff complement oversaw the profitable growth of services with retention of overall quality.

Overall analytical performance

The performance of the laboratory has been compared on a year by year basis in Table 12. The performance has been satisfactory, with the number of export certificates comparable to last year, and comparable overall numbers of routine analyses in the analytical lab. There was a significant overall rise in many of the trace analytical laboratory tests, of which many generated a large number of data points, see for example the test for 4-ethylphenol–this test has been adopted by many winemakers as an essential tool in the fight against *Brettanomyces* spoilage of wines.

Analytical Laboratory

Matt Holdstock successfully completed a vintage in the US. In the new year, he accepted a position in the Industry Services group as Chemist but has remained available to endorse NATA reports in the analytical laboratory. Matt Cream, who has been with the Analytical Service for nine years, has assumed the supervisory position. He has also assumed the responsibilities held by Matt Holdstock at the Interwinery Analysis Group.

The change in the number of export certificates issued has been minor. Enzymatic tests such as malic acid and glucose/fructose declined slightly, while acetic acid determinations rose significantly. Other tests such as sterility, volatile acidity (by distillation) and sulfur dioxide measurements increased.

Table 12: Comparison of number of tests performed by year

	99/00	00/01	01/02	02/03	03/04
Total number of tests	44846	46037	48314	53932	56181

The high volume measurements for sulfur dioxide and volatile acidity have been performed manually with no inexpensive automated procedure in place capable of meeting OIV requirements. The flow injection analyses (FIA) procedures currently being validated will allow both methods to be performed in an automated manner, permitting very fast turnround times and excellent accuracy. Most importantly, there will be considerable increase in capacity to meet peak demand during vintage. As the labour requirements will be greatly reduced with the FIA, the price of the services will be less dependent on labour costs and unit costs will drop with the number of analyses performed on the equipment. The reagent costs for volatile acidity analyses will be lower than for enzymatic acetic acid test kits.

A FOSS Winescan 2000 was installed in late 2002. The calibrations for the first stage have been completed on over 600 wines ranging from sweet whites to dry reds and fortified wines. We have almost completed the next step, which is the validation using wines from the general laboratory analyses. Other than labour savings, many advantages of using the Winescan can be envisaged, e.g. very high residual sugar distorts the alcohol results obtained from the NIR and it appears likely that the Winescan will provide superior results. Once accredited, the methods developed for the FOSS Winescan should provide a less labour intensive option to some of the slower old methods.

Following the extensive published closure trial conducted by our Industry Services team (AWRI publication #666) many suppliers of cork-based products approached the AWRI to undertake evaluations of 'new' closures on a purely 'commercial-in-confidence' basis. Accordingly, commercial closure trial testing was conducted for the 12 and 18 month time points and went well.

Trace Analytical Laboratory (TAL)

The duties of supervision of the trace analysis laboratory have been divided between Randell Taylor and Greg Rueidger. Randell has taken on all routine day to day operations, while Greg is now concentrating on GLP and non-routine tests.

The work in the trace analytical laboratory has grown significantly, with increased utilisation having occurred by 'organic' growth. Assays such as 4-ethylphenol (*Brettanomyces*), oak flavours, chloroanisoles, ethyl carbamate and the multiresidue screen (listed above) are conducted using our gc/ms instruments. Of these, the 4-ethylphenol and multiresidue screens have shown large increases in sample numbers. To a large degree, the growing awareness of *Brettanomyces* contamination problems and the solutions provided by the AWRI's Industry Services team have provided an impetus for industry to identify and control problems that might arise. We should note that the decline in sample numbers for TCA was fully expected, as one of our major customers had ordered a headspace gc/ms instrument and were recruiting a chemist at the end of last year to do TCA analyses 'in-house'.

A number of analyses we offer are HPLC-based. These include: the HPLC multi-residue analyses (principally fungicides, which are thermally unstable and cannot be run on the gc/ms), ochratoxin A, amino acid profiles and the biogenic amines histamine and tyramine. Resveratrol is a stilbene found in grapes and wines. It supposedly can play a role as an anti-cancer agent and interest in the measurement has grown. An analysis for resveratrol (and piceid, the glycosylated form) has therefore been developed to run on the platform.

Sample preparation is less of a problem than it was 12 months ago. First, we have an additional part-time staff member (Stella Kassara) to assist in this task and second, the Gerstel SPME autosampler acquired recently reduces the labour requirements quite dramatically. In brief, manual preparation of a sample required extraction with a solvent, or passing a sample down a conditioned solid phase extraction (SPE) cartridge. If an SPE cartridge is used, then the analyst must elute off the compound(s) of interest. A cleanup step may be used to remove some potential interfering compounds, which damage the chromatography or co-elute in the final step. It is then necessary to concentrate the resulting sample to a small volume, before it is placed onto the gc. While this method is still preferred for some analytes, many volatile compounds can be prepared for analyses by simply placing a small amount of wine into a special vial, capped and extracted from the headspace by a coated fibre in an automated manner. This latter method significantly reduces the amount of preparation time. Several methods have been validated to use this advanced technology.

New methods developed and validated include: headspace TCA (cork taint) and headspace 4-ethylphenol (*Brettanomyces* taint), associated with potential ethyl carbamate and resveratrol. We attempted to use SPME for the ethyl carbamate method, but this was not successful. Instead we were able to modify the existing method to a small scale format which saves time, solvents and reagents.

Several staff members have attended specialised instrument training courses to ensure new equipment is well maintained and used to its maximum capability. The most recent course attended addressed using the peak lock feature of the Agilent software. Heather Brooks has developed quickly and has attended training courses on the Agilent HPLC operating software (Chemstation). David Boehm has attended a method validation course run by AGAL (now called the National Measurement Institute). Many aspects of this software is common to the Agilent gc/ms platform and so we expect Heather will gradually be able to run sequences on this instrument too.

The Analytical Service has, for a number of years, conducted trials for agrochemical companies. The chemical company trials have again progressed well, with all completed and another currently underway. The value of such services for chemical companies can be further enhanced if we achieve good laboratory practice (GLP) accreditation. To achieve GLP accreditation, we have modified and expanded the quality system documentation to meet the stringent requirements of GLP. Additionally, we have installed roll-down screens which can be locked and placarded as a means of ensuring the integrity of the samples during the various phases of the trials. Secure archival storage and archival procedures are now in place. It is anticipated that GLP accreditation inspections will be completed by the end of 2004.

Analytical work performed here and overseas for a cork closure company was completed during early in this financial year.

Finally, an extensive industry survey with the Australian Wine and Brandy Corporation (AWBC) was commenced in early December 2003. We coordinated the program with Helen Berden (AWBC), and were able to complete the task in early May (well ahead of the agreed schedule). The analytical program in the next financial year is expected to be on a smaller scale.

Administration and Client Services

Administration of the Analytical Service is being lead by Sandra Lloyd-Davies, Customer Service Manager, who has taken on the role of ensuring all database issues are addressed with our external IT contractor. Some of the problems encountered will be covered later in the section on the LIMS system. Both Sandra and Maria Mills, Customer Service Officer, attended a Workplace Conflict conference.

Fees

New fees were introduced in April 2004, which reflected small increases (*ca* 3.8%) across the board largely due to increased freight and handling costs. A number of savings that were expected to flow on from the rise of the Australian dollar did not materialise.

General activities

Advertising

A new suite of advertisements promoting our key services has been created by Geoffrey Reed Communications. These striking advertisements use a common 'theme' and have begun to appear in wine industry publications.

Wine Show Trophy

The Analytical Service continued to support the Royal Adelaide Wine Show through sponsorship of the Trophy for the Best Riesling, in September 2003.

Twelfth Australian Wine Industry Technical Conference

Extensive preparations for the Twelfth Australian Wine Industry Technical Conference were completed. We have sponsored the 1,700 satchels for delegates and included branded bottle openers, Dropstop, and jelly beans to promote the Analytical Service. Exhibition space has been taken at the Trade Exhibition (run in conjunction with the 12AWITC). Staff members of the Analytical Service will attend to visitors at the booth and provide information on the services provided. A variety of taints will be presented as a way of allowing attendees to become familiar with the aroma properties of the specific taints and to allow Analytical Service staff to market the AS analyses which can quantify the levels present in customers' wines.

Legal matters

Procedures have been initiated to ensure further the integrity of all data and results, especially where the analytical results are likely to form part of a legal action or insurance claim. Document trails have been improved by placing documentation in a lockable archival cabinet and samples stored in a dedicated lockable compactus with movements recorded. These procedures will enable us to deal with complex queries more easily and provide an auditable trail should questions of sample integrity arise. Legal work utilising analytical results were successfully concluded.

Provisor Pty Ltd

A good working relationship has commenced between Provisor and the Analytical Service.

External environmental audit

No issues relating to the environmental impact of our operation were identified. The major waste materials are packaging materials which are collected for recycling and excess wine from analyses which we neutralise using sodium bicarbonate.

Publications

During the year, one research article and a short technical review article were published while a second research article for publication has been submitted. A poster was presented at the 19th Conference of Residues Chemists (Canberra, September 2003) (Appendix 1) and two new posters have been prepared for the 12th AWITC (Melbourne, July 2004).

STATEMENT OF FINANCIAL PERFORMANCE FOR THE YEAR ENDED 30 JUNE 2004

	2004 $	2003 $
Revenue from operating activities		
Grape and Wine Research and Development Corporation		
Project funds	4,975,689	4,324,916
Equipment	295,932	438,420
CRCV project funds	601,716	580,282
Commercial research collaborations	54,812	96,170
Analytical Service	1,628,230	1,507,854
Assistance provided by SA Government for Provisor Pty Ltd	0	700,000
Other revenue	430,794	340,644
Expenses from operating activities		
Employee benefit expense	4,785,401	4,281,915
Analytical and project operating expenses	1,381,041	1,194,694
SA Government assistance to Provisor Pty Ltd	0	700,000
Administration and general service expenses	607,650	557,898
Depreciation and amortisation expenses	749,248	471,029
Travel expenses	141,779	161,791
Profit from operating activities	322,054	620,959
Net gain (loss) on disposal of assets		
Motor vehicles	8,493	0
Equipment	(4,004)	(12,720)
Profit from ordinary activities	**326,543**	**608,239**
Total changes in equity	**326,543**	**608,239**

STATEMENT OF FINANCIAL POSITION AS AT 30 JUNE 2004

	2004 $	2003 $
Current assets		
Cash assets	1,412,016	1,416,954
Commercial bills	2,013,437	1,253,916
Receivables	354,477	511,693
Other current assets	101,695	68,745
Total current assets	3,881,625	3,251,308
Non current assets		
Leasehold buildings	1,485,052	1,522,048
Plant and equipment	1,802,870	2,070,026
Investment In Provisor Pty Ltd	650,000	650,000
Australian Wine Industry Chair of Oenology	840,000	840,000
Total non current assets	4,777,922	5,082,074
TOTAL ASSETS	8,659,547	8,333,382
Current liabilities		
Payables and accruals	1,849,867	1,482,678
Project funds not expended and repayable		
GWRDC	55,023	191,589
CRCV	55,320	17,345
Unpaid investment in Provisor Pty Ltd	0	323,186
Provisions	682,109	624,457
Total current liabilities	2,642,319	2,639,255
Non current liabilities		
Provisions	63,969	67,411
Total non current liabilities	63,969	67,411
TOTAL LIABILITIES	2,706,288	2,706,666
NET ASSETS	5,953,259	5,626,716
EQUITY		
Reserves	966,750	966,750
Retained profits	4,986,509	4,659,966
TOTAL EQUITY	5,953,259	5,626,716

Rachel Edwards

Hans Muhlack

STATEMENT OF CASH FLOWS
FOR THE YEAR ENDED 30 JUNE 2003

	2004 $	2003 $
CASH FLOWS FROM OPERATING ACTIVITIES		
Grants and other income	7,946,686	7,665,762
Interest received	150,489	112,541
Payments to suppliers and employees	(6,578,799)	(6,719,029)
Net cash provided by operating activities	1,518,376	1,059,274
CASH FLOWS FROM INVESTING ACTIVITIES		
Assistance provided by SA Government for Provisor Pty Ltd	0	700,000
Payments for commercial bills	(759,521)	(61,916)
Payments for plant and equipment	(465,016)	(682,215)
Proceeds from sale of plant and equipment	24,409	300
Payments for investment in Provisor Pty Ltd	(323,186)	(326,814)
SA Government Assistance to Provisor Pty Ltd	0	(700,000)
Net cash used in investing activities	(1,523,314)	(1,070,645)
Net increase (decrease) in cash held	(4,938)	(11,371)
Cash at 1 July	1,416,954	1,428,325
Cash at 30 June	1,412,016	1,416,954
Reconciliation of net cash provided by ordinary activities with profit		
Profit from ordinary activities	326,543	608,239
Non cash flows in operating profit		
Amortisation and depreciation	749,248	471,029
(Profit) loss on the sale of plant and equipment	(4,489)	12,720
Charges to (reduction in) provisions	54,210	73,502
Changes in assets and liabilities		
(Increase) decrease in inventories	14,264	(4,965)
(Increase) decrease in receivables and repayments	110,002	(209,983)
Increase (decrease) in creditors and accruals	268,598	108,732
Net cash provided by ordinary activities	**1,518,376**	**1,059,274**

External seminars and talks

Staff	Title of talk	Where and to whom	Date
M.J. Herderich	Red wine colour and mouth-feel: analysis of phenolic pigments and tannins	E. & J. Gallo Winery, Modesto USA	2 Jul 03
G.M. Elsey	The formation of naturally occurring damascenone	The 19th Royal Australian Chemical Institute Organic Conference (19RACI-OC), Cumberland Conference Resort, Lorne Vic	6-11 Jul 03
K.L. Wilkinson	Precursors to oak lactone: synthesis of four β-D-glucosides of 3-methyl-4-hydroxyoctanoic acid		
J.M. Eglinton, A.J. Heinrich, A.P. Pollnitz, P.A. Henschke, M.A. de Barros Lopes	Investigating the link between yeast genes and wine flavour	XXI International Conference on Yeast Genetics and Molecular Biology, Göteborg, Sweden	7-12 Jul 03
I.S. Pretorius	Novel approaches to winemaking – tailoring wine yeast for future challenges	Southcorp Winemakers seminar, AWRI, Urrbrae SA	7 Jul 03
P.A. Henschke	Yeast fermentation efficiency		8 Jul 03
P.W. Godden	*Dekkera/Brettanomyces* and the production of negative sensory compounds in wine (presentation and tasting)		
I.L. Francis, J. Field[1], M. Gishen, A.D. Coulter, P. Valente, K.A. Lattey, P.B. Høj, E.M.C. Robinson, P.W. Godden	Assessment of the performance of various types of wine bottle closures during storage of white wine - results to three years post-bottling	American Society of Enology and Viticulture (Eastern Section) Wine Closures symposium, Corning New York, USA	9 Jul 03
G.K. Skouroumounis, I.L. Francis, M.J. Kwiatkowski, H. Oakey[2], M.A. Sefton, E.J. Waters	The effect of ascorbic acid, closure type and storage conditions on the composition and sensory properties of a Chardonnay and a Riesling wine		
I.L. Francis	Screw cap closures in the Australian wine market		10 Jul 03
D. Cozzolino, M.J. Kwiatkowski, M. Parker, W.U. Cynkar, R.G. Dambergs, M. Gishen, M.J. Herderich	Prediction of phenolic compounds in red wine fermentations by near infrared spectroscopy	3rd Symposium *In Vino Analytica Scientia*, Aveiro, Portugal	10–12 Jul 03
A.D. Coulter, P.W. Godden, E.M.C. Robinson	An overview of the AWRI *Dekkera/Brettanomyces* spoilage project	ASVO Seminar *Grapegrowing at the edge; Managing the wine business; Impacts on wine flavour,* Tanunda, SA	11 Jul 03
M.A. de Barros Lopes	Ascertaining the degree of genetic variation between strains of *Dekkera/ Brettanomyces* yeast isolated from different Australian wineries and regions		
H.E. Smyth, D. Cozzolino, I.L. Francis	Identification of key flavour compounds in Australian Riesling and unwooded Chardonnay wines		
A.P. Pollnitz, D.L. Capone, M.C. Caldersmith[13], M.A. Sefton	The effect of various wine bottle closures and fining agents on flavour and aroma compounds in wine		
A.D. Coulter	A tasting of wines containing key *Dekkera/ Brettanomyces* spoilage compounds		
M.J. Herderich	Wine science and careers in chemistry	RACI careers night, Flinders University, Adelaide, SA	11 Jul 03
M.A. de Barros Lopes	Genetic improvement of wine yeast	Joint Australia-Taiwan Food Biotechnology workshop, Sydney NSW	20-22 Aug 03
P.A. Henschke	Controlling the microbial formation of flavour in wines	Australian Institute of Food Science and Technology Conference, Melbourne Vic	25 Aug 03
S.-J. Bell	Spray diaries and new chemicals	Grapegrowers from the Adelaide Hills Wine Region, Lenswood Research Station, Lenswood SA	26 Aug 03
J.M. Eglinton, I.L. Francis, P.B. Høj, P.A. Henschke	Winemaking properties and potential of *Saccharomyces bayanus* wine yeast	Romeo Bragato Conference 2003, Wellington, New Zealand	27-30 Aug 03
P.W. Godden, P.A. Henschke	*Dekkera/Brettanomyces* and the production of negative sensory compounds during winemaking	Seminar and tasting: BRL Hardy technical staff, Reynella, SA	4 Sep 03
E.J. Bartowsky	Identification of wine bacteria using molecular and classical methodology	GWRDC Microbiological identification techniques workshop, CSIRO, Waite Precinct, Urrbrae SA	9 Sept 03

Staff	Title of talk	Where and to whom	Date
M.J. Herderich	The 'Tannin Project': research relevance	CRCV Review, The University of Adelaide, Urrbrae SA	10 Sept 03
M. Birse	Contribution of anthocyanin-derived pigments to red wine colour		
M.J. Herderich	The 'Tannin Project': research overview and future directions		11 Sept 03
S.-J. Bell	Agrochemical issues for grapegrowers and winemakers	Barossa Valley Grapegrower Technical Group Meeting, Seppelts Winery, Barossa Valley, SA	19 Sept 03
P.W. Godden	*Dekkera/Brettanomyces* and the production of negative sensory compounds during winemaking	Seminar and tasting: 15th Rutherglen Wine Show Technical Seminar, Rutherglen Vic	25 Sep 03
M. Gishen, P.W. Godden	The wine industry — a new frontier for chemical engineering in Australia	CHEMECA2003 (31st Australasian Chemical Engineering Conference), Stamford Plaza, Adelaide, SA	28 Sept - 1 Oct 03
G.K. Skouroumounis, M. Kwiatkowski, Z.K. Peng, I.L. Francis, M.A. Sefton, E.J. Waters	Oxidation of bottled wine: non-destructive measurement of white wine colour, the role of ascorbic acid, and bottle storage conditions	Orlando Wyndham, Rowland Flat, SA	1 Oct 03
H.E. Smyth, M.J. Herderich, M.A. Sefton, I.L Francis	Riesling wine aroma: relating volatile composition to sensory data using PLS regression models	6th Annual Scientific meeting of the Australasian Association for Chemosensory Science, Rutherglen, Vic	4 Oct 03
B. Stummer[4], I.L Francis, K.A. Lattey, A.J. Markides[3] S.J. Clarke[4], C. Day[5], E.S. Scott[4]	Sensory properties of Chardonnay wines made from powdery mildew affected grapes		
P.W. Godden	Trouble free winemaking – the identification, management and avoidance of common wine instabilities	Roadshow Wodonga, Vic	7, 8, 9 Oct 03
A.D. Coulter, E.M.C. Robinson, G.D. Cowey, P.W. Godden	Tasting of wines with simulated faults		
G.D. Cowey	Laboratory analysis and quality management		
E.M.C. Robinson	Introduction to *AWRI Solutions Website*		
A.D. Coulter, E.M.C. Robinson, G.D. Cowey	Laboratory techniques – theory and practical demonstrations		
A.D. Coulter, E.M.C. Robinson, G.D. Cowey	Microbiological techniques – practical demonstrations		
A.D. Coulter	Isolation of hazes/deposits and use of microscope		
	What bug is that?		
R.E. Day	New ways with old varieties	Riverland Wine Industry Technical Seminar *Some like it hot*	16 Oct 03
M.J. Herderich	Polyphenols, pigmented polymers and red wine colour and the importance of seeds in the phenolic composition of red wine	Orlando Wyndham Viticulturists' annual meeting	16 Oct 03
P.W. Godden	Update on the AWRI trial of the technical performance of wine closures	Roadshow, Hunter Valley, NSW	20 Oct 03
	Dekkera/Brettanomyces, and the production of 4-ethylphenol during winemaking		
I.L. Francis	Tannins and mouth-feel: terminology and methods for sensory evaluation of red wines. (Mouth-feel wheel, and the results of some recent tannin sensory studies)		
	Major influences on red wine mouth-feel – the effect of tannins, anthocyanins, ethanol and polysaccharides		
M. Gishen	The link between bentonite requirements and vineyard and winemaking practices		
	Quality management systems (ISO 9000, HACCP) – what are they, do you need them, how do you do it? - cheaply!		
E.J. Bartowsky, P.A. Henschke	Which bacterial strains are conducting your MLF? Flavour aspects of MLF – control of the 'buttery' diacetyl character in wine		
E.J. Bartowsky, D. Xia[12], G.H. Fleet[12], P.A. Henschke	Microbiological induced oxidative spoilage of bottled red wine – role of acetic acid bacteria		
M.A. Sefton	White wine ageing: the role of ascorbic acid, and bottle storage conditions		
	Non destructive measurement of white wine colour		
	Authentication of juice (and wine?) by characterisation of the proteins present		

Staff	Title of talk	Where and to whom	Date
M.J. Herderich	Polyphenols, pigmented polymers and red wine colour: results from the Tannin Project: the 2001 large-scale winemaking trial	Roadshow, Hunter Valley, NSW	20 Oct 03
	The importance of seeds in the phenolic composition of red wine		
P.W. Godden	Update on the AWRI trial of the technical performance of wine closures	Roadshow, Griffith, NSW	22 Oct 03
	Dekkera/Brettanomyces, and the production of 4-ethylphenol during winemaking		
M.A. Sefton	White wine ageing: the role of ascorbic acid, and bottle storage conditions		
	Non destructive measurement of white wine colour		
	Authentication of juice (and wine?) by characterisation of the proteins present		
M. Gishen	The use of NIR for the measurement of grape, juice and wine components, including colour		
	The influence of deficit irrigation on salt accumulation in grapes, and wine quality		
	Quality control of corks		
I.L. Francis	Identification and measurement of key wine flavour compounds		
	Major influences on red wine mouth-feel – the effect of tannins, anthocyanins, ethanol and polysaccharides		
E.J. Bartowsky, P.A. Henschke	Flavour aspects of MLF – control of the 'buttery' diacetyl character in wine		
J.M. Eglinton, P.A. Henschke, E.J. Bartowsky	Winemaking with alternative yeasts: modification of wine composition, flavour and colour		
M.J. Herderich	Polyphenols, pigmented polymers and red wine colour: results from the Tannin Project: the 2001 large-scale winemaking trial		
	The importance of seeds in the phenolic composition of red wine		
P.W. Godden	Managing stuck fermentation	Roadshow, Mudgee, NSW	24 Oct 03
	Dekkera/Brettanomyces, and the production of 4-ethylphenol during winemaking		
M.A. Sefton	White wine ageing: the role of ascorbic acid, and bottle storage conditions		
	Non destructive measurement of white wine colour		
	Authentication of juice (and wine?) by characterisation of the proteins present		
E.J. Bartowsky, D. Xia[12], G.H. Fleet[12], P.A. Henschke	Microbiological induced oxidative spoilage of bottled red wine – role of acetic acid bacteria		
J.M. Eglinton, P.A. Henschke, E.J. Bartowsky	Winemaking with alternative yeasts: modification of wine composition, flavour and colour		
I.L. Francis	Tannins and mouth-feel: terminology and methods for sensory evaluation of red wines. (Mouth-feel wheel, and the results of some recent tannin sensory studies)		
	Major influences on red wine mouth-feel – the effect of tannins, anthocyanins, ethanol and polysaccharides		
M. Gishen	The use of NIR for the measurement of grape, juice and wine components, including colour		
M.J. Herderich	Polyphenols, pigmented polymers and red wine colour: results from the Tannin Project: the 2001 large-scale winemaking trial		
	The importance of seeds in the phenolic composition of red wine		
	The ability of various wine bottle closures and fining agents to remove flavour and aroma compounds from wine		
P.A. Henschke	The use of novel yeasts and fermentation technologies to understand better the role of micro-organisms in wine flavour formation	Chemistry Faculty, Republican University of Uruguay, Montevideo, Uruguay	30 Oct 03
I.S. Pretorius	Development of superior wine yeast: current status and future opportunities to meet the consumer challenge	Waite Precinct Plant Researchers Seminar Series, Plant Research Centre, Urrbrae SA	19 Nov 03
P.W. Godden	*Dekkera/Brettanomyces*, and the production of negative sensory compounds during winemaking	Victorian Pinot Noir Workshop, Acheron, Vic	20 Nov 03
P.W. Godden (joint presentation with P.A. Leske[6])	Cleaning them up – fining, finishing and filtration (seminar and tasting)		
P.A. Henschke	Life Sciences Programs: microbial modulation of wine sensory characteristics – an overview	GWRDC Review of AWRI Life Sciences, AWRI, Urrbrae, SA	24 Nov 03
M.A. de Barros Lopes	Life Sciences Programs: molecular tools for the evaluation and improvement of wine fermentations- an overview		
R.G. Dambergs	NIRS in the wine industry	Renmark Agricultural Bureau Annual General Meeting, Renmark, SA	24 Nov 03
P.W. Godden	Tasting: International benchmark Cabernet Sauvignon wines	Langhorne Creek Benchmarking and Educational Seminar: Cabernet Sauvignon, Langhorne Creek, SA	26 Nov 03

Staff	Title of talk	Where and to whom	Date
P.W. Godden	Cabernet Sauvignon – the wines that Australia can make, and the wines the world wants to drink	Langhorne Creek Benchmarking and Educational Seminar: Cabernet Sauvignon Langhorne Creek, SA	26 Nov 03
P.W. Godden	Managing stuck fermentation	Roadshow, Canberra, ACT	1 Dec 03
	Dekkera/Brettanomyces, and the production of 4-ethylphenol during winemaking		
E.J. Bartowsky, P.A. Henschke	Flavour aspects of MLF – control of 'buttery' diacetyl character in wine		
P.A. Henschke, K.S. Howell, E.J. Bartowsky, G.H. Fleet[12]	'Natural fermentation': potential of alternative inoculation strategies?		
J.M. Eglinton, P.A. Henschke	The role of volatile acidity in re-starting stuck fermentation		
M.J. Herderich	Polyphenols, pigmented polymers and red wine colour: results from the Tannin Project: the 2001 large-scale winemaking trial		
	The importance of seeds in the phenolic composition of red wine		
S-J. Bell	Managing *Botrytis* in the vineyard		
	The link between bentonite requirements and vineyard and winemaking practices		
I.S. Pretorius	Strategies for successful induction of malolactic fermentation		
	Winemaking with alternative yeasts: modification of wine composition, flavour and colour		
M. Gishen	The use of NIR for the measurement of grape, juice and wine components, including colour		
	White wine ageing: the role of ascorbic acid, and bottle storage conditions		
	Non destructive measurement of white wine colour		
P.W. Godden	Trouble free winemaking – the identification, management and avoidance of common wine instabilities	Roadshow, Bunbury, WA	9, 10, 11 Dec 03
A.D. Coulter, E.M.C. Robinson, G.D. Cowey, M. Gishen, P.W. Godden	Tasting of wines with simulated faults		
M. Gishen	Laboratory analysis and quality management		
E.M.C. Robinson	Introduction to AWRI Solutions Website		
A.D. Coulter, E.M.C. Robinson, G.D. Cowey	Laboratory techniques – theory and practical demonstrations		
A.D. Coulter, E.M.C. Robinson, G.D. Cowey	Microbiological techniques – practical demonstrations		
A.D. Coulter	Isolation of hazes/deposits and use of microscope		
	What bug is that?		
P.W. Godden	Trouble free winemaking – the identification, management and avoidance of common wine instabilities	Roadshow seminar, Manjimup, WA	20-22 Jan 04
G.D. Cowey, E.M.C. Robinson, M. Gishen, P.W. Godden	Tasting of wines with simulated faults		
M. Gishen	Laboratory analysis and quality management		
E.M.C. Robinson	Introduction to *AWRI Solutions Website*		
E.M.C. Robinson, G.D. Cowey, M. Gishen	Use of a microscope, laboratory techniques for the isolation and identification of hazes and deposits in wine – theory and practical demonstrations		
A.D. Coulter, E.M.C. Robinson, G.D. Cowey	What bug is that? – Identification of common wine microflora - theory and practical demonstrations		
C.D. Curtin, W. Zhang[12], C. Franco[14]	Role of transcriptional regulation in variability of *Vitis vinifera* L. cell culture-based anthocyanin production	International Workshop on Anthocyanins, NSW Stamford Plaza, Sydney, NSW	28 Jan 04
M.J. Herderich	Anthocyanins, anthocyanin-derived pigments and the mysteries of red wine colour		29 Jan 04
Y. Hayasaka	Confirmation of pigmented polymers present in grape skin and wine		29 Jan 04
T.E. Siebert, H.E. Smyth, D.L. Capone, K.S. Howell, M.J. Herderich, A.P. Pollnitz	Stable isotope dilution analysis of wine fermentation products by SPME-GC-MS	Eighth International Symposium on Hyphenated Techniques in Chromatography (HTC-8), Brugge, Belgium	5 Feb 04
S.J. Dillon, E.J.C. Bartowsky, K.A. Lattey, I.L. Francis, A. Julien[3], A.J. Markides[3], L. Dulau[3], P.B. Høj, P.A. Henschke	A quantitative chemical and sensory approach to characterising wine yeast for improved red wine colour and flavour	3rd Yeast: Products and Discovery meeting, Nuriootpa, SA	4-6 Apr 04

Staff	Title of talk	Where and to whom	Date
D. Cozzolino, L. Flood, M.A. de Barros Lopes, R.G. Dambergs, W.U. Wynkar, L.J. Janik, M. Gishen, P.B. Høj	Yeast identification by infrared spectroscopy	3rd Yeast: Products and Discovery meeting, Nuriootpa, SA	4-6 Apr 04
K.S. Howell, M. Klein[7], H. Swiegers, G.M. Elsey, T.E. Siebert, Y. Hayasaka, E.J.C. Bartowsky, G.H. Fleet[12], I.S. Pretorius, M.A. de Barros Lopes	A genetic study to characterise the release of volatile thiols by *S. cerevisiae*		
M.A. de Barros Lopes	Wine yeast gene technology		
D. Cozzolino, R.G. Dambergs, W.U. Cynkar, L.J. Janik, M. Gishen, P.B. Høj	An overview of the use of near infrared spectroscopy in the Australian grape and wine industry	11th Australian Near Infrared Spectroscopy Group conference, Fremantle, WA	19-21 Apr 04
H.E. Smyth, D. Cozzolino, M.J. Herderich, M.A. Sefton, I.L. Francis	Identification of key aroma compounds in Australian Riesling and unwooded Chardonnay wines	7th Wartburg Symposium on Flavour Chemistry & Biology, Eisenach, Germany	21 Apr 04
K.L. Wilkinson	Formation of oak lactone from 3-methyl-4-hydroxy-octanoic acid and its 4-O-β-D-glucopyranosides		
L.J. Janik, M. Gishen	FOSS 'Winecan' – calibration and protocols for wine analysis	Wine Industry User's Group, FOSS Directions 2004, Swan Valley, WA	22-23 Apr 04
C.S. Stockley	Recommendation of alcohol in moderation: an international comparison	15th International Conference on the Reduction of Drug Related Harm, Melbourne Vic	23 Apr 04
H.E. Smyth, D. Cozzolino, M.J. Herderich, M.A. Sefton, I.L. Francis	Key aroma compounds in white wine	Department of Dairy and Food Science, The Royal Veterinary and Agricultural University, Copenhagen, Denmark	26 Apr 04
M.J. Herderich	Winegrape tannin and colour specifications	Visiting group of Italian winemakers, AWRI, Urrbrae SA	27 Apr 04
H.E. Smyth, D. Cozzolino, M.J. Herderich, M.A. Sefton, I.L. Francis	Key aroma compounds in white wine	Department of Analytical Chemistry, University of Zaragoza, Zaragoza, Spain	29 Apr 04
E.J.C. Bartowsky	The buttery attribute of wine, diacetyl, desirability, spoilage and beyond – butter or no butter	XVI scientific meeting held by the Lallemand group, Porto, Portugal	4-5 May 04
S.-J. Bell	A tale of two seasons – impact of viticultural practice on red grape phenolics	7th International Symposium on Grapevine Physiology and Biotechnology, California, USA	24 Jun 04
P.A. Henschke, J.R. Bellon, D. Capone, A.D. Coulter, G.D. Cowey, D. Cozzolino, C.D. Curtin, J.B. Field[1], M. Gishen, P. Graves, K.A. Latey, E.M.C. Robinson, I.L. Francis, P.B. Høj, M.A. de Barros Lopes, P.W. Godden	Incidence and control of *Brettanomyces*: the Australian perspective	55th annual meeting of the American Society for Enology and Viticulture held in San Diego, California, USA	29 Jun to 2 Jul 04

Workshops

Author	Title	Location	Date
S.-J. Bell	Sugar Acids and pH for quality / Research to Practice™ Grapevine Winegrape Quality Management	Langhorne Creek, SA	11 Sept 03
S.-J. Bell	Sugar Acids and pH for quality / Research to Practice™ Grapevine Winegrape Quality Management	McLaren Vale, SA	17 Nov 03 25 Nov 03
R.G. Dambergs	Spectral analysis of commercial graded red wines	Riverlink Cabernet Quality workshop, Merbein Vic	29 Jul 03
P.W. Godden	Trouble free winemaking – causes and prevention of common wine instabilities	Roadshow workshops, *Trouble free winemaking and troubleshooting wine instability problems*, to winemakers and grapegrowers, Wodonga Vic	7, 8 and 9 Oct 03
A.D. Coulter, E.M.C. Robinson, G.D. Cowey, P.W. Godden	Tasting of simulated faulty wines		
G.D. Cowey	Laboratory analysis and quality management		
E.M.C. Robinson	Introduction to AWRI technical manual website		
A.D. Coulter	Isolation of hazes/deposits and use of microscope		
	What bug is that?		
P.W. Godden	Trouble free winemaking – causes and prevention of common wine instabilities	Roadshow workshops, *Trouble free winemaking and troubleshooting wine instability problems*, to winemakers and grapegrowers, Canberra, ACT	2 Dec 03
A.D. Coulter, E.M.C. Robinson, G.D. Cowey, M. Gishen, P.W. Godden	Tasting of wines with simulated faults		
M. Gishen	Laboratory analysis and quality management		
E.M.C. Robinson	Introduction to AWRI Solutions Website		
A.D. Coulter	Isolation of hazes/deposits and use of microscope		
	What bug is that?		
P.W. Godden	Trouble free winemaking – causes and prevention of common wine instabilities	Roadshow workshops, *Trouble free winemaking and troubleshooting wine instability problems*, to winemakers and grapegrowers, Bunbury, WA	9, 10, 11 Dec 03
A.D. Coulter, E.M.C. Robinson, G.D. Cowey, M. Gishen, P.W. Godden	Tasting of wines with simulated faults		
M. Gishen	Laboratory analysis and quality management		
E.M.C. Robinson	Introduction to AWRI Solutions website		
A.D. Coulter	Isolation of hazes/deposits and use of microscope		
	What bug is that?		
P.W. Godden	Trouble free winemaking – causes and prevention of common wine instabilities	Roadshow workshops, *Trouble free winemaking and troubleshooting wine instability problems*, to winemakers and grapegrowers, Manjimup, WA	20, 21 and 22 Jan 04

Author	Title	Location	Date
G.D. Cowey, E.M.C. Robinson	Tasting of simulated faulty wines	Roadshow workshops, *Trouble free winemaking and troubleshooting wine instability problems*, to winemakers and grapegrowers, Manjimup, WA	20, 21 and 22 Jan 04
M. Gishen	Laboratory analysis and quality management		
E.M.C. Robinson	Introduction to AWRI *Practical solutions* website		
G.D. Cowey, E.M.C. Robinson	Isolation of hazes and deposits, theory and practical		
	What bug is that?		
P.W. Godden	Conclusion		
Y. Hayasaka, G.K. Skouroumounis, E. Meudec[8], V. Cheynier[8], S. Vidal[9], J. Kennedy[10]	Confirmation of pigmented polymers present in grape skin and wine	3rd International Workshop on Anthocyanins, Sydney NSW	27-29 Jan 04

Posters presented

Author	Title	Organisation and location	Date
M.A. Daniel, G.M. Elsey, M.A. Sefton	The consumption of ß-damascenone in wine	The 19th Royal Australian Chemical Institute Organic Conference (19RACI-OC), CumberlandConference Resort, Lorne Vic	6-11 Jul 03
J.M. Eglinton, A.J. Heinrich, A.P. Pollnitz, P.A. Henschke, M.A. de Barros Lopes	Investigating the link between yeast genes and wine flavour	21st International Conference on Yeast Genetics and Molecular Biology (ICGMB) Göteborg, Sweden	7-12 Jul 03
I.S. Pretorius	Rme1p induces FLO11 expression through an 11 bp Rme1p response element		
	Ras regulates the carnitine shuttle in yeast		
	Maltotriose transport in yeast		
	Factors affecting fructose consumption in wine yeast		
	Influence of flocculation on ethanol production by recombinant yeast		
R.L. Taylor, G.A. Ruediger	Determining the impact of pesticide residues on the winemaking process	19th Conference of Residues Chemists, Canberra ACT	16-18 Sept 03
D. Torrea, T.E. Siebert, B. Liebich[5], C. Ancin[11], I.L. Francis, P.A. Henschke	Effect of ammonium supplementation of a Chardonnay must on wine aroma	3rd Yeast: Products and Discovery meeting, Nuriootpa SA	4-6 Apr 04
J.R. Bellon, C.D. Curtin, G.D. Cowey, J. Field[1], P.J. Graves, E.M.C. Robinson, P.B. Høj, P.W. Godden, P.A. Henschke, M.A. de Barros Lopes	Genetic diversity of *Brettanomyces/Dekkera* strains isolated from Australian wines		
K.S. Howell, M. Klein[7], H. Swiegers, G.M. Elsey, T.E. Siebert, Y. Hayasaka, E.J. Bartowsky, G.H. Fleet[12], I.S. Pretorius, M.A. de Barros Lopes	A genetic study to characterise the release of volatile thiols by *Saccharomyces cerevisiae*		
R.G. Dambergs, B.E. Stummer[4], T. Zanker[4], D. Cozzolino, M. Gishen, E.S. Scott[4]	Detection of powdery mildew in grapes by near infrared spectroscopy	11th Australian Near Infrared Spectroscopy Group conference, Fremantle, WA	19-21 Apr 04
H.E. Smyth, D. Cozzolino, M.J. Herderich, M.A. Sefton, I.L. Francis	Identification of key aroma compounds in Australian Riesling and unwooded Chardonnay wines	7th Wartburg Flavour Symposium, Wartburg, Germany	21-23 Apr 04
C.D. Curtin, W. Zhang[12], C. Franko[14]	Regulation of anthocyanin biosynthesis in *Vitis vinifera* cell culture following treatment with sucrose, jasmonic acid, and continuous white light irradiation	55th Annual Meeting of American Society for Enology and Viticulture, San Diego, USA	30 Jun-2 Jul 04

Other activities Staff	Activity	Date
P.W. Godden, E.M.C. Robinson, K.A. Lattey, G.D. Cowey, A.D. Coulter, M. Parker, P. Jorgensen, C. Day[5], W. Cynkar	Advanced Wine Assessment Course, Adelaide, SA	22-25 Jul 03
P.W. Godden	Associate Judge, Royal Adelaide Wine Show, Adelaide, SA	29 Sep - 2 Oct 03

1 Field Consulting Services
2 Biometrics SA
3 Lallemand
4 School of Agriculture and Wine, The University of Adelaide
5 Provisor Pty Ltd
6 Nepenthe Vineyards
7 The Aachen University of Technology
8 Unite Mixté de Reserche Sciences pour l'Oenologie, INRA France
9 Inter Rhône, France
10 Department of Food Science and Technology, Oregon State University, USA
11 Universidad Publica de Navarra
12 Department of Food Science, University of New South Wales
13 Hollicks Wines
14 Flinders University

APPENDIX 2. Teaching responsibilities of AWRI staff during 2003/04

Subject	No. of lectures	Institute staff
Adelaide University		
2003 – Semester 2		
3045WT/7048WT Advances in oenology	2	I.L. Francis
	1	P.W. Godden
	4	M.J. Herderich
	4	P.A. Henschke
	3	E.J. Bartowsky
3003WT Wine packaging and quality management	1	P.W. Godden
Viticulture Production B	2	S-J. Bell
Flinders University		
CPES7001 Aromaticity and Pericyclic Reactions (Honours course)	12	G.M. Elsey
MMED 3921 Industrial and pharmaceutical microbiology	1	P.A. Henschke
2004 – Semester 1		
The University of Adelaide		
2580 Stabilisation and clarification	3	E.J. Waters
	1	A.D. Coulter
	4	P.W. Godden
3009WT Advanced sensory practice	4	P.W. Godden
3011WT/3011WA/7013WT Winemaking	2	P.A. Henschke
2001WT and 7030WT Wine and Society	2	C.S. Stockley
3005WT Grape Industry Practice Policy and Communication	Approx. 50 hours	C.S. Stockley and P.B. Høj

APPENDIX 3. Graduate and Honours student supervision responsibilities of AWRI staff for 2003/04

Student	Supervisor/s	Source of funds
PhD		
M. Birse	M.J. Herderich, I.L. Francis, A.P. Pollnitz	CRCV
A. Cox	G.M. Elsey, M.A. Sefton, M.V. Perkins	GWRDC/CRCV
M.A. Daniel	G.M. Elsey, M.A. Sefton, M.V. Perkins	GWRDC/CRCV
J.M. Eglinton	P.A. Henschke, P.R. Langridge[3]	AWRI staff
A. Grimaldi	E.J. Bartowsky, V. Jiranek[3]	GWRDC/School of Agriculture and Wine, University of Adelaide
K.S. Howell	P.A. Henschke, E.J. Bartowsky, G.H. Fleet[6], M.A. de Barros Lopes	University of NSW/GWRDC
F. Lloyd	E.J. Waters, C.B. Colby[1], B. ONeill[1], A.Lim[2]	School of Chemical Engineering, University of Adelaide/GWRDC
0.J. McIntyre	E.J. Waters, I.S. Pretorius, C.B. Colby[1], B. ONeill[1], A. Lim[2]	School of Chemical Engineering, The University of Adelaide/GWRDC
R.A. Muhlack	E.J. Waters, P.B. Høj, C.B. Colby[1], B. ONeill[1], A. Lim[2]	School of Chemical Engineering, University of Adelaide/GWRDC/ The Hardy Wine Company
A. Oberholster	I.L. Francis, E.J. Waters, P. Iland[3,4], G.P. Jones[3]	own
C.J. Puglisi	G.M. Elsey, M.A. Sefton, R.H. Prager[5]	GWRDC/CRCV
R. Ristic	P.G. Iland[3,4], M.J. Herderich, I.L Francis	GWRDC
H.E. Smyth	I.L. Francis, M.J. Herderich, M.A. Sefton	GWRDC
M. Ugliano	L. Moio[7], P.A. Henschke, E.J. Bartowsky	Universitá di Foggia scholarship
K. L. Wilkinson	G.M. Elsey, M.A. Sefton, R.H. Prager[5]	GWRDC
Honours		
G. Alexander	P.R. Jones, E.J. Waters, P.B. Høj	School of Agriculture and Wine, University of Adelaide/GWRDC
E. Dennis	P.J. Smith, M. Perkins[5]	GWRDC, Flinders University
L-H. Ly	G.M. Elsey	SoCPES-Flinders Uni.
C. Sarnekis	P.A. Smith, P.R. Jones, E.J. Waters, M.J. Herderich	Flinders University
M.H. Turner	G.M. Elsey	SoCPES-Flinders Uni.

Theses completed

Student	Hon/PhD	Title of thesis	Supervisors
S. Brown	PhD	Investigation into the mechanism of action and biological role of *Saccharomyces cerevisiae* mannoproteins which reduce visible haziness in white wine	E.J. Waters, M.A. de Barros Lopes, P.B. Høj
F. Carrau	PhD	Selection of native wine yeasts with low nitrogen demand characterisation, competition and aroma profile composition of wines	E. Dellacassa[8], P.A. Henschke
R.C. Brown	Hons	The stereoselective synthesis of γ-lactone-based aroma compounds	G.M. Elsey, D.K. Taylor[5]
J.C. Crossman	Hons	Synthesis and hydrolytic studies of glycosylated precursors to TDN	G.M. Elsey
N.R. Sleep	Hons	The development of stable isotope dilution assays for the quantification of Important aroma precursors in wine	G.M. Elsey
D. Coates	MAgSc	The physiochemical and sensory properties of Shiraz wine following malolactic fermentation	E.J. Bartowsky

1 School of Chemical Engineering, The University of Adelaide
2 The Hardy Wine Company
3 School of Agriculture and Wine, The University of Adelaide
4 Now Patrick Iland Wine Promotions
5 Flinders University
6 Department of Food Science and Technology, University of New South Wales
7 Universitá di Foggia
8 University of the Republic of Uruguay

735	Francis, L.; Field, J.; Gishen, M.; Coulter, A.; Valente, P.; Lattey, K.; Høj, P.; Robinson, E.; Godden, P. The AWRI closure trial: sensory evaluation data 36 months after bottling. *Aust. N.Z. Grapegrower Winemaker* (475): 59–60, 62–64; 2003.
736	Pretorius, I.S. The genetic analysis and tailoring of wine yeasts. de Winde, J.H. (ed.) Functional genetics of industrial yeasts. Berlin: Springer-Verlag; 2003: 99–142. (Topics in Current Genetics; 2).
737	de Barros Lopes, M.; Eglinton, J.; Henschke, P.; Høj, P.; Pretorius, I. The connection between yeast and alcohol reduction in wine: managing the double-edged sword of bottled sunshine. *Aust. N.Z. Wine Ind. J.* 18(4): 17–18, 20, 22; 2003.
738	Verstrepen, K.J.; Van Laere, S.D.M.; Vanderhaegen, B.M.P.; Derdelinckx, G.; Dufour, J-P.; Pretorius, I.S.; Winderickx, J.; Thevelein, J.M.; Delvaux, F.R. Expression levels of the yeast alcohol acetyltransferase genes ATF1, Lg-ATF1, and ATF2 control the formation of a broad range of volatile esters. *Appl. Environ. Micrbiol.* 69 : 9; 5228-5237; 2003.
739	Stockley, C.S. A component of wine called resveratrol. *Aust. N.Z. Grapegrower Winemaker* (477): 114–117; 2003.
740	Stockley, C.S. The importance of diet in conjunction with wine consumption. *Aust. N.Z. Grapegrower Winemaker* (477): 118–119; 2003.
741	Asenstorfer, R.E.; Morgan, A.L.; Hayasaka, Y.; Sedgley, M.; Jones, G.P. Purification of anthocyanins from species of Banksia and Acacia using high-voltage paper electrophoresis. *Phytochem. Anal.* 14: 150–154; 2003.
742	Smith, B.P.C.; Tyler, M.J.; Williams, B.D.; Hayasaka, Y. Chemical and olfactory characterization of odorous compounds and their precursors in the parotoid gland secretion of the green tree frog, *Litoria caerulea*. *J. Chem. Ecol.* 29: 2085–2100; 2003.
743	Hayasaka , Y.; Kennedy, J.A. Mass spectrometric evidence for the formation of pigmented polymers in red wine. *Aust. J. Grape Wine Res.* 9(3):210–220; 2003.
744	Capone, D.; Sefton, M.; Pretorius, I.; Høj, P. Flavour 'scalping' by wine bottle closures — the 'winemaking' continues post vineyard and winery. *Aust. N.Z. Wine Ind. J.* 18(5): 16, 18–20; 2003.
745	Siebert, T.; Herderich, M.; Francis, L.; Pollnitz, A. No evidence of 'atypical ageing taint' in Australian white wine. *Aust. N.Z. Wine Ind. J.* 18(5): 55–56, 58; 2003.
746	Gockowiak, H.; Henschke, P.A. Interaction of pH, ethanol concentration and wine matrix on induction of malolactic fermentation with commercial 'direct inoculation' starter cultures. *Aust. J. Grape Wine Res.* 9: 200–209; 2003.
747	Sutherland, C.M.; Henschke, P.A.; Langridge, P.; de Barros Lopes, M. Subunit and cofactor binding of *Saccharomyces cerevisiae* sulfite reductase—towards developing wine yeast with lowered ability to produce hydrogen sulfide. *Aust. J. Grape Wine Res.* 9:186–193; 2003.
748	Becher, J.V.W.; Armstrong, G.O.; van der Merwe, M.J.; Lambrechts, M.G.; Vivier, M.A.; Pretorius, I.S. Metabolic engineering of *Saccharomyces cerevisiae* for the synthesis of the wine-related antioxidant resveratrol. *FEMS Yeast Res.* (4) 79–85; 2003.
749	Verstrepen, K.J.; Derdelinckx, G.; Dufour, J.-P.; Winderickx, J.; Pretorius, I.S.; Thevelein, J.M.; Delvaux, F.R. The *Saccharomyces cerevisiae* alcohol acetyl transferase gene ATF1 is a target of the cAMP/PKA and FGM nutrient-signalling pathways. *FEMS Yeast Res.* 4: 285–296; 2003.
750	Cozzolino, D.; Smyth, H.E.; Gishen, M. Feasibility study on the use of visible and near-infrared spectroscopy together with chemometrics to discriminate between commercial white wines of different varietal origins. *J. Agric. Food Chem.* 51: 7703–7708; 2003.
751	Janusz, A.; Capone, D.L.; Puglisi, C.J.; Perkins, M.V.; Elsey, G.M.; Sefton, M.A. (E)-1-(2,3,6-Trimethylphenyl)buta-1,3-diene: a potent grape-derived odorant in wine. *J. Agric. Food Chem.* 51: 7759–7763; 2003.
752	Pretorius, I.S. The genetic improvement of wine yeasts. Arora, D.; Bridge, P.D.; Bhatnagar, D., (eds.) Handbook of fungal biotechnology 2nd ed. New York, USA: Marcel Dekker Inc.; 2004: 209–232.
753	Pollnitz, A.P.; Capone, D.L.; Caldersmith, M.C.; Sefton, M.A. The effect of various wine bottle closures and fining agents on flavour and aroma compounds in wine. Bell, S.M.; de Garis, K.A.; Dundon, C.G.; Hamilton, R.P.; Partridge, S.J.; Wall, G.S., (eds.) Grapegrowing at the edge; Managing the wine business; Impacts on wine flavour: proceedings of a seminar; 10–11 July 2003, Barossa Convention Centre, Tanunda, S.A. Adelaide, S.A: Australian Society of Viticulture and Oenology; 2003: 59–63.
754	Smyth, H.E.; Cozzolino, D.; Francis, I.L. Identification of key aroma compounds in Australian Riesling wines. Bell, S.M.; de Garis, K.A.; Dundon, C.G.; Hamilton, R.P.; Partridge, S.J.; Wall, G.S., (eds.) Grapegrowing at the edge; Managing the wine business; Impacts on wine flavour: proceedings of a seminar; 10–11 July 2003, Barossa Convention Centre, Tanunda, S.A. Adelaide, S.A: Australian Society of Viticulture and Oenology; 2003: 56–58.
755	Bellon, J.; Robinson, E.; Cowey, G.; Graves, P.; Field, J.; Godden, P.; de Barros Lopes, M. The degree of genetic variation between strains of *Dekkera/Brettanomyces* yeast isolated from different Australian wineries and regions. Bell, S.M.; de Garis, K.A.; Dundon, C.G.; Hamilton, R.P.; Partridge, S.J.; Wall, G.S., (eds.) Grapegrowing at the edge; Managing the wine business; Impacts on wine flavour: proceedings of a seminar; 10–11 July 2003, Barossa Convention Centre, Tanunda, S.A. Adelaide, S.A: Australian Society of Viticulture and Oenology; 2003: 51–55.
756	Coulter, A.; Robinson, E.; Cowey, G.; Francis, I.L.; Lattey, K.; Capone, D.; Gishen, M.; Godden, P. *Dekkera/Brettanomyces* yeast—an overview of recent AWRI investigations and some recommendations for its control. Bell, S.M.; de Garis, K.A.; Dundon, C.G.; Hamilton, R.P.; Partridge, S.J.; Wall, G.S., (eds.) Grapegrowing at the edge; Managing the wine business; Impacts on wine flavour: proceedings of a seminar; 10–11 July 2003, Barossa Convention Centre, Tanunda, S.A. Adelaide, S.A: Australian Society of Viticulture and Oenology; 2003: 41–50.
757	Vidal, S.; Cheynier, V.; Waters, E.; Francis, L. The effects of tannins, anthocyanins, ethanol and polysaccharides on red wine mouth-feel. Allen, M.; Bell, S.; Rowe, N.; Wall, G., (eds.) Use of gases in winemaking: proceedings of a seminar; 10 October 2002, Australian Mineral Foundation, Glenside, S.A. Adelaide, S.A: Australian Society of Viticulture and Oenology; 2002: 34–38.
758	Eglinton, J.; Henschke, P.; Høj, P.; Pretorius, I. Winemaking properties and potential of *Saccharomyces bayanus* wine yeast—harnessing the untapped potential of yeast biodiversity. *Aust. N.Z. Wine Ind. J.* 18(6): 16–19; 2003.
759	Vidal, S.; Francis, L.; Williams, P.; Kwiatkowski, M.; Gawel, R.; Cheynier, V.; Waters, E. The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium. *Food Chem.* 85: 519–525; 2004.
760	Vidal, S.; Hayasaka, Y.; Meudec, E.; Cheynier, V.; Skouroumounis, G. Fractionation of grape anthocyanin classes using multilayer coil countercurrent chromatography with step gradient elution. *J. Agric. Food Chem.* 52: 713–719; 2004.
761	Francis, L.; Cynkar, W.; Kwiatkowski, M.; Høj, P. The Bioprep5 robotics system: the development of an automated grape analysis sample preparation unit. *Aust. N.Z. Wine Ind. J.* 19(1): 12–16; 2004.
762	D'Incecco, N.; Bartowsky, E.; Kassara, S.; Lante, A.; Spettoli, P.; Henschke, P. Release of glycosidically bound flavour compounds of Chardonnay by *Oenococcus oeni* during malolactic fermentation. *Food Microbiol.* 21: 257–265; 2004.
763	Vidal, S.; Courcoux, P.; Francis, L.; Kwiatkowski, M.; Gawel, R.; Williams, P.; Waters, E.; Cheynier, V. Use of an experimental design approach for evaluation of key wine components on mouth-feel perception. *Food Qual. Pref.* 15: 209–217; 2004.

764	Howell, K.S.; Bartowsky, E.J.; Fleet, G.H.; Henschke, P.A. Microsatellite PCR profiling of *Saccharomyces cerevisiae* strains during wine fermentation. *Lett Appl. Microbiol.* 38: 315–320; 2004.
765	Lynd, L.R.; von Blottnitz, H.; Tait, B.; de Boer, J.; Pretorius, I.S.; Rumbold, K.; van Zyl, W.H. Converting plant biomass to fuels and commodity chemicals in South Africa: a third chapter? *S. Afr. J. Sci.* 99: 499–507; 2003.
765 a	Ruediger, G.A.; Pardon, K.H.; Godden, P.W.; Pollnitz, A.P. Removal of pesticides from red and white wine by the use of fining and filter agents. *Aust. J. Grape Wine Res.* 10: 8–16; 2004.
766	Baldock, G.A.; Hayasaka, Y. Screening method for petroleum-derived aromatic hydrocarbons in wine. *Aust. J. Grape Wine Res.* 10: 17–25; 2004.
767	I.S. Pretorius, I.S. Tailoring new grapevine varieties for the wine industry. *Acenologia* (May 2004; http://www.acenologia.com/ciencia67_1ang.htm)
768	Vidal, S.; Francis, L.; Noble, A.; Kwiatkowski, M.; Cheynier, V.; Waters, E. Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. *Anal. Chim. Acta* 513: 57–65; 2004.
769	Cozzolino, D.; Kwiatkowski, M.J.; Parker, M.; Cynkar, W.U.; Dambergs, R.G.; Gishen, M.; Herderich, M.J. Prediction of phenolic compounds in red wine fermentations by visible and near infrared spectroscopy. *Anal. Chim. Acta* 513: 73–80; 2004.
770	Bartowsky, E.J.; Henschke, P.A.; Høj, P.B.; Pretorius, I.S. Chasing wine aroma — Does *Oenococcus oeni* have the potential to release aroma compounds from authentic grape precursors? *Aust. N.Z. Wine Ind. J.* 19(2): 24–26, 28, 30–31; 2004.
771	D'Incecco, N.; Bartowsky, E.; Kassara, S.; Lante, A.; Spettoli, P.; Henschke, P. Release of glycosidically bound flavour compounds of Chardonnay by *Oenococcus oeni* during malolactic fermentation. *Food Microbiology* 21: 257–265; 2004.
772	Pollnitz, A.P.; Pardon, K.H.; Sykes, M.; Sefton, M.A. The effects of sample preparation and gas chromatograph injection techniques on the accuracy of measuring guaiacol, 4-methylguaiacol and other volatile oak compounds in oak extracts by stable isotope dilution analyses. *J. Agric. Food Chem.* 52: 3244–3252; 2004.
773	Alexandre, H.; Costello, P.J.; Remize, F.; Guzzo, J.; Guilloux-Benatier, M. *Saccharomyces cerevisiae–Oenococcus oeni* interactions in wine: current knowledge and perspectives. *Int. J. Food Microbiol.* 93: 141–154; 2004.
774	Vidal, S.; Courcoux, P.; Francis, L.; Kwiatkowski, M.; Gawel, R.; Williams, P.; Waters, E.; Cheynier, V. Use of an experimental design approach for evaluation of key wine components on mouth-feel perception. *Food Qual. Pref.* 15: 209–217; 2004.
775	Jones, P.R.; Kwiatkowski, M.J.; Skouroumounis, G.K.; Francis, I.L.; Lattey, K.A.; Waters, E.J.; Pretorius, I.S.; Høj, P.B. Exposure of red wine to oxygen post-fermentation — If you can't avoid it, why not control it? *Aust. N.Z. Wine Ind. J.* 19(3): 17–20,22–24; 2004.

APPENDIX 5. Media Interviews

Date	Staff	Discussed	Media
2 Jul 03	Peter Godden	*Dekkera/Brettanomyces*, particularly sensory thresholds for aroma compounds	Tim White, *The Australian Financial Review*
9 Jul 03	Peter Godden	Article on up-coming AWRI Roadshows	Mark Osborne, *National Grapegrower*
9 Jul 03	Peter Godden	Testing of juice and wine proteins as an aid to varietal identification	Max Allen, *The Weekend Australian, Divine*
14 Jul 03	Peter Godden	*Dekkera/Brettanomyces*, background, particularly sensory thresholds for aroma compounds	Jeremy Oliver
22 Jul 03	Sakkie Pretorius	GMOs	Jamie Good, *Harpers*
28 Jul 03	Peter Godden	Information for article on wine closures	Peter Brady, *National Grapegrower*
1 Aug 03	Peter Godden	Discuss issues related to Heather Smyth's work on wine flavour	Tim White, *The Australian Financial Review*
10 Aug 03	Sakkie Pretorius	GMOs	Andrea Schumacher, *Bild der Wissenschaft*
12 Aug 03	Peter Godden	Update on results from the AWRI closure trial	Birgitt Baader, *Choice*
12 Aug 03	Paul Henschke	Yeast and wine flavour	ABC Radio Newcastle
13 Aug 03	Paul Henschke	Yeast and wine flavour	Chris Coleman, ABC Radio Sydney
16 Aug 03	Paul Henschke	Controlling the microbial formation of flavour in wines	Caitlin Fitzsimmons, *The Australian* (article published page 3, 19 Aug 03 *Grape new yeasts win by a nose*)
18 Aug 03	Peter Godden	Information on oak sourced from regions outside of France and the USA	Guy Woodward, *Decanter*
20 Aug 03	Paul Henschke	Novel yeast and wine flavour diversity	Paul Bevan, ABC Radio Newcastle
21 Aug 03	Peter Godden	Information regarding the technical performance of a branded wine closure	Tim White, *The Australian Financial Review*
21 Aug 03	Peter Godden	Information regarding the technical performance of a branded wine closure	Chris Snow, *Drinks Bulletin 'Booze News'*
26 Aug 03	Peter Godden	Regarding AWRI *Bayanus* sp. yeast, and the flavour profiles produced Wine flavour in general	Craig Zonker, *ABC Radio*, Rockhampton
26 Aug 03	Paul Henschke	Novel yeast and wine flavour diversity	*Herald Sun* 'Mmmm & taste the peel in that' *Adelaide Advertiser* 'Fruit of the vine' *Daily Telegraph* 'Strange fruit for new drops' Ferret.com.au 'Honey and apricot wine flavours soon on menu'
28 Aug 04 29 Aug 04	Creina Stockley	Nature article *Small molecule activators of sirtuins extended* S. cerevisae *lifespan* by Howitz et al. 2003	Craig Zonker ABC Radio Rockhamption Michael Condon ABC Radio Country Derek ABC Radio Melbourne Skye McCarthy ABC Radio Western Australia
18 Sept 03	Sakkie Pretorius and Peter Godden	The role of The Australian Wine Research Institute within the Australian wine industry	Nicolas Glimois, France 5 TV
22 Sep 03	Peter Godden	Cork issues – particularly TCA incidence	Helen Matherson, *The Australian*. Article published on 30 Sept 03
25 Sep 03	Peter Godden	*Brettanomyces*	Martin Moran, *Wine Republic* (Republic of Ireland)
7 Oct 03	Sakkie Pretorius	GMOs	Adam Lechmere, *Decanter*
15 Oct 03	Peter Godden	The current state of knowledge concerning the ageing of wine post bottling, and the most important factors that might determine how a wine ages	Jenni Port, *The* (Melbourne) *Age*. Article published on 18 Nov 03
16 Oct 03	Sally-Jean Bell, Creina Stockley, Peter Høj	CCA-treated posts in vineyards	Margaret Paton (nee Jakovac) Blue Mountains based freelance writer, writing for *Town and Country Farmer* magazine
27 Oct 03 29 Oct 03 6 Nov 03	Peter Høj	Wine aroma and flavour research survey release	Stewart Stansfield, ABC Mt Gambier Kim Fuhrmann, ABC WA Kathryn Attwood, ABC Orange Skye McCarthy, ABC Radio WA Danny Kennedy, ABC Western Queensland
29 Oct 03	Peter Godden	Closure issues	Claire Konkes, *Tasmanian Country* (newspaper)
11 Nov 03	Peter Godden	Contribution for article on the McLaren Vale wine region	Jane Ferguson, *Country Style magazine*
27 Nov 03	Helen Holt and Leigh Francis	Science of creating the perfect drop	Tim White, *The Australian Financial Review*
5 Dec 03	Peter Godden	Development of wine in bottle; and the possibility of TCA taint from screw caps	Sally Easton, Winewisdom, freelance journalist, UK
11 Dec 03	Peter Godden	The concept of 'extractable' or 'releasable' TCA from cork	Sally Easton, Winewisdom, freelance journalist, UK
23 Dec 03	Peter Godden	AWRI's historical data on the composition of Australian wine, with special focus on Langhorne Creek Cabernet Sauvignon seminar presentation	Anita Donaldson, *The Australian and New Zealand Grapegrower and Winemaker*
6 Jan 04	Peter Godden	Up to date information on *Brettanomyces* for use in a 'consumer's guide'	Jeremy Oliver
7 Jan 04	Peter Godden	Sabate 'Diamond' technical corks	Tim White, *The Australian Financial Review* (Article published 9 and 10 Jan 04)
9 Jan 04	Peter Godden	Effects of temperature on the volatility of wine aroma compounds, and overall perception of wine aroma	Tim White, *The Australian Financial Review*
12 Jan 04	Peter Godden	The use of SO_2 in wine	Jamie Goode, *Harper's* and for a book on wine science for Mitchell Beazley publishers
21 Jan 04	Peter Godden	The AWRI, particularly the purpose of Roadshows	Virginia Egerton-Warburton, *Manjmup-Bridgetown Times*

Date	Staff	Discussed	Media
29 Jan 04	Peter Godden	Possible sources of TCA in wine	Hank Behar, *Beverage Aisle* Magazine (USA)
2 Feb 04	Peter Godden	The use of added tartaric acid in Australian winemaking	Brendan O'Keefe, *The Australian*
6 Feb 04	Peter Godden	Comments on potential quality of upcoming vintage and research trials with a vintage focus	Mark Osborne, *Australian Vignerons,* and *National Grapegrowers*
16 Feb 04	Peter Godden	The potential effects of smoke from fires on grapes and wine, with reference to fires in Bridgetown, WA, in December 2003	Skye McCarthy, ABC Radio, WA
5 Mar 04	Sakkie Pretorius	The AWRI and its contribution to the Australian wine industry	Jacqueline Jensen, *Wines & Spirits – Gastronomi* (Denmark)
10 Mar 04	Peter Godden	Perception of vintage conditions to date, with a focus on contact received from WA industry	Jackie Granger, ABC Radio South West (WA)
15 Mar 04	Peter Høj	Role of innovation in Australian wine industry	Cees van Casteren, *Wijn plein* (The Netherlands)
6 Apr 04	Peter Godden	Request for image of closure trial bottles for article on closures	Gael Arthur, *Good Life in Vancouver*
27 Apr 04	Peter Godden	General discussion for an article on Riesling, and styles of Riesling wines	Nick Stock, *Adelaide Review*
27 Apr 04	Peter Godden	Article on Champagne: how to calculate the rate of chilling of wine in various size bottles when placed in iced water?	Jeremy Chun, *Mens Style* magazine
19 May 04	Peter Godden	Alternative closures, particularly screw caps	Alan Dick, *The Land* newspaper
19 May 04	Peter Godden	Issues relating to taints in grapes and wines caused by smoke from bushfires and prescribed burning	Ron Tate, ABC Radio, Bunbury

APPENDIX 6. Institute Committees

Staff member	Management Advisory	Research Steering	Industry Services Steering	Communication Steering	Analaytical Service Steering	Information Technology	Biosafety	Occupational Health and Safety	Staff Code Negotiation	Chemistry Laboratory Management Committee
E.J.C. BARTOWSKY						X		X	X	
S.-J. BELL	X	X	X	X	X					
R.J. BLAIR	X			C						
D. CAPONE									R	
A.D. COULTER	X		X		X					
G.D. COWEY								X		
C.G. DANIEL				X						
M.A. DE BARROS LOPES	X	X					X			
R.L. EDWARDS						X				
J.M. EGLINTON				X		C	C		R	
P.C.H. EICHINGER	X		X		X		X			
I.L. FRANCIS	X	X	X							
M. GISHEN	X	X	X		C					
Y. HAYASAKA	X	X	X							
P.A. HENSCHKE	X	X			X			C		
M.J. HERDERICH	X	X								
P.B. HØJ	C	X	X	X	X	X			X	
H. GOCKOWIAK									X	
P.W. GODDEN	X	X	C	X	X				X	
H.E. MUHLACK	X								X	
I.B.M. OATS									X	
K.F. POCOCK					X		X	X		
A.P. POLLNITZ						X			R	X
I.S. PRETORIUS	X	C								
M.A. SEFTON	X	X			X					
G.K. SKOUROUMOUNIS									X	X
C.S. STOCKLEY		X		X						
R.L. TAYLOR						X				
D. TYNAN								X		
E.J. WATERS	X	X								

C = Denotes holder of Chair
R = revolving representative

The staff of The Australian Wine Research Institute

Front row (left to right):	Sakkie Pretorius, Rae Blair, Kate Lattey, Julie McConnell, Rachel Edwards, Peter Høj
Second row:	Creina Stockley, Kevin Pardon, Hans Muhlack, Pauline Jorgensen, Catherine Daniel, Gordon Elsey
Third row:	Robyn Willmott, Gayle Baldock, Yoji Hayasaka, Bob Dambergs, Melissa Francis, George Skouroumounis, Maria Mills, Holger Gockowiak
Fourth row:	Kate Beames, Narelle D'Costa, David Boehm, Mango Parker, Wies Cynkar, Heather Donnell, Richard Muhlack, Peter Eichinger
Fifth row:	Jane McCarthy, Kate Howell, Heather Smyth, Cristian Varela, Helen Holt, Heather Brooks, Randell Taylor, Chris Curtin
Sixth row:	Sandra Lloyd-Davies, Les Janik, Miguel de Barros Lopes, Jenny Bellon, Ella Robinson, Paul Smith, Greg Ruediger, Peter Godden, Alan Pollnitz
Seventh row:	Paul Henschke, Sally-Jean Bell, Mariola Kwiatkowski, Maria Birse, Katryna van Leeuwen, Carol Sigston, Ingrid Oats, Markus Herderich, Daniel Tynan, Jelena Jovanovic
Eighth row:	Leigh Francis, Jeff Eglinton, Tracey Siebert, Eveline Bartowsky, Elizabeth Waters, Ken Pocock, Matthew Holdstock, Hentie Swiegers
Back row:	Slavko Bekavac, Anthony Heinrich, Matthew Cream, Mark Gishen, Geoff Cowey, Dimitra Capone, Sue Milnes
Absent:	Belinda Bramley, Adrian Coulter, Daniel Cozzolino, Patrik Jones, Danielle Leedham, Rhonda Packer, Mark Sefton