

48TH ANNUAL REPORT

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30 JUNE 2002

PRESENTED TO
THE AUSTRALIAN WINE INDUSTRY

Among all the happenings of the past year, a number have a special significance for the Australian Wine Research Institute. Three of these, in particular, have profound and far reaching implications.

The first, was the publication of a paper in *Science* co-authored by the Director, Professor Peter Høj. One may well ask, so what? The Institute has papers published regularly in top quality scientific journals. It is not every week, however, that an Institute publication appears in *Science* – in fact this is the first one ever and it is only the second Institute publication to appear in an absolute 'A-list' journal following a publication in *Nature* in 1966. This is the Institute's equivalent of an icon wine – it doesn't necessarily contribute enormously to the bottom line but it has real importance for the image of the Institute. While it is very obvious that the core pursuit is science not marketing, the current difficult climate for recruiting quality research staff means that publication of papers such as this are very important factors in helping to attract quality staff.

The second very significant event was the publication by GWRDC of an independent consultant's evaluation of the value of R & D conducted by the Australian wine industry. For the first time we have a detailed quantification of the value of research and considering the current discussions on R & D levies, it could hardly have come at a better time. I commend as compulsory reading, the detailed report on this evaluation in Peter Høj's Director's report. Hopefully, every technical manager in the industry will ensure that their CEO's and financial officers are appraised of its contents.

The third significant event which I would like to mention is perhaps a little more obvious – the successful bid for a major national research facility in which the Institute and CSIRO Plant Industry are the major shareholders along with The University of Adelaide and the South Australian Research and Development Institute (SARDI). The new facility which will be known as the National Wine Industry Research Cluster (NWIRC) has far greater significance than just being another building with instruments and salaried researchers operating them. It actually represents a major conjunction of the vine and wine and as its stature rises with its outputs being noted, many of us would have hopes that it first acts as a bridge bringing these two cultures together and then as a cement to bind them together inseparably. We are delighted to have been able to appoint Mr Peter Wall as Chairman of NWIRC Pty Ltd, as he possesses the experience and capability to guide this facility particularly through the difficult 'start-up' phase.

During the year Institute staff put substantial effort into the organizing and the running of the Australian Wine Industry Technical Conference. This was the eleventh technical conference and was certainly one of the most successful ever attracting 1448 Australian and 226 international delegates. Conference Manager Rae Blair, Workshop Coordinator Peter Godden and the industry-based Conference Planning Committee are to be congratulated on another very professionally organized conference.

It is instructive to consider how Analytical Services have become the vanguard of an important new project for the Institute. Through the background work done by the oak flavour group, analysis of 4-ethylphenol has been added to the list of routine analyses available to winemakers. For the last year or so, the 4-ethylphenol analysis has been running at about double the budgeted figures effectively becoming the signal to embark on a detailed project on *Brettanomyces* incidence and control. Because of the large amount of anecdotal experience on this subject, Institute staff will be endeavouring to capture as much information from industry as possible as a guide to research on this subject.

Two Council members have retired during the past year. Richard Gibson, who has made valuable contributions since 1995 has moved on from the corporate wine world to establish his own consultancy. Our former Chairman, Doug McWilliam merits special mention. Doug has been a major factor in moulding the current shape of the Institute having spent five years on Council before assuming the role of Chairman in 1992. During his nine year period as Chairman, Doug provided an enthusiastic but balanced influence for staff and Council. He successfully combined attention to financial detail with a practical focus on maximizing research outcomes. Doug's Chairmanship was indeed a job well done.

I would also thank the remaining members of Council for their continued contribution and support and welcome the future contributions of new Councillors, Jim Brayne and Peter Dawson.

Institute staff members have again been very diligent and enthusiastic not only in their core research activities but many have put a great deal of effort into extending the message into the industry via roadshows, seminar and conference presentations and workshops. Many staff have also been forebearing in their acceptance of the reporting strings attached to our research funding, when their burning desire is to research not report.

The unflagging energy and task orientation of the Director Professor Peter Høj deserves special mention. On behalf of the Institute's Council and staff I would like to thank Peter for his outstanding efforts to drive the Institute for the benefit of the industry. We look forward to enhancing Peter's capacity even further by the appointment of a Director of Research in the future.

Finally, the recent Five-Year Planning task for the Institute notes two significant areas which will enable the Institute to improve its outputs for industry benefit.



Robin Day
Chairman of Council

In the medium term, more energy will be put into harnessing industry experience to improve the usefulness of research outcomes and more effort will also be put into communication of research results so that the industry as the owner of the AWRI, continues to derive maximum benefit from its investments. In the long-term, we will continue to build capacity by creating an environment to which good people are attracted because of an appropriate balance between longer-term strategic research and shorter-term problem based research, with near immediate flow-on benefits to industry.

Robin Day
Chairman of Council

Chairman

At the Council meeting held on 6 November 2001, Mr R.E. Day was elected Chairman of Council.

Members of the Executive Committee

Mr R.E. Day
Mr P.F. Hayes
Professor P.B. Høj
Mr T.W.B. James
Mr G.R. Linton
Professor S.D. Tyerman

Deputy Members of Council

Mr N.P. Blieschke
Mr L.P. Deans
Dr P.R. Dry
Mr A.M. Kennedy
Mr D.J. McWilliam
Mr J. Northey
Dr N.S. Scott

Meetings

Ordinary General Meeting

The 47th Ordinary (Annual) General Meeting was held on 6 November 2001.

Council

The Council of the Institute met on the following dates: 24 July 2001, 6 November 2001, 26 February 2002, 9 May 2002. Executive members of Council met on 3 August 2001 and 4 December 2001.

Funding

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

Appreciation

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

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



Members of Council

Stephen Herman

Jim Brayne

Peter Dawson

Peter Hayes

Geoff Linton

Tim James

Nigel Scott (deputy for Robert Walker)

Robin Day

Geoff Scollary

Peter Høj

Robert Walker

Our lives would be unimaginable without science – what would our industry be like without science and innovation?

*'Wherever we turn in our daily lives, we constantly encounter reminders of the contributions of science and technology For the most part they could not have been foreseen decades earlier, when the research was being done, and would not have been available without vigilance and patience in R&D funding. The technologies we enjoy today – e.g. those dealing with information, global positioning, and biomedical advancement, to pick just three – are really the fruits of research seeds planted decades ago'*¹

Companies whether large or small naturally seek to contain costs to maximize profits of benefit to its owners and as a means to secure further growth. It should, therefore, be a natural part of the wine industry's *modus operandi* to ascertain whether its investment in R&D delivers value for the buck.

The benefits from investment in R&D are dynamic in nature and derived over different time periods and the returns are often hard to measure using traditional economic indicators. Nevertheless, empirical evidence typically shows that a one per cent increase in the stock of R&D leads to a rise in output of 0.05 – 0.15 per cent of GDP.²

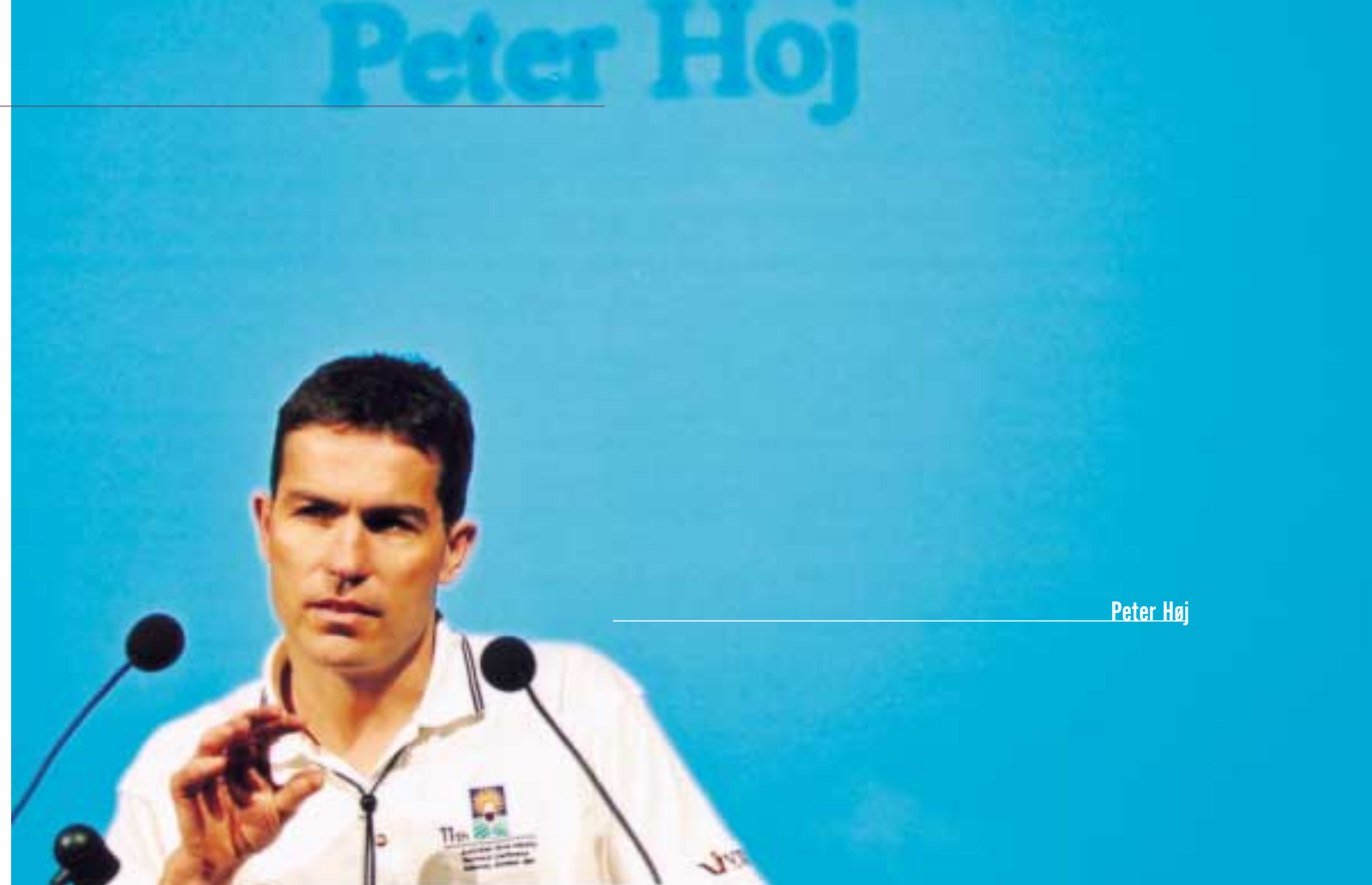
Notwithstanding the difficulties in estimating the returns on R&D investment, the Grape and Wine Research and Development Corporation (GWRDC) nevertheless undertook a detailed and systematic cost benefit analysis of its research portfolio. The result showed that the likely benefit to cost ratio of the GWRDC investment was 8.4, not a bad return on investment and from an industry point of view even more impressive given that a maximum of 50% of the input was derived from collected grape and wine levies. A less rigorous but by no means less revealing 'back of the envelope' cost benefit analyses can also be performed as follows: the progress in our understanding has been phenomenal and most people I have spoken to would readily agree that R&D over the past 30 or so years would have contributed 10 cents to the profit that can be made from a 750 mL bottle of wine through increased vineyard productivity, prevention of product spoilage and through genuine increase in quality. With Australia's annual winegrape harvest of approximately 1.6 million tonnes this would translate into an annual contribution to net income of \$160 million per annum. Such a figure compares favourably with an annual cash contribution from industry of less than \$10 million per annum and illustrates the significant return on investment that strategic R&D conducted within an application framework is able to generate. It can thus be argued, that the current size, the contribution to the Australian economy, the global influence and the market share of the Australian wine industry would be only a shadow of what it is today, had the industry not committed itself to a concerted financial and human contribution to R&D in the past 50 years.

Having realized the contribution innovation has made to the industry so far, the question arises as to whether future returns will be as spectacular and indeed whether enhanced investment over and above current levels are warranted? The answer to the latter question is almost certainly yes.

It is estimated that more than 50% of GDP in the major OECD economies is knowledge-based³ and, at the same time, knowledge is accumulating at an ever increasing rate. It is postulated that, thirty years ago, knowledge doubled every fourteen years – now it doubles every seven years, a phenomenon that many wine industry practitioners might recognize in the form of an ever increasing pile of unread reports, papers and books. Knowledge itself does not translate into economic advantage – for this to happen the process of innovation must kick in; innovation being the people-dependent process that turns knowledge into outcomes much like photosynthesis uses light to turn water and carbon dioxide into sugar.

It is, therefore, my contention that the Australian wine industry will be well served by continuing and possibly further enhancing its long-term investment in R&D, especially where the R&D is conducted according to certain principles by which the Institute seeks to operate:

- As a research organisation, we recognize that customers are people with problems and money. Our activities must strike an appropriate balance between the pursuit of scientific excellence and the meeting of customer needs.
- We should do research as part of an agenda to provide knowledge and technology for the improvement of existing, and development of new, products. We should provide objective information which allows our industry to overcome ill-founded technical barriers to the trade of its products.
- As a research provider, we must take a portfolio approach to our offerings: providing both long-term research and short-term problem solving to our customers, and linkages between the two. A pointer to success is our addressing of problems that include elements which, if solved immediately, give the industrial partner(s) a near immediate return, together with elements that offer long-term potential for applied research in the decades to come.
- As a research provider, we understand that research without a commitment to knowledge transfer is inconsequential. Knowledge transfer has been said to be 'a body-contact sport' – we agree.
- As a research provider, our role is not to dictate what style and type of wine our industry should be making, but to provide the tools for individual practitioners to achieve the ability to produce the product they wish to present to their defined target customers.
- As a research provider, we should make 'discoveries by observing what everyone else has seen, but think about it in a way that nobody else has done before.'



Peter Høj

Throughout its 47 years of history, the Institute has broadly adhered to these principles and delivered numerous tangible outcomes. It is not the place and time to outline all of our achievements here as they are best assessed through perusal of the 700 Institute publications listed on our web-site (www.awri.com) and the annual highlights listed elsewhere in this report. It is, however, the place and time to remind ourselves that the average investment in R&D by all industries in Australia is 0.64% of GDP compared with the OECD average of 1.53% (2.08% in the US and 1.47% in Canada) and that even a doubling of Australian industry investment in R&D will bring us nowhere near the OECD average. The question to answer is perhaps not where our industry would have been without innovation, but whether we can imagine how much further we can go if we decide to increase wine industry investment eight-fold to match the OECD average?

Peter Høj
Director

Turning now to less abstract issues, it is again a great pleasure to note the significant contributions that past and present staff as well as past and present Council members have made to the Institute over many years. These contributions are continuing and evidence was in 2001/2002 exemplified by the Institute's publication of its 'Closure Trial' and the conduct of the 11th Australian Wine Industry Technical Conference with its record number of attendees and record number of workshop offerings to ensure technology transfer is maximized. A special thanks should go to our immediate past Chairman, Mr Doug McWilliam. Doug joined the Council of the Institute in 1987 and had a distinguished and unselfish career as Chairman from January 1992 until his retirement from the Chairmanship in November 2001.

'New ideas, like seedlings, must be well watered if they are to survive'

P. K. Shaw

¹ Dr Neal Lane, Assistant to the President of the United States of America for Science and Technology, from a speech at the Australian National Press club, October 31, 2000.

² Cameron, G. *Innovation and Growth: A survey of the empirical evidence*, Oxford: Nuffield College.

³ Stevens C, 'The Knowledge-Driven Economy', *OECD Observer*, 200:6-10, 1996

- Publication of the outcomes of the Institute's major wine closure trial in July 2001 resulted in unprecedented media attention, both from industry publications and mainstream print and electronic media in Australia and overseas. The published data has provided objective information on which media commentators have been able to pursue the debate on the relative merits of various types of wine closure.

- Using stable isotope labelled analogues of important wine flavour/aroma compounds, the issue of flavour scalping by closures is being addressed. A preliminary analysis of data for wines that have been stored in bottle for two years has indicated that different types of closures vary considerably in their capacity to absorb flavour compounds from wine.

- Thirty stable isotope labelled analogues of important fermentation volatiles have been synthesised for use in developing new analytical methods for compounds which contribute to wine flavour.

- A new precursor to trihydrodimethylnaphthalene (TDN), which confers kerosene-like characters to aged Riesling wine, has been identified. This knowledge is a prerequisite to better control the development of this character in Riesling.

- A rapid and non-destructive method to measure wine colour was developed. The sample port of a common UV-Vis spectrophotometer was inexpensively modified to enable the insertion of different bottle sizes. In non-coloured glass bottles (Flint) the full visible spectrum range of 350 to 700 nm can be determined. Whilst the full range cannot be determined in the coloured glass bottles, it is possible to determine A 420 nm in French green (pale yellow glass), emerald green and cobalt blue bottles, and by correlation with A 540 nm, in amber (brown glass) and antique green (dull yellow brown glass) bottles.

- Visual comparisons between pairs of white wines to which ascorbic acid was or was not added at bottling have called into question the universal value of A 420 nm ('yellow') as an indicator of brown colour in wines. Wines to which ascorbic acid was added had the higher instrumentally measured browning (A 420 nm) but were not visually assessed as the browner wine. This information points to a need for a careful reassessment of conclusions based on A 420 measurements in relation to the pros and cons of ascorbic acid use in white winemaking.

- Using HPLC and mass spectrometric methods, numerous potentially hitherto unknown red pigments could be detected in wine samples and the presence of hitherto unknown large tannins in grape seeds was demonstrated.

- In the 2002 vintage, the first highly integrated large scale winemaking trials were performed that successfully linked our tannin and anthocyanin research with microbiological studies focused on the fermentation properties of *S. bayanus*, and included experiments on the feasibility of Near Infrared Spectroscopy (NIRS) for fermentation monitoring.

- The application of the 'mouth-feel wheel' was extended to the formal descriptive sensory analysis of several wines from viticultural and winemaking trials. The sensory studies included trials on the effect of canopy architecture, sun exposure and irrigation practices on Shiraz wine, as well as assessments of the impact of powdery mildew infection on the sensory properties of Chardonnay wine, and the identification of mouth-feel differences between Cabernet Sauvignon wines made in the 2001 vintage with *S. cerevisiae* and *S. bayanus* yeasts.

- With advanced sensory studies based on an incomplete factorial design the primary and interactive effects of proanthocyanidin ('tannin') concentration, ethanol, anthocyanins and wine polysaccharides on the mouth-feel perception of wine like media were explored.

- The NIRS technique continues to show promise as a useful tool for the industry and several companies are furthering their adoption of the technology into their business activities. Institute staff are assisting the Cooperative Research Centre for Viticulture's commercialisation activities in further field-proving trials to assist transfer to the broader industry. Commercialisation trials conducted during the 2002 vintage highlighted the significant challenges with both standardisation of the instruments and transfer and robustness of calibrations.

- The evaluation of an NIRS instrument that can rapidly scan whole intact grape berries indicated that the technique was feasible but with the cost of lower accuracy compared to scanning of homogenates of grape berries.

- The NIRS technique has shown promise as a means of rapid measurement of the concentrations of malvidin 3-glucoside, pigmented polymer, tannin, and gallic acid for monitoring their evolution during red wine fermentations.

- Preliminary trials with the FT-IR (Fourier Transform Infrared) spectroscopy technique using a novel sample presentation device (attenuated total reflectance, ATR) showed promise in providing correlative measurement of compounds that are present in relatively low concentration in grapes.

- Further analysis of the role of a *Saccharomyces bayanus* yeast in red wine production compared with a conventional *Saccharomyces cerevisiae* yeast has shown that *S. bayanus* can modify wine flavour not only by affecting the aroma profile, but also by affecting palate and colour through altering the anthocyanin and pigmented 'polymers' content. Appraisal of wines from the 2001 vintage by several winemaking teams has led to a more extensive evaluation during the 2002 vintage.

- Application of the laboratory model system for predicting the effect of fermentation yeast on malolactic bacteria to 84 combinations of 21 yeasts and four bacteria has shown that: 1) Compatibility between yeast strain and bacteria strain depended on the combination of strains involved; and 2) The majority of yeasts were compatible with the four bacteria strains, however, one bacteria strain revealed a higher proportion of incompatibility reactions than the other three strains, suggesting that specific combinations of yeast and bacteria could be chosen for promoting or retarding MLF.



- A study of the precursor compounds required to produce mousy off-flavour by *Dekkera/Brettanomyces* yeast and heterofermentative lactic acid bacteria has revealed several factors that could be used to control off-flavour formation in addition to those which prevent biomass formation, and include controlling residual sugar and oxygen in the case of *Dekkera/Brettanomyces* yeast and ferrous ions in the case of heterofermentative lactic acid bacteria.

- A laboratory-scale red wine fermentation system that can produce wine with comparable colour measurements as achieved on the winery scale has been developed. Using this fermentation system it could be shown that yeast strain clearly had an effect on wine colour, irrespective of the region from which the grapes were sourced.

- Interspecies hybrids between commercial *Saccharomyces cerevisiae* wine strains and strains from all the other *Saccharomyces sensu stricto* species, including *Saccharomyces bayanus*, have been produced. Several of these hybrids have been used in laboratory scale winemaking and commercial winemaking trials where they have been shown to ferment grape juice at rates comparable to commercial wine strains. Sensory analysis by Institute staff and company winemakers indicates that the hybrids have the potential to impart diverse aromas and flavours to wine.

- The identity of genes that code for haze protective factors (HPFs: mannoproteins with haze protective activity) has been unambiguously confirmed. This now gives us the ability to prepare larger quantities of these active materials for pilot scale trials. Studies with these 'HPF' genes also show that these genes may have some impact on the ability of yeast to tolerate cold and ethanol. This is an unexpected bonus and illustrates the serendipitous nature of research.

- Combined heat and proteolytic enzymatic treatment of white wines to remove heat unstable protein and reduce bentonite requirements appears to be a promising procedure for commercial application. Wines with and without enzyme addition were heated at 90°C for 1 minute, then immediately cooled to 16°C to 19°C in a tubular heat exchanger. This combined heat and enzyme treatment, or heat treatment alone, reduced the protein levels and bentonite-fining requirements but had negligible effect on aroma and no effect on palate characteristics.

- 72 workshops held at AWITC were coordinated by Institute staff, with several workshops being presented by Institute staff (see Appendix 1).

- A 'Roadshow' visit was made to Queensland in November 2001. Six senior Institute staff presented a full-day seminar, and Industry Services staff presented two full day seminars concurrently, on each of two days.

- 546 separate wine industry technical problems were investigated during the year, resulting in the analysis of 1223 individual samples, and formal reports on the investigations and advice on avoiding re occurrence of the problems, was provided to the industry practitioners concerned.

- The eleventh Australian Wine Industry Technical Conference™ was successfully held in Adelaide with a record number of delegates registered (1,678 delegates). The formal program was presented by 57 speakers and Chairs, 72 workshops were held, 134 posters were displayed and 178 exhibitors participated in the Trade Exhibition.

- 56% increase in requests for information was fielded by staff of the John Fornachon Memorial Library (3,363 requests).

- The Library's local searchable database now indexes over 44,000 scientific and technical reprint articles.

- The web-accessible database (available only to Australian wine and grape levy payers) doubled in size during the year and now comprises approximately 20,000 records from relevant trade and research publications and proceedings.

- The Institute published 28 papers on Institute activities in refereed and non-refereed publications within Australia and internationally.

- Institute staff gave 78 invited oral presentations in Australia and overseas.

- 29 workshops and 44 posters were presented in Australia and internationally.

- Institute staff presented 50 lectures and coordinated a six week subject to undergraduate students.

- Institute staff supervised 27 postgraduate students and supervised the completion of five theses.

- Institute staff provided a free advice and problem-solving service to Australian winemakers including responding to some 1,600 enquiries from industry.

- Overall, Institute staff responded to 5,720 requests for information during the 2001/2002 year or, to put the statistics into perspective, 22 people contacted the Institute seeking information on every working day of the year. This figure does not include request for work through the Analytical Service.

- The Viticulturist responded to 566 enquiries of which approximately 75% were related to agrochemicals.

- 9000 copies of the Institute's annual publication, *Agrochemicals registered for use in Australian Viticulture 2002/2003* were produced and increased demand has required an additional print run.

- The Institute, in conjunction with The Alfred and Monash University, secured funding from the GWRDC for a new two-year project entitled 'The identification and measurement of potential allergens in wine.'

- The Institute publication, *Analytical specifications for the export of Australian wine*, was republished with information for 47 export destinations.

- Institute staff assisted in the development of National Wine Centre exhibits.

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

Information on seminars, talks and poster papers given to outside organizations, academic lectures delivered, graduate students supervised, and the papers published is tabulated and can be found in Appendices 1–4 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)
- International Trade Advisory Committee (AWBC)
- Wine Industry Technical Advisory Committee (AWBC/WFA)
- NWIRC Pty Ltd Board (National Wine Industry Research Cluster)
- Cooperative Research Centre for Viticulture II 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 Eleventh Australian Wine Industry Technical Conference (7-11 October 2001, Adelaide) (Chair)
- Australian Organising Committee of the 26th World Congress and 81st General Assembly of the Office International de la Vigne et du Vin (OIV) (11-18 October 2001, Adelaide) and was Section President of the Oenology Sub-Commission for the Australian OIV Congress.

He is also the Institute's representative on Adelaide University's Department of Horticulture, Viticulture and Oenology Advisory Committee and the Management Committee of the Department of Horticulture, Viticulture and Oenology. Professor Høj is the current holder of the Australian Wine Industry Chair of Oenology at Adelaide University.

Hans Muhlack is the Public Officer of the Australian Wine Industry Technical Conference Inc. and NWIRC Pty Ltd.

Rae Blair was a member of the Conference Planning Committee for the 11th AWITC and is the Treasurer and Conference Manager of the Australian Wine Industry Technical Conference. She was also a member of the Australian Organising Committee of the 26th World Congress and 81st General Assembly of the OIV.

Creina Stockley is a member of the Australian Wine and Brandy Corporation's Legislation Review Committee, Technical liaison Officer of the Wine Industry Technical Advisory Committee of the Winemakers' Federation of Australia/Australian Wine and Brandy Corporation, a member of the National Reference Committee – Environment Strategy Development (South Australian Wine and Brandy Industry Association), is Vice-President of the Nutrition and Wine Expert Group for the OIV and is on the Advisory Board of *Vinsalud Chile 2002*, Wine and Health International Congress.

Elizabeth Waters is Manager of Program 1 of the Cooperative Research Centre for Viticulture II (CRCVII).

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.

Markus Herderich is leader of project 1.2 of the CRCVII and is an 'Affiliate Associate Professor' at The University of Adelaide.

Leigh Francis is an Editorial Board member of the *Journal of the Science of Food and Agriculture*.

Paul Henschke serves on the Editorial Review Board of the following journals: *Australian Journal of Grape and Wine Research*; *South African Journal of Enology and Viticulture*, *Mitteilungen Klosterneuburg*; and *Australia and New Zealand Wine Industry Journal*.

Eveline Bartowsky serves on the Faculty of Sciences (Waite Campus) Occupational Health and Safety Committee.

Peter Godden was a member of the Conference Planning and Program sub-Committees and was the Workshop Coordinator of a program of 72 workshops held at the Eleventh Australian Wine Industry Technical Conference.

Mark Gishen is leader of project 1.4 of the CRCVII.

Don Buick is a voluntary assessor for the National Association of Testing Authorities in the field of chemical analysis of food and wine.

Matthew Holdstock serves on the Interwinery Analysis Committee.



Visitors to the Institute

Australia

Dr Robert Kagi, Professor of Applied Chemistry, Acting Head, Department of Applied Chemistry, Dean of Science, Curtin University & Director, Centre for Petroleum and Environmental Organic Geochemistry (10 July 2001)

Dr Chris Ford and 12 undergraduate students, University of Adelaide (3 August 2001)

Tony Begley, St Peter's College (7 August 2001)

Peter Kane, Austrade National Manager, Centre Europe (16 August 2001)

Michael Kenneally, Chairman Spatial Australia (16 August 2001)

Nicolo Voeckler, Flinders University (6 September 2001)

Melanie Cooper, *New Scientist* (18 September 2001)

Vivian Boghassian, Food Science Australia (24 September 2001)

Professor O.H. Blake, Vice Chancellor, University of Adelaide (5 October 2001)

Dr Chris Ford and 11 undergraduate students, University of Adelaide (11 October 2001)

The Hon. Peter McGauran, Federal Minister for Science (29 November 2001)

Tony Ryan, Executive Producer Lifelong Learning 5UV Radio, Adelaide University (30 November 2001)

Michel Meunier (wine production lecturer) and **Leigh Schmidtk** (wine microbiology lecturer), Charles Sturt University (5 February 2002)

Brenton Baker, Group Viticulturist and **Alex Sas**, BRL Hardy (8 February 2002)

Members of the Prime Minister's Science Engineering and Innovation Council (Acquaculture): **Dr Wendy Craik**, Chair, Australia Fisheries Management Authority, **Brian Jeffries**, President Tuna Boat Owner's Association, **Professor George Kailis**, Executive Director, MG Kailis Group, **Ron Reichbert**, CEO CRC Reef Research, **Peter Rothlisberg**, Program Leader, CSIRO Division of Marine Research (11 February 2002)

Stephen Strachan, Winemakers' Federation of Australia (13 February 2002)

Tony Martin, Chairman CRCV II and **Dr Jim Hardie**, Chief Executive Officer CRCV (19 February 2002)

Dr Paul Chambers, School of Life Sciences, Victoria University of Technology (21 February 2002)

Members of CSIRO Land and Water Divisional Executive Committee (5 March 2002)

Dr Kees Versteeg, Food Science Australia (8 March 2002)

Dr Greg Organ, Flavour Chemist, Lion Nathan (21 March 2002)

Violetta Misiorek, CSIRO Mathematical and Information Services (24 April 2002)

16 Certificate in Food Technology students from Gilles Plains TAFE accompanied by **Adrian Griffith**, lecturer (6 May 2002)

Michael Burleigh, Foss Australia (13 May 2002)

Board members for Cooperative Research Centre for Viticulture (17 May 2002)

Group of grapegrowers through a coordinated visit from Elders (21 May 2002)

11 Certificate in Food Technology students from Regency Park TAFE accompanied by **Karma Pearce**, lecturer (28 May 2002)

Charles Drew, Scholefield Robinson Horticultural Services (6 June 2002)

International

Brazil

Julio Meneguzzo, - Manager Cave de Pedra Winery, **Cleber Andrade** - Enologist, President Brazilian Association of Enology, **Alberto Miele** – Researcher, **Antonio Santin** - Agronomist Engineer, **Romenilson Souza** - Enologist, Manager Organizacao Leao do Norte, **Jorge Tonietto** – Researcher, **Clovis Boscato** - Enologist, Manager Boscato Ind. de Vinhos Winery, **Ademir Brandelli** - Enologist, Manager Wines Don Laurindo Winery, **Antonio Czarnobay** - Enologist, Cooperativa Vinicola Aurora Winery, **Alexandre Miolo** - Manager Miolo Winery, **Antonio Mioranza** - Manager Mioranza Winery, **Arnaldo Passarin** - Manager Uniao de Vinhos Winery, **Cristiane Passarin** - Manager Uniao de Vinhos Winery, **Werner Schumacher** - Manager Coatec Company, **Lidio Ziero** - Manager Casa Cordelier Winery, President, Association Vale dos Vinhedos (Vineyard Valley), **Mrs Natercia Ziero**, **Alberto Miele**, Ecofisiologia da videria, Embrapa, **João Seibel**, Presidente Executivo, Ibravin, **Lucindo Copat**, Director Tecnico, Salton Vinhos, **Jean Pierre Rosier**, Dr. em Enologia, Epagri, Brazil (11 October 2001)

British Columbia

Allan Markin, Regional Principal at Okanagan University College in British Columbia (19 March 2002)

France

Bertrand Thuillier, ABT Informatique, France (5 July 2001)

Dr Dominique Deltiel, ICV, France (18 October 2001)

Chris Chervin, ENSAT Toulouse, France (5 March 2002)

Germany

Dr Stefan Maus, Freelance Journalist, Germany (5 October 2001)

Professor Rainer Wittkowski, BGVV, Berlin Germany (18 October 2001)

Professor Helmut Guth, University of Wuppertal, Germany (18 October 2001)

Dr Juliane Feurle, Leader Analytical Services, 45C AG, Germany (20 December 2001)

Jochen Basler, Deutscher Weinbauverband E.V., Germany (24 January 2002)

Jürgen Hoffmann, Director of winemaking at Reh Kendermann Winery, Bingen, Germany (12 April 2002)

Japan

Professor Hiroshi Takesako, Meiji University and six postgraduate agricultural science students, Japan (4 September 2001)

New Zealand

Tony Pritchard, Montana Wines and nine winemakers, Hawkes Bay, New Zealand (12 October 2001)

Paul Eden, Team Leader: **Graeme Bradshaw**, Allan Scott Wines, Blenheim; **Quintin Broad**, Waipara; **Peter Kerdemelios**, Nelson Marlborough Institute of Technology; **Stephen McNally**, **Andrew Moore**, Waipara Springs Winery; New Zealand (29 January 2002)

Norway

Frihthof Nicolaysen, DG adjoint & CQ, Arcus Produkter AS, Norway (18 October 2001)

Portugal

Dimatino Da Souza, DRS, Portugal (14 February 2002)

România

Alexandru Radocea, Secretary General of the Ministry of Public Administratin and President National Office for Cadastre, Geodesy & Cartography; **Mirela Iorgulescu**, Director General Bucharest Cadastre Office, **Silviu Soare**, Account Manager Government, ICL Romania (16 August 2001)

South Africa

Professor Isak Pretorius, University of Stellenbosch, **Dr Pierre van Rensburg**, University of Stellenbosch, **Dr Melanie Vivier**, University of Stellenbosch, **Dr Markus Lambrecht**, Distell, Stellenbosch, **Professor Leopold van Huysteen**, University of Stellenbosch (18 October 2001)

Anthony Meduna, **Bosman Bonthuys**, **Carel Hugo**, **David Smit**, **Deon Boshoff**, **Eduard Malherbe**, **Esmarie Smuts**, **Inus van der Westhuizen**, **Jean van Rooyen**, **JG Auret**, **Jonathan Snashall**, **Jurgen Siebritz**, **Lauren Dalton**, **Kathleen Krone**, **Nevin du Toit**, **Rolainie Lotz**, **Takkies Cloete**, **Pieter de Wet**, Eisenburg College, South Africa (23 November 2001)

Spain

Professor Santiago Minguéz, Catalanian Institute for Grape and Wine Research, Spain (18 October 2001)

USA

Jeffrey McCord, Director of Research and Technical Sales, StaVin Incorporated, Sausalito, CA USA (30 July 2001)

David Plumb, Fine Northern Oak, Minesota, USA (4 October, 2001)

M. Carmo Vasconcelos, Associate Professor Viticulture, Oregon State University, USA (29 November 2001)

Dennis Hill, Director of Winemaking, Codera Wine Group, California, USA (7 February 2002)

Leslie Norris, Dry Creek, E & J Gallo, Modesto, USA (7-8 February 2002)

Paul Stafford, Director of Table Wines, E & J Gallo, California, USA (6 March 2002)

Marcello Monticelli, Director of Winemaking, Sonoma Estate Wines, California, USA (6 March 2002)

Terry Lee, Vice President, Research, E & J Gallo, California, USA (6 May 2002)

Ken Ford, President Winery Operations, The Wine Group, California, USA (27 May 2002)

Other

A group of international delegates attending the 81st International Congress and 26th General Assembly of OIV (18 October 2001)





Carolyn Grant

Rae Blair

Trudy Weber

Narelle D'Costa

The Eleventh Australian Wine Industry Technical Conference (7-11 October 2001)

The Australian Wine Research Institute is a co-member with the Australian Society for Viticulture and Oenology of the Australian Wine Industry Technical Conference Inc. (AWITC). Held every three years, the AWITC is the Australian wine industry's premier technical conference, and a major extension effort opportunity for The Australian Wine Research Institute.

Institute staff feature in many of the key roles in the conduct of the Conference, viz: Professor Peter Høj is the Chair, Conference Planning Committee; Rae Blair is the Treasurer and Conference Manager and participated on the Conference Planning Committee; Peter Godden participated on the Conference Planning Committee and was responsible for the coordination of the 72 workshops; Ella Robinson and Trudy Weber assisted with the coordination of the workshops; a large number of staff from the Institute worked some very long hours to assist with the workshops; Jeremy Hack coordinated the poster display, and Hans Muhlack is the Public Officer of the AWITC Inc.

The eleventh Conference, held in Adelaide, enabled the Australian wine industry to come together at the Adelaide Convention Centre to be part of the Centre's first major conference and trade exhibition in its newly expanded facilities. Despite some local and international delegates being unable to attend the event due to the problems with flight cancellations at the time, over 1,650 delegates attended the event from 7-11 October 2001, and took part in the activities.

Around 200 international visitors attended the conference, with the majority of these delegates being from New Zealand and South Africa. Whilst the program, workshops and posters were of immense value to delegates, the ability to meet and informally exchange information with so many national and international colleagues over four days was vitally important. This type of interaction allows delegates to broaden their outlook and facilitate an understanding of local and global issues with consequent opportunities to fine-tune operations in their own organisations.

A valued aspect of the conference was the high quality and variety of the 130+ technical posters. The posters covered a range of oenological and viticultural categories, and poster prizes were awarded to the following posters, with Institute staff co-authoring many of the winning posters:

- *Non-destructive measurement of white wine colour*, authored by G. Skouroumounis, M. Kwiatkowski and E. Waters from The Australian Wine Research Institute;
- *Mechanisms of copper mediated oxidation of (+)- catechin in synthetic white wine* authored by A. Clarke and G. Scollary from Charles Sturt University; and
- a range of wine microbiology posters authored by J. Eglinton, E. Bartowsky, J. Bellon, P. Costello, G. Currie, M. de Barros Lopes, G. Fleet, L. Francis, A. Heinrich, P. Henschke, K. Howell, V. Jiranek, M. Kwiatkowski, P. Langridge, J. McCarthy, A. Pollnitz, A. Markides and B. van Wegen from a range of Australian institutions.

The 72 workshops were well attended with over 1,900 places being sold. It was a massive undertaking to mount 72 workshops, and the input from convenors and presentors was greatly valued. Many delegates found one or more workshops that they wished to attend, and many delegates were able to attend multiple workshops. The conduct of a vast range of workshops proved to be a huge logistical task, from clearing international wines through customs to washing the cardboard smell from thousands of wine glasses, to ensuring delegates were out of the formal sessions, fed and onto one of the many buses for their trip to the workshop venue, just to name a few. Many of the workshops sparked lively debate amongst attendees. Some workshops such as the *Ethical issues facing winemakers – a discussion* will result in minutes being prepared,

which will be circulated to the attendees and to the major wine industry bodies. It was a satisfying outcome that the organisers were able to provide workshops that filled an industry need, and may have a positive impact on the industry's future.

Presentations in the formal program were well attended in the main auditorium which was held from Sunday, 7 October to Wednesday, 10 October. Members of the Conference Planning Committee were pleased overall with the way the sessions had turned out, and quality presentations were evident in all sessions. However, based on the views of many delegates one could probably identify Session 6 (Chaired by Louisa Rose from Yalumba) *Application of sensory science for product development* as the most thought-provoking session, overall. Former Institute Director, Dr Terry Lee (now with E. & J. Gallo), and Jane Robichaud, from Beringer Blass Wine Estates, gave presentations with theories of mapping consumers by their taste/preference, not just simply by their usual demographics, which would appear to be a valuable commercial tool. The Institute's Dr Leigh Francis presented *Characterising 'in mouth' sensory properties of red wine*, which came from research undertaken by The Australian Wine Research Institute, in collaboration with Adelaide University. From this research, a 'mouth-feel wheel' has been developed which identifies and defines standard terminology to describe the feel of a wine in-mouth. Professor Helmut Guth from the University of Wuppertal presented aspects of his seminal work on the reconstruction of varietal flavour in white wines



Delegates viewing the Poster Display



Jeremy Hack

and provided further very hard evidence for the 'dulling' effect alcohol has on the perception of individual aroma compounds. Paradoxically, we might find that in our quest to ripen fruit to maximise the concentration of fruit characters, this benefit is annulled by the consequent increase in ethanol concentration in the final wine. The session was completed by an eloquent presentation by Brian Walsh from Yalumba Wines on the Australian wine show system, and its relevance to our modern industry.

The formal program was supplemented with an oenological and a viticultural colloquium. These colloquia were developed from excellent poster abstracts received which enabled two colloquia to be formed with topical presentations.

The social program at the Conference comprised a Welcome Reception function on the Sunday evening, and a Conference dinner which was held on the Tuesday evening with 820 attendees.

Run alongside each Technical Conference is a trade exhibition, and this year's exhibition was the largest ever held with a Technical Conference. Some 178 exhibitors completely filled the newly expanded facility in Adelaide and, unfortunately, some exhibitors were turned away due to lack of exhibition space. The exhibition was open from Sunday night through to Thursday evening, and was open to delegates and non-delegates. It was pleasing to see so many of the suppliers participating in the exhibition, and the companies endeavouring to achieve maximum information flow to delegates. Many went to great lengths

to ensure their display was distinctive, which created a festival-like atmosphere. The trade exhibition is an important component of the whole event, and it provides a great opportunity for delegates and non-delegates alike to view, in the one venue, a wide range of products and the latest innovations available that support the wine industry.

This type of event is impossible to mount at the low registration fees charged, without the strong support of sponsors. Additionally we believe it is important for students to attend the event, as they are the future of the industry, therefore sponsors money was applied to allow student registration fees to be heavily subsidised. The following sponsors were valuable contributors to the event: Netafim Australia, Vinpac International, Amorim, Seguin Moreau, Chr Hansen, The Stephen Hickenbotham Memorial Research Trust, Winetitles and the Grape and Wine Research and Development Corporation.

Being able to bring together such a large number of Australia's wine industry technicians, and present to them a feast of knowledge, in a variety of forms, was remarked upon by many international visitors as there are few comparable events on the world wine calendar.

Wine grape tannin and colour specification

Staff

Dr Markus Herderich, Dr Leigh Francis, Dr Elizabeth Waters, Dr Zhong Kui Peng, Yoji Hayasaka, Dr George Skouroumounis, Dr Stéphane Vidal, Mariola Kwiatkowska, Kevin Pardon, Gayle Baldock, Kate Lattey, Mango Parker, Maria de Sa, Heather Donnell
Collaborators Dr Patrick Iland, Dr Graham Jones, Stephanie Lambert, David Lee, Renata Ristic (The University of Adelaide)

This project was initiated as collaboration between The Australian Wine Research Institute and The University of Adelaide at the request of industry; an Industry Reference Group composed of twelve senior winemakers advises it. Funding for this project is provided by the GWRDC as well as by the CRCV.

Since the importance of grape anthocyanins to wine colour is well established, effort in the research program on red wine colour has been on the formation of new and more stable wine pigments from the anthocyanins, and the influence of tannins and other phenolic compounds on either stabilising wine colour or augmenting colour through copigmentation interactions. In addition, focus is on methods for thorough structure evaluation of tannins, and on qualitative and quantitative changes of polyphenols during viticulture and winemaking. The studies on red wine mouth-feel concentrated on the sensory evaluation of purified grape tannins and have been extended to evaluate the influence of polysaccharides, anthocyanins, and ethanol concentration on tannin perception.

The ambitious objectives of this challenging multi-disciplinary project are:

- To gain an understanding of the types of compounds important to red wine colour and mouth-feel and of conditions favouring their presence in wine.
- To determine whether the interaction of tannins with other grape and wine compounds modifies their sensory properties.
- To establish viticultural and oenological techniques that enhance wine colour and desirable mouth-feel.

Research staff involved in the Tannin project

It remained particularly difficult to attract Australian researchers with a strong science background for the CRCV-funded post-doctoral and PhD positions. All tannin-related research projects will, however, significantly benefit from the contributions of the new team members: Tangerine 'Mango' Parker (research assistant, polyphenol analysis and Shiraz 'pepper flavour'), Kate Lattey (sensory assistant), and Maria de Sa (CRCV PhD student, 'red wine colour') who commenced in early 2002. In addition, Markus Griesser and Christian Landmann, two post-graduate Food Chemistry students from Germany, had joined the Tannin team for six months as 'occupational trainees' and provided substantial analytical back-up for the 2002 winemaking trials, assisted with method development, and initiated studies on yeast-mediated formation of stable red pigments. Other fluctuations of the involved research staff

included Stephanie Lambert, student at The University of Adelaide, who finished the experimental part of her PhD research on copigmentation and submitted her thesis for the award of the degree of Doctor of Philosophy, and Dr Ewald Swinney (Industrial Research Limited, NZ), who has accepted the position as post-doctoral fellow at The University of Adelaide under the daily supervision of Associate Professor Graham Jones (The University of Adelaide).

Wine pigments

Research in Dr Graham Jones' group (The University of Adelaide) has demonstrated that copigmentation reactions are strongly influenced by pH, with colour enhancement attributable to copigmentation being greatest at higher pH values. Ethanol decreases the copigmentation interaction. Self-association of Malvidin-3-glucoside also appears to be a major cause of colour enhancement. As model experiments show that the extent of intermolecular copigmentation depends on the concentration of anthocyanins, more is likely better in young red wines. Conversely, aged products feature significantly reduced concentrations of anthocyanins and the contribution of intermolecular copigmentation to the colour of older red wines remains to be established.

Stephanie Lambert, a University of Adelaide PhD Scholarship holder, has completed her PhD research on copigmentation and has submitted her PhD thesis entitled *Copigmentation and its impact on the stabilisation of red wine pigments*.

Robert Asenstorfer completed his PhD research at The University of Adelaide on new wine pigments derived from anthocyanins in 2001. Robert has demonstrated that the new wine pigments are more resistant to oxidation as well as to SO₂ bleaching and are much more long-lived than the simple grape anthocyanins. (Asenstorfer, R.E.; Hayasaka, Y.; Jones, G.P. Isolation and structures of oligomeric wine pigments by bisulfite-mediated ion-exchange chromatography. *J. Agric. Food Chem.* 49: 2001; 5957-5963 – Institute publication # 683).

To extend our knowledge on anthocyanin derived stable pigments, the conditions mediating the formation of vitisin A, one prominent red pigment in wine, have been studied in detail by Robert. A manuscript describing the formation of vitisin A in experimental Shiraz wines has been submitted for publication to the *Australian Journal of Grape and Wine Research*.

In collaboration with Robert, Yoji Hayasaka developed and optimised methods for the detection of stable wine pigments by Electro spray ionisation mass spectrometry (ESI-MS/MS). Applying tandem mass spectrometric experiments numerous potential red pigments could be detected in wine samples. With this approach we also identified four novel polyphenol derivatives for the first time as natural products. (Hayasaka, Y.; Asenstorfer, R.E. [2002] Screening for potential pigments derived from anthocyanins in red wine using nano-electrospray tandem mass spectrometry. *J. Agric. Food Chem.* 50: 756-761 – Institute publication # 678).

Anders Hakansson, visiting postgraduate student from Denmark has synthesized two of these Malvidin-derived pigments and confirmed their chemical structure. Their resistance to SO₂ induced bleaching and relative insensitivity to pH induced 'fading' was also demonstrated.

Linking the astringency and mouth-feel properties of wines to tannins and other polymer fractions in wine

For the first time purified tannin preparations have been profiled for their astringency sub-qualities. The sensory studies clearly demonstrated that the intensity of astringency increased with the degree of polymerisation, i.e. with larger molecular mass. However, no bitter properties were associated with the tannins under study.

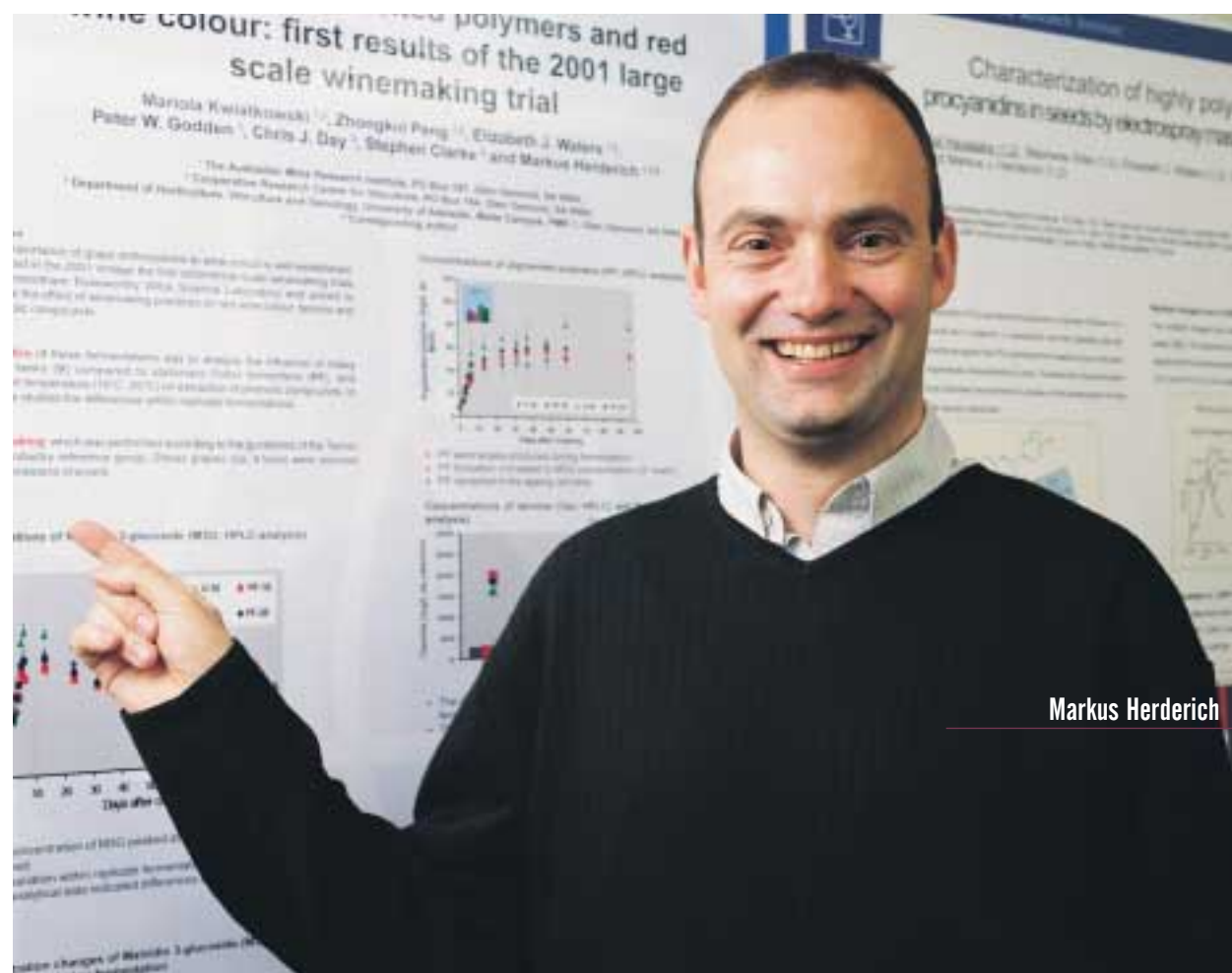
Galloylated tannins such as seed tannins tended to be coarser while the presence of epigallocatechin units that can be found in skin tannins decreased the perception of roughness. It should be noted that the extent to which those grape tannins under study exist in wine is largely unknown and requires further investigation.

These outcomes have been drafted for publication by S. Vidal, I.L. Francis, S. Guyot, M. Kwiatkowska, R. Gawel, V. Cheyrier and E. Waters and the manuscript *The mouth-feel properties of grape and apple proanthocyanidins in a wine like medium* has been submitted for publication.

In addition, the main neutral and acidic wine polysaccharides were proven to significantly enhance the 'fullness' perception. The acidic polysaccharides were also involved in decreasing the astringency properties of a model wine.

A complex sensory study, based on an incomplete block experimental design, was conducted to evaluate the influence of polysaccharides, anthocyanins, and ethanol concentration on tannin perception. The mouth-feel properties of the samples were mainly determined by the tannin concentration, but also strongly influenced by all the other factors. An increase of ethanol concentration was responsible for an increased perception of bitterness. Neutral polysaccharides seemed to lower bitterness but not the astringency perception whereas acidic polysaccharides reduced astringency, specifically its coarse, chalk and pucker sub-descriptors. Presence of anthocyanins contributed both fullness and coarseness/chalkiness.

These studies demonstrated that tannin concentration was the major factor responsible for the mouth-feel differences observed within the set of samples tasted in our experiments. In addition, not only grape tannins but also wine polysaccharides can play an important role in the mouth-feel properties of wine. Neutral and acidic polysaccharides are likely to contribute a 'fullness' sensation to some wines, especially to those with low levels of tannins such as white wines. When present in wine with tannins, as in a red wine, neutral polysaccharides may lower bitterness perception while acidic polysaccharides may reduce astringency and more specifically its coarse, chalk and pucker sub-qualities. Our results also indicate that mouth-feel perception is a highly complex



Markus Herderich

process depending on the presence of each individual component and on interactions between components.

A manuscript summarizing these outcomes, entitled *The mouth-feel properties of polysaccharides and anthocyanins in a wine like medium*, by S. Vidal, I.L. Francis, P. Williams, M. Kwiatkowska, R. Gawel, V. Cheyrier and E.J. Waters was submitted for publication.

Drs Stéphane Vidal and George Skouroumounis started the adoption of Multilayer Coil Countercurrent Chromatography (MLCCC) techniques for the preparative isolation of anthocyanins and related 'small' polyphenols required for extended sensory evaluation. They made good progress with the separation of anthocyanin and related anthocyanin derivatives, and developed the method further by using gradient elution with different solvent systems.

During the 2001/2002 season, Renata Ristic, PhD student at The University of Adelaide, and Dr Patrick Iland continued studies on canopy exposure with Shiraz vines at the Nuriootpa Viticultural Research Station, South Australia. With this on-going trial they compared the physical and chemical composition of berries with chemical composition and sensory properties of wines made from these vines grown under varying degrees of canopy shading. The viticultural trial featured four treatments with eight replicates for each

treatment: 1) vines wrapped in bird netting (shaded treatment), 2) shoots positioned up and down (open canopies) and 3) shoots positioned up and down (open canopies) and 4) a reference treatment, which consisted of vines in another row and represented a typically low yielding, well balanced, open canopy Barossa Valley vineyard.

This exposure trial forms the major part of the PhD research conducted by Renata Ristic who has now commenced writing her thesis. To date, most of the chemical analyses including skin anthocyanins and phenolics, seed catechins and tannins of grapes and wines have been completed, all wines from the 2000 and 2001 vintages have been assessed by sensory studies, and the data is now being analysed by statistical techniques. The results have been presented at the Eleventh Australian Wine Industry Technical Conference (11AWITC) and a draft manuscript on *Grape seed development and its relationship to berry development for Shiraz* has been prepared for publication.

The major outcome of this exposure trial to date is that berries from shaded canopies grown in this experiment at Nuriootpa have lower concentrations of skin anthocyanins and phenolics, and higher concentrations of seed catechins and tannins. The sensory studies at the Institute have revealed that wine made from fruit from the strongly shaded treatment was rated, by a trained sensory panel, as more 'herbaceous' in aroma and more 'viscous' in mouth-feel than the

sun exposed treatments. The sun exposed treatments were rated higher in 'overall fruit flavour' and 'plum' and 'licorice' aroma, they were also more 'drying', 'coarse grain', and 'adhesive' than the shaded counterparts, and deeper in colour. A separate quality assessment panel, evaluating the wines in duplicate, rated the shaded treatment as lower in overall quality, as indicated by the 20 point score system.

During the 2001/2002 growing season, a research collaboration has been initiated between Renata Ristic and Dr Patrick Iland (The University of Adelaide), the Tannin team at the Institute, and Mark Downey and Dr Simon Robinson from CSIRO Plant Industry to study the impact of sun exposure and shade on anthocyanin and tannin properties. The viticultural trial takes advantage of boxes developed at the CSIRO to protect bunches from light exposure without any further modification of the canopy. After fruit set, 400 bunches were enclosed in boxes at a McLaren Vale Shiraz vineyard. At this stage the fruit has been harvested and used for small-lot winemaking. Preliminary data indicate that the total colour of berries from boxed bunches was significantly lower than that of berries from exposed bunches. However, since the size of berries from the sun-protected boxed bunches was lower, the concentration of berry colour (expressed as mg anthocyanins per gram berry weight) was similar for each treatment. Interestingly, the colour parameters of the finished young wine were

lower in the wine from sun-protected boxed bunches. Detailed analysis of anthocyanins, flavonols and flavanols will be carried out on berries and wines to investigate possible causes for this phenomenon.

Under the guidance of Dr Patrick Iland, Institute tannin project members including Christian Landmann and Mango Parker were involved with small lot winemaking and HPLC analysis of fruit sourced from a Cabernet Sauvignon pruning trial at the Clare Valley. This well designed viticultural trial provided a unique opportunity to study the impact of viticultural techniques on grape and wine composition with wines made on an experimental 1 kg scale as well as in commercially relevant 1 tonne fermenters. Conditional on future funding and the on-going support of the involved company (BRL Hardy/Leasingham), it is envisaged that this well designed viticultural trial may continue as a new research element within the CRCV and a preliminary project application will be submitted to CRCV by Dr Patrick Iland (as consultant), Dr Chris Ford (The University of Adelaide) and Dr Markus Herderich.

Methods to quantify the levels of tannins in seed extracts and in wine have been developed, and the identity of polymeric tannins has been rigorously demonstrated by mass spectrometric analysis. (Peng, Z.; Hayasaka, Y.; Iland, P.G.; Sefton, M.; Høj, P.; Waters, E.J. [2001] Quantitative analysis of polymeric procyanidins [tannins] from grape [*Vitis vinifera*] seeds by reverse phase high-performance liquid chromatography. *J. Agric. Food Chem.* 49: 26-31 – Institute publication # 660).

A method to determine anthocyanins and pigmented 'polymers' in wine samples has been published. (Peng, Z.; Iland, P.G.; Oberholster, A.; Sefton, M.A.; Waters, E.J. [2002] Analysis of pigmented polymers in red wine by reverse phase HPLC. *Aust. J. Grape Wine Res.* 8: 70-75 – Institute publication # 686).

At present, the analytical protocols are adopted and validated as routine methods for the determination of oligomeric and polymeric polyphenols in grape, seed and wine samples from numerous viticultural and winemaking trials. We established chromatographic techniques that allowed significantly reduced analysis time and resulted in the higher throughput required for the substantially increased sample numbers from fermentation trials. Continuing our method development for the analysis of anthocyanins and pigmented 'polymers', we optimize the accelerated methods and aim for compatibility between our routine HPLC systems and the research HPLC-MS techniques to facilitate identification and structure elucidation.

A method for characterising the composition of grape tannins using acid hydrolysis in presence of phloroglucinol has been developed, and the analytical protocol has been published. (Kennedy, J.A., Jones, G.P. [2001] Analysis of proanthocyanidins cleavage products following acid-catalysis in the presence of excess phloroglucinol. *J. Agric. Food Chem.* 49: 1740-1746 Institute publication and Kennedy, J.A.; Hayasaka, Y.; Vidal, S.; Waters, E.J.; Jones, G.P. [2001]



Composition of grape skin proanthocyanidins at different stages of berry development. *J. Agric. Food Chem.* 49: 5348-5355 – Institute publication # 682).

With focus on the study of wine tannins the original hydrolysis method is now adopted and validated at the Institute. Subsequently, it will be applied to characterise several tannin fractions from grape seeds and skins, and wine. Preliminary results indicate that wine tannins are more resistant to acid hydrolysis than grape tannins. In addition, it could be demonstrated that pigmented 'polymers' did not yield significant amounts of coloured and easy-to-analyse breakdown products, most likely because pigmented 'polymers' remained intact upon hydrolysis.

Following the publication of the phloroglucinol method, an ESI-MS/MS technique developed by Yoji Hayasaka allowed for the first time the detection of individual tannins on a molecular level. This represents a major progress for our studies aiming to understand the chemical properties of grape tannins used for sensory evaluation and the results have been drafted for publication by Y. Hayasaka, M. Herderich, E. Waters, V. Cheynier, P. Høj and S. Vidal (*Characterization of proanthocyanidins in grape seeds using electrospray mass spectrometry*).

Winemaking practices that effect tannins levels and sensory properties of wine

To study the effect of winemaking practices on colour and mouth-feel, the first commercial scale winemaking trials have been conducted in 2001 at the Hickinbotham Roseworthy Wine Science Laboratory of The University of Adelaide. With these initial 20 fermentations we aimed to analyze the influence of red grape varieties, fermentation tanks and fermentation temperatures on red wine phenolic compounds.

We completed the HPLC analysis of 300+ wine samples from the 2001 Shiraz and Cabernet Sauvignon winemaking trials. Together with the outcomes of the 2002 winemaking trials, these data sets form the basis of chemometrical studies with focus on colour extraction, anthocyanin degradation and the formation of anthocyanin-derived red pigments.

As expected, the concentration of the major grape derived red pigment Malvidin-3-glucoside (M3G) peaked at pressing and then declined immediately. The variation of M3G within replicate fermentations was rather small and demonstrated that the winemaking which followed a protocol developed in close collaboration with our Industry Reference Group, as well as the sampling scheme and the HPLC analysis yielded consistent analytical results. In contrast to the grape-skin derived M3G, the stable pigmented 'polymers' (PP) were largely produced during fermentation and their concentration could be correlated to the amount of Malvidin-3-glucoside and tannin present. Importantly, no significant PP degradation could be observed in the ageing wines. Please note that pigmented 'polymers' is a generic term describing pigmented compounds which are most likely formed from anthocyanins. Pigmented 'polymers' represent non-polar species of higher molecular weight than typical grape-skin anthocyanins which typically elute 'late' at high acetonitrile concentrations upon HPLC analysis. The extraction of tannins and gallic acid was fastest until pressing and was largely finished after the first racking. After fermentation the gross concentration of tannins appeared to remain constant over time.

Dr Bob Damberg performed extensive chemometrical analysis of large data sets derived from HPLC analysis and Dr Chris Somers UV-measurements (e.g. Institute publication # 151) of Shiraz wines made during the 2001 winemaking trials. The outcomes demonstrated that the concentrations of tannin and anthocyanins (expressed as Malvidin-3-glucoside) were the only variables studied that could be clearly correlated with the formation of pigmented 'polymers' during fermentation. In addition, a mathematical model was developed to predict formation of pigmented 'polymers' based on the concentrations of anthocyanins and tannins.

That initial mathematical model to predict formation of pigmented 'polymers' was verified with data obtained by Drs Eveline Bartowsky and Paul Henschke from the Lallemand yeast trial: HPLC analysis of twelve young Shiraz wine samples prepared from grapes sourced at Langhorne Creek and Adelaide Hills with six different yeast strains demonstrated that the model developed with Coonawarra Shiraz for large scale (700 – 800 kg) ferments convincingly described the formation of pigmented 'polymers' during small lot winemaking as well.

Taken together, it was demonstrated that formation of pigmented 'polymers' is significantly enhanced by fermentation and can be directly linked to concentrations of both anthocyanins and tannins in the ferments. Therefore, it can be predicted that a better understanding and greater control of the reactions responsible for PP formation during fermentation can contribute to increased colour stability of ageing red wine. The model also explains the anecdotal evidence that addition of oenotannins before or during fermentation is likely to support the formation of stable colour in red winemaking.

To study the relevance of viable or actively fermenting yeast for the formation of pigmented 'polymers', the Tannin team initiated a research cooperation with the Institute's wine microbiology team. Jeff Eglinton and Markus Griesser, a visiting postgraduate student from Germany, have developed a suitable model system, using chemically defined media, purified anthocyanins and grape derived oenotannins to establish the contribution of yeast to the formation of anthocyanin derived red pigments. This work, still in its early stages, shows considerable promise in regard to explaining the fermentation characteristics which are important for the formation of stable red pigments in red wine fermentations.

The collaboration between the Tannin team and the Institute's wine microbiology team was extended to the 2002 winemaking trials at the Hickinbotham Roseworthy Wine Science Laboratory (HRVSL). By comparing wines made from *S. bayanus* and *S. cerevisiae* with one batch of fruit under controlled and replicated conditions, our focus is on yeast effects on anthocyanin and tannin extraction, pigmented 'polymer' formation, colour stability, and mouth-feel properties.

We operated six rotary fermenters to replicate the outcomes of the 2001 winemaking trials (3 x *S. bayanus*, 3 x *S. cerevisiae*, 20°C, ca. 610 kg of grapes), and a further set of six 1100 kg stationary Potter fermenters (3 x *S. bayanus*, 20°C and 28°C, ca. 700 kg of grapes) to yield additional information on the characteristics of large scale *S. bayanus* fermentations. The grapes for the 2002 trial (ca. 8 tonnes Cabernet Sauvignon) have been generously provided by Orlando Wyndham (Inca Lee and Russell Johnstone). The fruit was sourced from an homogenous Padthaway vineyard, it was carefully randomized by mechanically harvesting into several bins per row and further at the HRVSL during crushing. The 12 fermenting tanks have been sampled twice daily during fermentation until pressing and, besides standard winemaking analysis, all ferments have been analyzed by HPLC for anthocyanins, tannins and pigmented 'polymers', GC-MS for acetaldehyde, and by NIRS. Outcomes of this large trial are still being determined and will be the subject of subsequent reporting.

The Tannin team initiated studies on the application of NIR spectroscopy to monitor red wine fermentations and joined forces with the *Rapid instrumental techniques* project of CRCV Program 1. The preliminary results are promising for Malvidin-3-glucoside, pigmented 'polymers' and potentially tannin. These cooperative validation studies have been extended during the 2002 vintage within the framework of the joint Tannin-Micro winemaking trials.

The 'Mouth-feel Wheel' (Institute publication # 648) is applied routinely to the sensory evaluation of wines from small lot and large scale winemaking trials, and the descriptors for profiling astringency were proven to be efficient to distinguish between different tannin fractions in a qualitative and quantitative manner.

The results of the 2001 winemaking trials have been presented as a poster and at a colloquium at the 11th Australian Wine Industry Technical Conference (see Appendix 1). In addition, the experimental wines were presented to the participants of the two workshops on the *Impacts of grape and wine tannins, and enotannins on red wine structure* at the 11th Australian Wine Industry Technical Conference, organized by Dr Elizabeth Waters with assistance from Dr Markus Herderich and valuable contributions from Pete Bissell (Balnaves), Dr Roger Boulton (University of California Davis), and Dr Graham Jones (The Adelaide University).

The contributions of Dr Leigh Francis (*Characterising mouth-feel properties of red wines*), Renata Ristic (*Studies on seed composition of Shiraz grapes*) and Dr Markus Herderich (*Polyphenols, pigmented polymers and red wine colour: Results of the 2001 large scale winemaking trial*) to the research colloquium at the 11th Australian Wine Industry Technical Conference have been submitted for publication in the conference proceedings which will be available to conference delegates in October 2002.

The Institute's tannin panel performed extensive descriptive sensory analysis on various wine samples derived from viticultural trials, including Dr Sally Bell's trial on the effect of canopy architecture and irrigation practices on Shiraz wine and Dr Patrick Iland's exposure trials, and assessed the impact of powdery mildew infection on the sensory properties of Chardonnay wine, a CRCV project led by Drs Stummer and Scott of The University of Adelaide. The sensory panel also assessed Cabernet Sauvignon wines made in the 2001 vintage at the Hickinbotham-Roseworthy Wine Science Laboratory by the wine microbiology team with a conventional *Saccharomyces cerevisiae* yeast strain and a *Saccharomyces bayanus* strain. The *S. bayanus* wines were rated significantly lower in 'adhesive', 'pucker', 'drying', 'astringent persistence' and 'velvet' attributes than the wine made from the *S. cerevisiae* strain, and higher in 'blackcurrant' aroma. One of the *S. bayanus* wine replicates was rated significantly higher in a 'sawdust/grainy' mouth-feel attribute than the other wines. These observed differences provided evidence that use of this *S. bayanus* strain may confer a 'softening' effect on mouth-feel, without apparently inducing any undesirable characteristics. The results of this study will be validated by the expanded large-scale winemaking study carried out in 2002.

Rapid instrumental techniques

Staff: Mark Gishen, Dr Leigh Francis, Dr Bob Damberg, Professor Peter Høj, Dr Wies Cynkar, Dr Elizabeth Waters, Les Janik, Peter Godden, David Boehm
Collaborators: Bruce Kambouris (BRL Hardy), Dr Graham Jones and Dr Patrick Iland (The University of Adelaide), Russell Johnstone and Inca Lee (Orlando Wyndham), Dr Andrew Kleinig and Eric Wilkes (Southcorp Wines)

Research into rapid instrumental methods has continued to concentrate on pursuing the promising technique of Near Infrared (NIR) spectroscopy. 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. Late in the year 2001, Dr Michael Esler left the Institute to take up a post in Europe and his replacement, Dr Daniel Cozzolino, commenced in July 2002. During the intervening period, Les Janik, formerly of CSIRO Land and Water, joined the team to assist in the processing and analysis of the now large dataset of spectra that has been collected over the past three years. During the year, the project team was heavily involved in the 11th Australian Wine Industry Technical Conference contributing two posters, convening two workshops, and also an invited paper (See Appendix 1). The team also published a paper in a refereed journal on the use of NIRS for the measurement of methanol in grape spirit (Institute publication # 687), and is currently finalising a contributed chapter in a monograph on applications of near infrared spectroscopy in agriculture.

This project has had a primary focus in the last year of furthering the development of a calibration for the rapid prediction by near infrared spectroscopy of red grape colour. This development firstly requires the elucidation of the necessary conditions in order to transfer the calibration that has been developed on the Institute's research grade instruments to others, of both similar and different types, in the field. 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 with assistance from Mr Greg Macpherson, Centre for Innovation.

CRCV Technologies Ltd., with the assistance of a commercialisation consultant, have engaged in negotiations with several instrument manufacturers in their management of trial commercial release of instruments provided with calibrations developed by the Institute. Firstly, with a relatively cheap and portable instrument manufactured in Australia, there have been some difficulties securing hardware that consistently meets the Institute's performance requirements at a price deemed affordable to smaller wine companies.

A second trial using the Institute's NIRSystems6500 instrument, evaluated the performance of developed calibrations after transfer to several standardised instruments. This has also encountered some difficulties. Three 'slave' instruments were standardised to the Institute's 'master' and a calibration dataset (vintages 1999-2001) was then transferred to the 'slaves'. Although the prediction errors appeared promising when determined at the time of standardisation, the results obtained from the transferred calibration when evaluated with 2002 season grape samples were not as accurate as required. The reasons for this, including the atypical nature of the 2002 vintage, are under investigation, but this experience highlights the degree of care required to ensure accuracy and precision before relying on NIR based instrumentation. It is likely that accuracy will be improved with inclusion of 2002 vintage data in the database.

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 (BRL Hardy, Orlando-Wyndham, Southcorp/Rosemount) has meant that more than 2300 berry samples from the 1999, 2000 and 2001 seasons (predominantly Shiraz, Cabernet Sauvignon, and Chardonnay from the Riverland, Barossa and South East SA regions) have been analysed by the conventional laboratory method and by scanning using research grade NIR instruments located at the Institute. The berry samples are scanned with no preparation except for homogenisation using a high-speed laboratory homogeniser. A further 1600 samples were also provided from the 2002 vintage and analysis is underway.

It has been found previously that, while the calibrations appeared to hold across seasons and a calibration could be developed that will give acceptable results for samples from multiple regions or from several varieties, best accuracy seems to be obtained from restricted variable (i.e. vintage, region or variety) data sets. Preliminary work with samples from the 2002 vintage, in which unusually high colour was generally observed, has highlighted the need to further examine the matrix dependency of the NIRS technique. Calibrations developed with the previous three years dataset were not able to predict well the colour of the new samples. This was improved by the addition of a relatively small number of the new samples to the dataset and it is hoped that the variation in the matrix introduced by these samples will improve the overall robustness of the calibration and make it more 'resistant' to vintage-to-vintage variation.

The project has continued to investigate the nature of the calibrations already developed, specifically, in evaluating robustness and various techniques of improving them. Now that the calibration dataset has become quite large it is apparent that non-linearity and matrix effects are important

considerations when using established partial least squares regression (PLS) calibrations as shown by the 'banana-shaped' curve in the left panel of Figure 1. Use of a locally weighted regression technique has shown improvement in the standard error of cross validation (Table 1) and linearity of the correlation curve (right panel of Figure 1) for red grape colour in samples scanned on the Institute's NIRSystems6500 instrument. An alternative approach of careful wavelength selection based on identification of regions of the spectra where there were clear differences in varieties, was also found to provide some relief from the non-linearity observed in the PLS calibration for red grape colour. Although the overall improvement in prediction error and correlation coefficient were not large, the method offers additional promise as a means of developing cheaper, robust, perhaps fixed-filter, field type instruments.

Further trials with two different instruments that are capable of scanning whole berries successfully demonstrated that scanning of whole fruit is feasible, but gives less accurate prediction calibrations compared with those developed by scanning of red grape homogenates (the

Table 1 Standard error of cross validation and correlation coefficient for calibrations developed using partial least squares regression and locally weighted regression.

	'global' PLS regression		locally weighted regression	
	SECV ^a	R ²	SECV	R ²
Colour (mg/g)	0.137	0.904	0.087	0.962
TSS (oBrix)	0.333	0.984	0.367	0.982
pH	0.078	0.813	0.068	0.914

Notes: a. All varieties and regions, full colour range; b. Standard error of cross validation

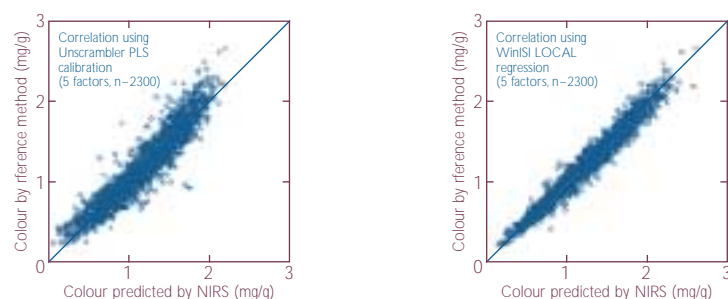


Figure 1 Correlations of colour measured by the reference method with those predicted by NIRS calibrations using partial least squares (PLS) and locally weighted (LOCAL) regression techniques.

Table 2 Correlation coefficient and standard error of cross validation for colour prediction using a Perten DA7000 instrument scanning Shiraz (n=283) and Cabernet Sauvignon (n=215) samples presented as whole berries and as homogenates.

Matrix	Correlation parameter for colour calibration	
	R ²	SECV ^a (mg/L)
Whole berries	0.81	0.15
Homogenates	0.89	0.11

Notes: ^a correlation coefficient between NIRS predicted value and the value derived by the reference method, ^b SECV= standard error of cross-validation.

performance statistics of one such instrument is shown in Table 2). Nevertheless, such instruments may find potential application for on-line or on-harvester measurement. It is likely that for laboratory applications, removing the relatively simple homogenisation step from the analysis is not likely to be beneficial as little time is saved, with substantially poorer accuracy of the analysis. The apparent high importance of the measurement of red grape colour in industry means that it is critical to have the reference method standardised across laboratories. In response to this need, any variations that may be applied to the method by other labs, such as sample handling (freezing/thawing), homogenisation (type and settings), standing times etc are being investigated. It is intended that a standard method will be recommended for the wider industry.

Some preliminary work using 145 samples of commercially made red wine (several varieties) from the Riverland and Sunraysia regions gave promising NIRS calibrations for the prediction of Somers' spectral measures of red wine colour density, hue, total anthocyanin content, degree of ionisation, and chemical age indices 1 and 2.

In collaboration with Institute staff working in GWRDC-funded project AWR 96/1, samples from red wine fermentations were analysed for phenolics and scanned using the FOSS NIRSystems6500. Promising calibrations were developed for the prediction of the concentrations of Malvidin-3-glucoside, pigmented polymer, tannin, and gallic acid. This will need to be scrutinised carefully to ensure that the correlations are robust and not simply co-correlations with more abundant and constantly changing compounds in the matrix such as ethanol. Further samples from trials undertaken in 2002 vintage are currently being analysed and this helps in providing more variation to allow for validation of the method.

Evaluation of the use of NIRS for the rapid determination of glycosyl-glucose (G-G) in white grape juice has been pursued and has proven more difficult than first anticipated. The most recent dataset comprises over 150 samples and the correlation achieved was relatively poor (R² = 0.645, standard error of cross validation = 61.5 μmol/L, over the range 200 to 600 μmol/L), suggesting that only an indicative (e.g. 'high/medium/low') result may be possible with the NIRS technique.

Work is continuing to identify and assess alternative methods and techniques for rapid measurement and some promising preliminary feasibility trials have been conducted using FT-IR (Fourier Transform Infrared) spectroscopy with a novel sample presentation device (attenuated total reflectance, ATR) to obtain scans. This has allowed grape homogenates to be scanned directly in a 'reflectance' type mode. It is hoped that this technique may provide better results for correlative measurement of compounds that have not been well predicted by NIRS and are present in relatively low concentration in grapes such as G-G, and amino acids.

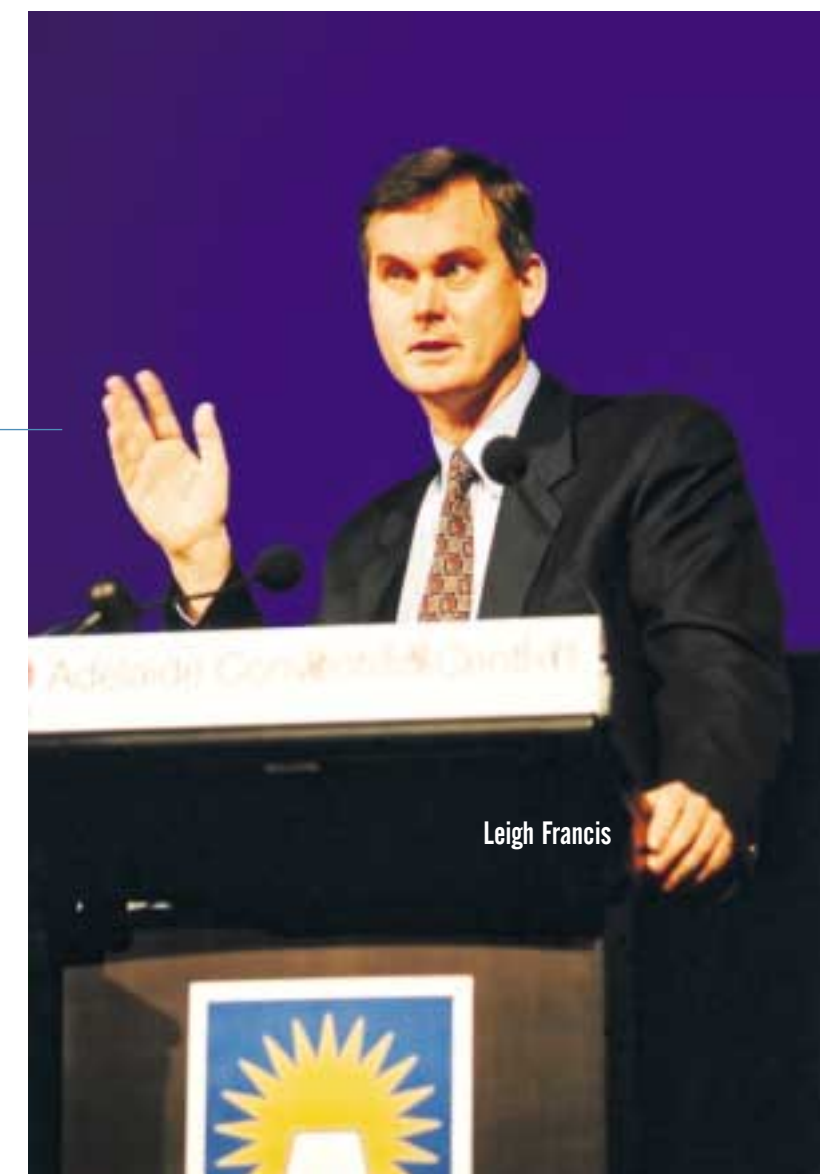
Grape composition and wine flavour

Staff
 Dr Mark Sefton, Dr Leigh Francis, Yoji Hayasaka, Professor Peter Høj, Gayle Baldock, Dr George Skouroumounis, Dr Alan Pollnitz, Dr Elizabeth Waters, Mariola Kwiatkowski, Kevin Pardon, Dimitra Capone

Analysis of wine components and their precursors

A large scale synthesis of the potent wine flavour compound 'wine lactone' as well as its deuterated analogue is well advanced. Wine lactone can impart coconut, woody and citrus-like characters to wine. A synthesis of the naturally occurring (optically active) form is also underway. The published methods to these compounds have been modified so as to improve yields in order to provide sufficient quantities to achieve several objectives. These are: a survey of Australian wines to determine which varieties should be focussed on for measuring wine lactone potential in the vineyard, a study on the rate of formation and yield of wine lactone from given quantities of two grape-derived precursors (already synthesised and discussed in the previous Annual Report), and sensory studies on the relationship between wine composition and wine aroma and flavour. The work on wine lactone forms part of our research portfolio aimed at making available analytical techniques for important flavour compounds and their precursors in grapes.

This project has the long-term objective of understanding how the combination of viticultural and winemaking practices determine the aroma and flavour characteristics of wine and is divided into three broad areas: the identification of grape-derived wine components that affect wine aroma and flavour; studies of the formation and degradation of these wine flavour constituents including the identification of their grape-derived precursors; and finally, studies on how viticultural variables affect the production of wine flavour compounds and their precursors in the grape. Where possible, early findings in this project will be employed to focussed short-term projects. A prime example of this is the so-called flavour-scalping project conducted as an extension to our closure trial as a sub-project to GWRDC-funded project AWR 11. The early outcomes of this sub-project are reported elsewhere in this report.



Leigh Francis

A program of preparing deuterium-labelled analogues of important fermentation esters, acids and alcohols has been successfully completed. Thirty labelled compounds are now available for development of rapid and accurate methods of analysis of the fermentation products using solid phase microextraction and gas chromatography/mass spectrometry. This development is already well advanced and will be applied in GWRDC-funded project AWR 2. We have also begun a collaboration with Professor Philip Marriott of RMIT University in Melbourne to employ two-dimensional gas chromatography techniques in wine analysis. Using synthetic standards supplied by the Institute, Professor Marriott has already achieved good separation of several fermentation volatiles that are not normally separable by conventional techniques. We anticipate that these new developments could open avenues in wine analysis not currently available to us.

The synthesis of labelled analogues of several additional important wine flavour compounds is also in progress. Among these are a group of grape-derived thiols that are key flavour impact compounds in many red and white wine styles.

With the completion of the analytical work on fermentation products we will have analytical methods using stable isotope dilution techniques for more than 50 wine flavour volatiles, and will continue to increase this number over the next twelve months.

Identification of new wine flavour components

Glycosidic extracts of Shiraz and Cabernet Sauvignon grape and vine leaf samples from vineyards in the Barossa Valley have been prepared and their composition investigated. Sensory studies on both mild acid and glycosidase enzyme hydrolysates prepared from the glycosidic extracts have shown that all of these hydrolysates contain unknown compounds which could contribute to wine flavour through their generation from glycosidic precursor forms during vinification or maturation. Although leaves are not used in winemaking, they contain individual glycosides at much higher concentration than do the berries, and the aromas from hydrolysates of these leaf glycosides are similar to those obtained from the fruit. Thus, isolating individual glycosides from leaves and studying the pattern of their hydrolysis products offers greater scope for identifying new flavour compounds than does studying hydrolysates of grape glycosides alone.

Work has begun on using the recently acquired high speed counter current chromatography (MLCCC) apparatus to separate grape glycosides of potential importance to wine flavour. Preliminary investigations have indicated that separation of individual grape glycosides from the main components of the extracts is possible. We expect to have several glycosides purified shortly, and will then be able to study their hydrolysates with the ultimate aim of identifying and developing measurements for new compounds of importance to grape and wine flavour/aroma.

Formation of important wine flavour compounds from grape-derived precursors

β -damascenone is a ubiquitous grape and wine flavour compound which, at low concentrations, gives rise to a stewed apple character. It is, therefore, important to understand the factors controlling its concentrations in grapes and wine. A small-scale synthesis of a glucosidic damascenone precursor has been successfully undertaken. The two isomeric forms of the glucoside can be separated by chromatography. The configuration of these two isomers is being determined and sufficient stocks are being prepared for hydrolytic studies. In the meantime, a preliminary hydrolytic study on a mixture of the two isomers of the glucosidic damascenone precursor has indicated that these isomers are converted exclusively to damascenone, but at a rate which is approximately ten times slower than the corresponding aglycone.

A new precursor to 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) has been identified. At wine pH, this precursor appears to give exclusively TDN, which has a kerosene-like aroma in aged Riesling wines. Kinetic studies are now underway to determine the rates of this conversion to TDN, and thus the relative importance of this, compared to other TDN precursors. The influence of winemaking techniques on the generation of TDN from this precursor will also be investigated with the ultimate aim of communicating whereby winemakers can modulate the concentration of TDN in wine.

Studies on unstable wine proteins involved in haze formation

Dr Elizabeth Waters, Dr Miguel de Barros Lopes, Professor Peter Høj, Dr Leigh Francis, Yoji Hayasaka, Gayle Baldock, Shauna Brown, Ken Pocock

The focus of work in the last 12 months in this project has been on developing alternative methods to prevent protein-induced haze formation and of exploiting the haze-forming properties of the proteins to discriminate juices and wines varietally.

Use of mass spectrometry to differentiate varieties

Since subtle differences between the molecular weights of proteins are readily detectable by electrospray mass spectrometry, it is theoretically possible to identify cultivars by the protein profile of berries, must and wine. Such an identification technique complements and extends the identification service currently available, since DNA fingerprinting is currently valid for berries and must only.

Like grape berries and juices, protein unstable (i.e. unfined) white wines contain the profile of proteins characteristic of the variety of grapes they were produced from. Our best data has been obtained from young white wines and it appears that the method struggles to obtain clear mass spectra from the proteins in older white wines. Only a very few red wines have been examined to date but it was not possible



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to obtain mass spectra from these wines and we predict that this method will not be applicable to bottled red wines. We have previously clearly established the ability to discriminate grape cultivars on the basis of differences in the molecular mass of berry proteins (Institute publication # 663). The challenge was then to extend this technology to wine.

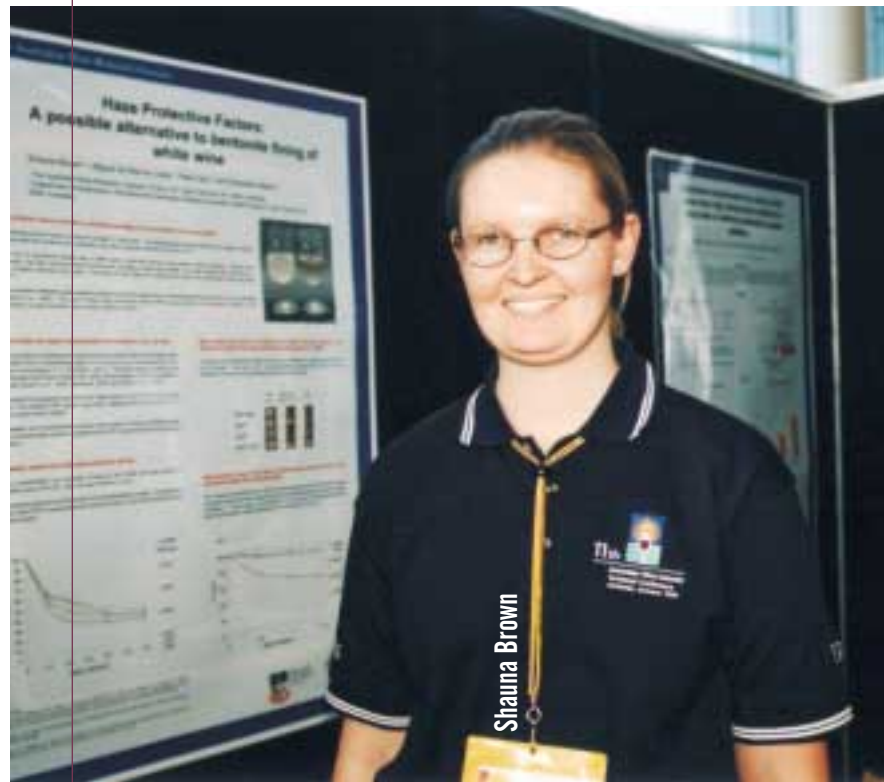
Bentonite fining presents a particular challenge to this method. White wines stabilised with bentonite contain low but detectable levels of the thaumatin-like proteins but do not contain detectable levels of chitinases, the most variably variable group of wine proteins. The differences in the Mr of thaumatin-like proteins alone are probably not sufficient for varietal differentiation but could be used to exclude the presence of certain varieties in a wine.

Work on this sub project is essentially complete. Unlike juices and berries, it seems unlikely that it will be possible to use the mass (Mr) profile of PR proteins to definitively identify the variety of grape that wines were vinified from but this information could be used to exclude the presence of certain varieties.

Proteolytic enzymes and/or heat as alternatives to bentonite

Whilst it is known from previous work at the Institute and elsewhere that proteolytic enzymes do not degrade PR proteins (wine proteins) at normal winemaking temperatures, a joint application of heat and enzymes has been demonstrated to be effective in a number of studies by others over the past 20 years. Nevertheless, proteases are not used and the use of heat treatment is not widespread in industry, possibly due to the perception that heating under any conditions is detrimental to wine quality. This appears to be unfounded because previous work (e.g. Institute publication # 462) has indicated that heating white wines at 90°C for 2 or 10 minutes had no effect and that heating at lower temperatures for longer periods conferred bottle aged characters to the wines that were appropriate for their origin and variety. A particularly important feature of these studies was the absence of any discernible oxidised, cooked or maderised aromas in any of the heated wines. Indeed, anecdotally we have heard that some winemakers heat premium juices to enhance flavour and also believe that this process increases wine protein stability. Thus, a study to confirm the published and anecdotal data on protease and heat treatment and extend it by determining the sensory effect of the treatment was initiated, as described in the 2001 Annual Report.

The study has shown that combined heat treatment with addition of proteolytic enzymes is effective in degrading a significant proportion of haze forming PR proteins in wine. Four wines and two heat treatment regimes were thoroughly investigated: 90°C for 1 minute and 45°C for several hours. Other temperature/time combinations were also successful. None of the treatments of any of the wines examined in the study completely eliminated the need to fine the wines with bentonite, but the rates required for stability were reduced to between 30 and 70% of the original



Shauna Brown



George Skouroumounis

requirements before the treatment. When heating at 90°C in the laboratory, degradation occurs as the wine heats up, rather than during the holding stage, so that further modifications to the procedure could be investigated.

Two enzymes were used in the study, Trenolin blank (Erbisloh Geisenheim Getränke-technologie, Germany) and pepsin (Sigma Aldrich). The former is already used for wine processing, but at a much lower recommended dosage than was used here. Separation of the active protease from this product would be an advantage for commercial use. Pepsin has not been approved for use in wine, and does not appear to offer any benefits over that of the presumed fungal pepsin like protease in Trenolin blank.

The fungal pepsin in Trenolin blank is not completely degraded by heating at 90°C, and is not completely removed by bentonite fining to produce heat stability. Pig pepsin and endogenous 'natural' wine proteases were also not completely eliminated by bentonite fining. The presence of this residual protein material did not, however, adversely affect the heat stability of the wines during storage.

The pilot scale experiments were not as effective as the laboratory trials, so that further work on a larger scale is necessary to optimise treatment parameters. Of particular industrial significance was the observation that the organoleptic qualities of the treated wines were not adversely affected by the treatments. There were some detectable effects on aroma in some cases from heating and from Trenolin blank, but the dosage rate of Trenolin blank was much higher than that recommended by the makers.

Combined heat and enzymatic treatment of white wines to remove heat unstable protein and reduce bentonite requirements clearly appears to be a promising procedure for commercial application. Further investigation by wineries on a large scale is warranted. A manuscript outlining these findings has been written and will be submitted for publication in October 2002.

Haze protective mannoproteins

Haze protective mannoproteins or factors (HPFs) are yeast cell wall mannoproteins present in wine and have the potential to prevent haze formation through their ability to change the particle size of haze. Two haze protective mannoproteins (*Hpf1* and *Hpf2*) have been isolated and their structural genes identified (*HPF1* and *HPF2*). *HPF1* has a homologue (*HPF1'*) thus there are three HPF genes. The project is currently focused on confirming that the genes identified do indeed code for haze protective factors and on producing enough HPF for pilot scale experiments to confirm their long term effectiveness at preventing haze.

The genes have been cloned and lab yeast strains containing extra copies of the genes fused to a strong promoter have been produced. Lab yeast strains with the genes deleted have also been constructed.

Under the growth conditions required for over expression, the *Hpf1p* and *Hpf1'p* over expressing strain secreted material with significantly more activity than the wild type strain. These data suggest that the *HPF1* gene and the *HPF1'* gene do indeed code for a haze protective factor. Further analysis of the material secreted by both the over expressers and deletion strains and of the material in the cell walls will be necessary to confirm these conclusions. Nevertheless the data is very encouraging as it indicates that we have successfully identified genes for HPFs.

Yeast strains in which the genes were deleted secreted material with less haze protective activity than that of the wild type strain. These results confirm that the genes almost certainly code for haze protective factors. The triple deletion strain (all three genes deleted) still, however, had some haze protective activity suggesting that haze protective factors other than *Hpf1p*, *Hpf1'p* and *Hpf2p* may also exist in yeast.

Phenotypic analyses have suggested that the HPF mannoproteins may also have some impact on the ability of yeast to tolerate cold and ethanol. This is an unexpected bonus and illustrates the serendipitous nature of research.

Yeast strains with the genes coding for a hexahistidine labelled form of *Hpf1p*, *Hpf1'p* and *Hpf2p* have also been produced. This will allow much more efficient purification of HPFs and greatly improve the purity of the product compared with conventional methods. Data that demonstrates that this pure product is active will be the conclusive proof that we have correctly identified a gene for HPF.

The progress into the molecular aspects of this research has only been possible through the expertise contributed by Dr de Barros Lopes and PhD student, Shauna Brown, again indicating the increased focus on a team-based approach to achieving outcomes not limited by individual researcher's skills set.

Studies on random oxidation of bottled wines

Staff: Dr Mark Sefton, Dr Leigh Francis, Dr George Skouroumounis, Dr Elizabeth Waters, Mariola Kwiatkowski

Random post-bottling oxidation shows itself in white wines as an obvious browning in a proportion of the bottled wine typically after six to 18 months' storage, and is accompanied by loss of SO₂ and ascorbate and oxidised flavour. It has been estimated that the problem costs the industry \$160 million per year in spoiled wine. We have worked in this area since 1995 and, excluding oxygenation of wine due to poor bottling procedures, have identified variable oxygen permeability of the closure as a major cause of the problem (Institute publications # 528, 534). It is possible that other winemaking techniques such as use of ascorbic acid, and upright storage, contribute to random oxidation. This aspect has continued to be the focus of the project during the past 12 months.

Non-destructive instrumental measurement of wine colour

The determination of A420 nm is commonly accepted as an estimation of wine browning. As part of the project, a rapid and non-destructive method to measure A420 in wine bottles was developed to facilitate data collection. The sample port of a common UV-Vis spectrophotometer was inexpensively modified to enable the insertion of different bottle sizes. In non-coloured glass bottles (Flint) the full visible spectrum range of 350 to 700 nm can be determined. Whilst the full range cannot be determined in the coloured glass bottles, it is possible to determine A420 nm in French green (pale yellow glass), emerald green and cobalt blue bottles, and by correlation with A540 nm, in amber (brown glass) and antique green (dull yellow brown glass) bottles.

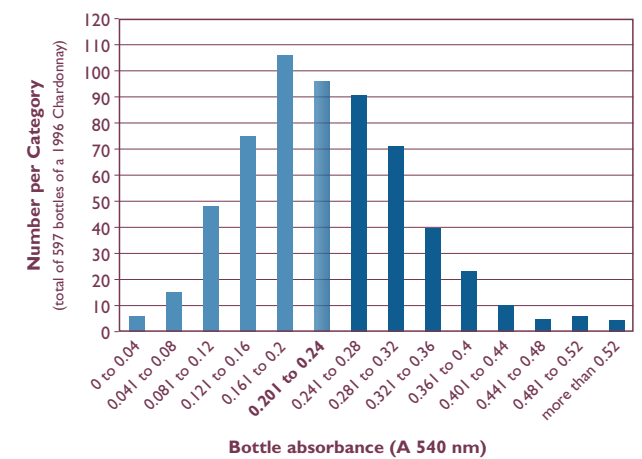


Figure 2 The range of A 540 nm values shown in April 2002 by 597 bottles of a 1996 Chardonnay. Number of wines in 14 categories of A 540 nm spanning the complete range of values measured are indicated. Example bottles from each category were tasted and those in the categories shown in yellow were judged to be commercially acceptable, and those in orange to be unacceptable. The "cutoff" A 540 nm value for commercial acceptability was set by the winemaker tasting panel at 0.24 (hatched bar). All measurements were made in situ in antique green Burgundy bottles, dull yellow brown coloured glass. It was not possible to visually assess the colour of this wine in the bottle.

An example that demonstrates the utility of this instrument is given here, but this instrument has also greatly facilitated analysis in the bottling trial described in the next section and in other projects undertaken at the Institute. The A420 nm values of a six year old Chardonnay wine in antique green bottles was estimated by measuring A540 nm of the bottles. This set of wines showed a high extent of random oxidation when opened, and because the wine was in antique green bottles, wine colour could not be visually assessed before opening. After scanning 600 bottles, a subset of 16 bottles were opened and tasted by a winemaker panel. On the basis of this tasting a commercial decision about the A540 nm value for the bottle above which wines were unacceptably oxidised could be set. The A540 nm values for all the wines are shown in Figure 2 and demonstrate the extent of the random oxidation problem by the spread of colour within this one set of wines was from almost water white to very dark.

The effect of ascorbic acid, bottle position and wine type on oxidation

As described in previous Annual Reports, a Riesling and a wooded Chardonnay wine were bottled in late August 1999. The wines are stored under relatively stable temperature and humidity conditions in the Hickinbotham Roseworthy Wine Science Laboratory (HRWWSL). Determination of A420 nm (an instrumental measure of browning) on at least 30 replicate bottles for each treatment has been taken throughout their storage period. The experiment has been designed to examine the effect of ascorbic acid (60 mg/L added at bottling or no addition), storage position (upright or inverted) and closure type (two natural cork corks and a synthetic closure) on wine oxidation.

The addition of ascorbic acid to both wine styles has increased the level of A420, a traditional measure of wine browning. This has been evident throughout the storage period. Preliminary visual assessment of browning of a small subset after two

years storage has not confirmed this analytical data. For the Chardonnay wines, pair wise comparisons of bottles of wines, stored for two years, with and without ascorbic acid addition, have consistently resulted in the wine with ascorbic acid added at bottling being picked as the less brown wine, not the more brown as expected from the A420 nm data and from previous work. For the Riesling wines, a similar visual comparison has not given such consistent results. Of 18 pairs, the panel was not able to pick one of the pair as browner out of ten pairs, picked the wine with ascorbic acid addition as browner in two pairs and the wine without ascorbic acid addition as the browner in six pairs.

Wines in the pairs with the higher instrumentally measured browning (A420 nm) were not always visually assessed as the browner wine. We have observed that the wines without ascorbic acid addition and picked by the panel as being browner generally had higher absorbances at wavelengths above about 500 nm (red and blue regions) and lower absorbances below about 450 nm (yellow region) than wine with ascorbic acid. The combination of increased 'red' and 'blue' absorbance in a yellow wine may give the appearance of increased brownness. **Notwithstanding the data shown in Figure 2, where a single absorbance value correlated well with visual assessment, these data call in question the universal value of A420 nm ('yellow') as an indicator of brown colour in wines, particularly when comparing the same wine with and without ascorbic acid added.** A more extensive colour evaluation of ten replicates of twelve bottles of each wine representing each of the combinations of the treatments (240 bottles in total) and involving more than 40 Institute staff and students was undertaken after two and a half years storage of the wines at HRWWSL. Panelists used five terms to describe the colour of the wines: overall colour intensity (from light to dark), brown, orange, yellow and green. The wines were scored on a scale of 0 to 10 in these attributes. Reference colour strips, based on

the Pantone® colour system, for the four colour terms were developed after input from the panelists and a consultant from a printing company. The statistically significant effects at the 5% level are summarised below. Helena Oakey, Biometrics SA, undertook the statistical analysis (ANOVA).

Chardonnay: The absence of ascorbic acid addition at bottling led to increased overall colour intensity, brownness, and orangeness and decreased yellow and green tints compared to wines to which ascorbic acid was added at bottling. Upright storage also increased intensity, brownness, and orangeness and decreased yellow and green tints of wines compared to inverted storage of wines for the natural closures only. The synthetic closure gave wines with the highest values for overall intensity, brown, and orange and lowest for yellow and green in the set. Upright storage without ascorbic acid gave the greatest values for brown, overall intensity and orange for the natural closures (see example in Figure 3).

Riesling: The effect of ascorbic acid on the colour rating of the Riesling wines was not as pronounced as that for the Chardonnay wines – at the 5% level only the effect on yellow was significant. The addition of 60 mg/L ascorbic acid at bottling led to increased yellow after storage compared to wines with no addition of ascorbic acid. There was, however, a trend (p = 0.09 and 0.11, respectively) for increased overall colour intensity and green tints in the presence of ascorbic acid. The effect of storage was, however, significant at the 5% level. For the natural closures, upright storage increased overall colour intensity, brownness, yellowness and orangeness and decreased green. As for the Chardonnay wines, storage position had no impact on the colour ratings for wines sealed with the synthetic closure. Also as seen with the Chardonnay wines, the synthetic closure, when compared with the two natural closures, gave Riesling wines with the highest values for overall intensity, brown, and orange.

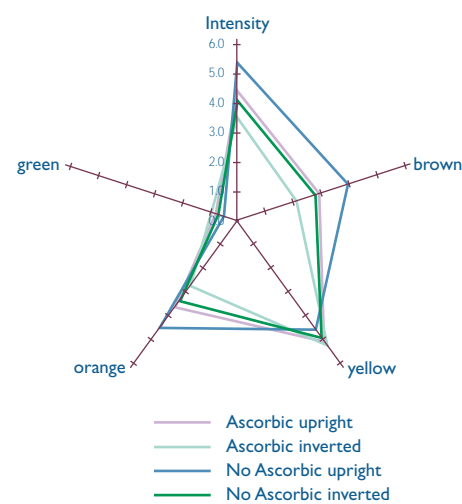


Figure 3 The colour attributes of Chardonnay wines sealed with natural closure 1 and stored at HRWWSL for 2.5 years either upright (pink) or inverted (green) and with either addition of 60 mg/L ascorbic acid at bottling (hatched) or not (solid).

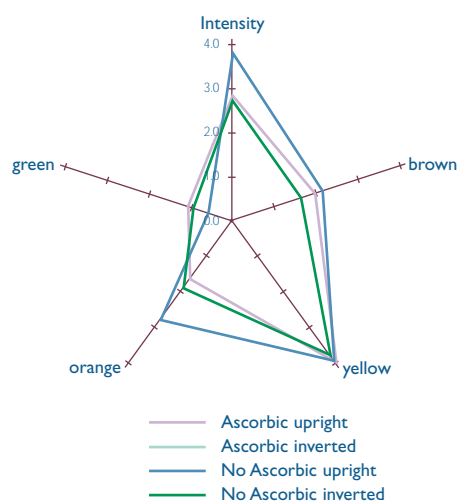
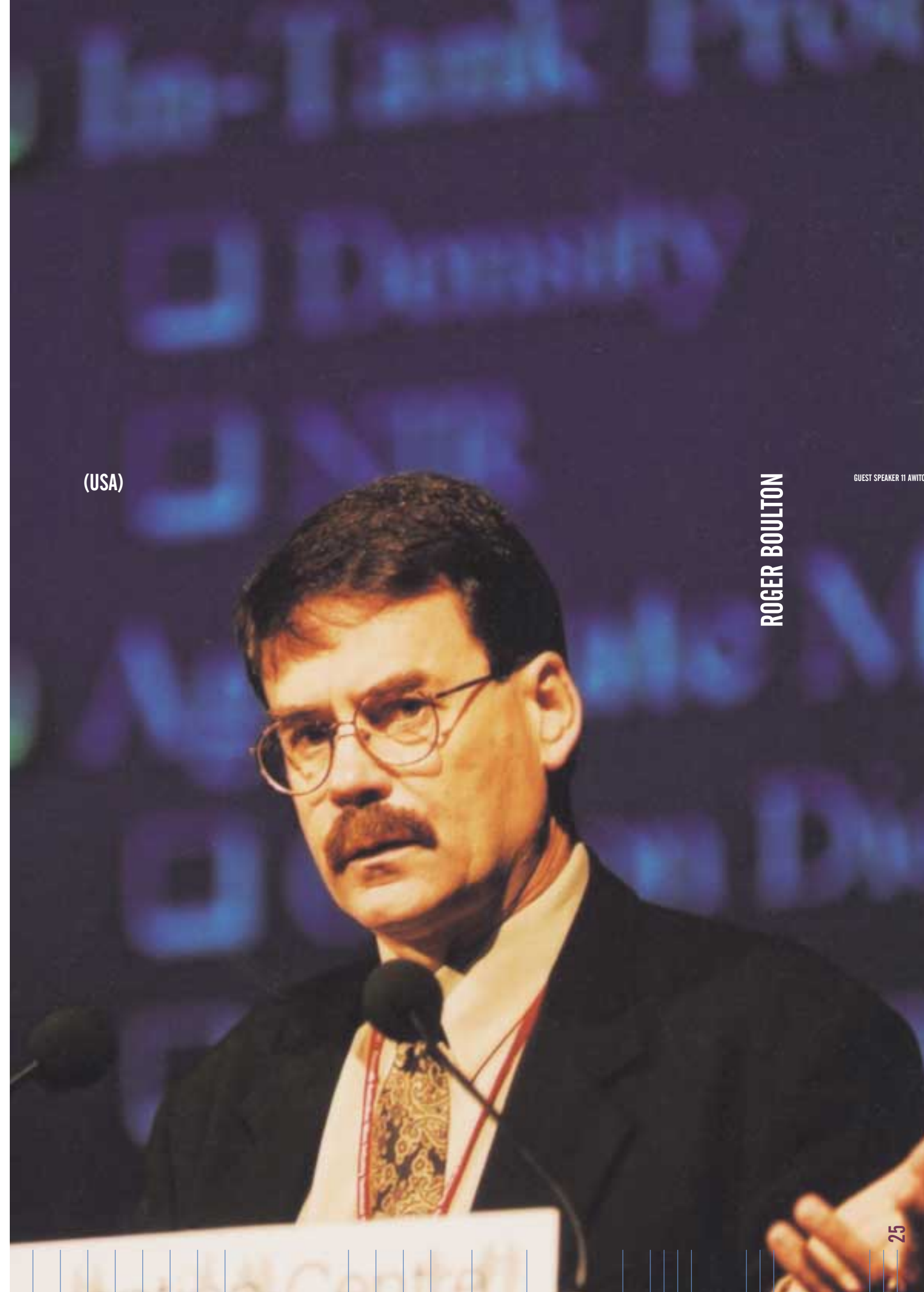


Figure 4 The colour attributes of Riesling wines sealed with natural closure 1 and stored at HRWWSL for 2.5 years either upright (pink) or inverted (green) and with either addition of 60 mg/L ascorbic acid at bottling (hatched) or not (solid).

(USA)

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For the natural closure, upright storage without ascorbic acid gave the greatest values for brown and orange (see example in Figure 4).

Summary: In general, the absence of ascorbic acid and upright storage for two and a half years under ideal conditions gave wines with the highest ratings for brown and orange. Whilst these wines could be described as appearing more oxidised than other wines in the sample set, we are yet to determine whether they taste more oxidised.

This taste evaluation will begin in late June using the same wines rated here for colour (10 replicates from each treatment). Analytical data will also be obtained when the wines are opened for tasting. In relation to prior studies at the Institute and elsewhere on the effect of ascorbic acid addition on wine composition, the present study underlines the crucial importance that researchers must place on the combination of instrumental and sensorial analyses. The global dependence on A420 measurements as a sole indicator of browning and inferred oxidation clearly has to be qualified and restricted to wines made in an identical fashion.

The effect of oxygen at bottling

An experiment was set up in April 2000 to examine the effects on wine of a large 'slug' of oxygen. Five bottles of Chardonnay wines with, and five without, ascorbic acid addition at bottling, all exhibiting similar browning levels as measured by A420 nm from the trial outlined above (nine months after bottling), were opened in the anaerobic hood, i.e. under nitrogen gas. Wines from the five bottles were pooled before an aliquot of 200 mL of wine was placed into 750 mL bottles. Each bottle was sealed with a rubber septum and either 0, 3.5 mL, 7 mL, 10.5 mL or 14 mL of air was added to the headspace of three replicate bottles. To another set of replicate bottles a total of 17.5 mL air was added in 3.5 mL aliquots over five weeks. These additions of air are equivalent to 0, 3.5 mL, 7 mL, 10.5 mL, 14 mL or 17.5 mL of O₂ per L. Approximately 5 mL of O₂ per L wine was originally present in the ullage of the wines sealed with Stelvin closures in the Southcorp trial (fill height of 52 mm and no dry ice 'snow dropping' used).

The bottles were stored in the anaerobic hood at 22°C for 15 months and browning measurements at 420 nm were taken every week. A sensory assessment of the level of visual browning was made at 6 and 12 months for selected wines and after 15 months storage for all wines. A panel of 60 was asked to pick the browner wine out of a pair, where the pair was wines with and without ascorbic acid at the same oxygen addition rate.

Clearly the kinetics of brown pigment formation and the influence of ascorbic acid on browning are complex and we are not yet at a position to interpret this data fully. In addition, it should be noted that the experiment is similar to a single ingress of oxygen some time after bottling. This situation is unlikely to occur commercially and thus the data should not be extrapolated to the commercial situation. Also only one wine type, a wooded Chardonnay, has been examined. Despite this, the data does demonstrate a few points:

- The effect of the added oxygen was not immediately obvious but took about three months to be evident as increased A420 nm values. One might, therefore, expect that oxygen pickup during wine transfer or problems at bottling which result in air in the ullage may not show up until some months later, at least not visibly.
- Greater oxygen exposure resulted in higher browning.
- Wines in the pairs with the higher instrumentally measured browning (A420 nm) were not always visually assessed as the browner wine. In addition, very small differences in A420 nm values of pairs were sometimes accompanied by big visual differences. These data call in question again the universal value of A420 nm as an indicator of brown colour in wines, particularly when wines of different compositions (e.g. absence or presence of ascorbic acid) are compared.

The influence of oak cooperage on wine composition

Staff Dr Mark Sefton, Dr Alan Pollnitz, Kevin Pardon

Good progress towards evaluating the importance of oak-derived thiols to wine flavour has been made. Two thiols, which have coffee-like aromas, have been synthesised and the deuterated analogues of both of them have also been prepared. These analogues will be used for wine analysis, and also for determining the rate at which the natural analogues can be formed in wine. Stable isotope labelled analogues of potential precursors to these thiols have also been prepared and some preliminary developmental work on their analysis has been undertaken. We are aiming to be able to detect these compounds in wine without the tedious isolation and purification steps that have been employed by others for this purpose.

Preliminary developmental work for the analyses has indicated that these thiols are able to react with other wine components and that these reactions affect the concentration of the thiols in wine. Such reactions are now being further investigated.

Following the successful synthesis of two gallate ester derivatives which were also considered as potential precursors of oak lactone (see Institute publication # 681), work has commenced on the synthesis of glucosidic derivatives of oak lactone in order to determine the status of these compounds as alternative precursors to oak lactone formation in wine. The key glycosylation and deprotection steps have been successfully carried out in high yield, and several other steps in the synthesis have been optimised. This target remains on schedule. These successes will enable us to obtain the target compounds by a much more direct route than originally anticipated. An additional 'spin-off' from these new reactions is that it will be possible to resolve the naturally occurring from the non-natural isomers of both cis- and trans-oak lactone. Such samples will enable us to better evaluate the sensory impact of these natural isomers in wine.

Chemical analysis of industry technical problems

Staff Dr Mark Sefton, Yoji Hayasaka, Gayle Baldock, Dr Alan Pollnitz, Kevin Pardon, Dimitra Capone

Problem solving work and research into the formation of 4-ethylphenol in wines is carried out by the group in collaboration with, and under the direction of the Industry Services section. Accordingly, the results of this work are given in the report by Industry Services. This team's role is to provide analytical support to these areas.

A detailed evaluation of the potential for a mass spectrometry-based 'electronic nose' to be used in mass screening of cork extracts for trichloroanisole (TCA) has been completed, but this technology proved insufficiently sensitive for the extremely low concentrations of TCA that can be detected by the human nose. No further work is planned in this area unless further developments in electronic nose technology indicate that a greatly enhanced sensitivity to TCA is possible.

As part of the program to develop additional taint screening methods for industry problem solving, we have started preparing samples of labelled analogues of chlorophenols which are precursors to chloroanisoles and taint compounds in their own right. Difficulties have been encountered in preparing labelled tetrachlorophenol and pentachlorophenol, but a 13C-labelled analogue of 2,4,6-trichlorophenol, the precursor to TCA, has been successfully prepared in high yield. Preparation of labelled analogues of mono- and dichlorophenols which are the most odorous of the chlorophenols is in progress.

A sample of deuterium-labelled 2-aminoacetophenone has been successfully synthesised. This compound is needed to determine whether a so-called 'untypical ageing' taint encountered in some German white wines also occurs in Australian white wines.

Waite Campus Mass Spectrometry Facility

Staff Yoji Hayasaka and Gayle Baldock

Role of the team

The four important roles of the Waite Campus Mass Spectrometry Facility are to act 1) as a leader in the application of mass spectrometry to grape and wine research; 2) as an investigator to solve the problems facing the wine industry and individual wine makers, using mass spectrometric techniques; 3) as a collaborator with the University of Adelaide in the research and teaching activities involving mass spectrometry and 4) as a provider of the versatile and advanced mass spectrometric technique and expertise to the scientific community including public as well as private institutions.



Dimitra Capone

Instruments in the Facility

The Facility accommodates three instruments for GC-MS analysis (2x Agilent GC-MS and 1x TSO GC-MS/MS) and one for LC-MS analysis (API LC-MS/MS).

The use of LC-MS (electrospray) has dramatically increased in the past decade because of the broad range of applications for the analysis of proteins, peptides, macromolecules, polar, involatile and labile compounds. In fact, the Institute's research is no exception, LC-MS is an indispensable tool for the protein and tannin projects and has considerable potential to expand its application to other projects. Therefore, the MS facility needs to have the additional electrospray capability to enhance the progress of the current projects and to prepare for future contributions to other projects.

The TSO GC-MS/MS and API LC-MS/MS are used for various purposes and appropriate financial arrangements for all users are in place to cover the running costs of the facility. The time usage of the TSO GC-MS/MS and API LC-MS/MS is detailed in Table 3.

Table 3 Percentage of time used of the Waite Campus Mass Spectrometry Facility's instruments by organisation

Organisation	Instruments	
	TSO GC-MS/MS	API LC-MS/MS
AWRI	100%	56.7%
The University of Adelaide	0%	40.6%
CSIRO	0%	2.1%
Flinders University	0%	0.6%

Note: the Agilent GC-MS systems are essentially used by Institute staff only.

Problem solving work

During the reporting period, ten cases of chemical analysis of either juice or wine were conducted using GC-MS, in collaboration with staff of the industry services. Some cases involved the analysis of wine or juice suspected of being contaminated with hydraulic oil. The contamination with hydraulic oil is difficult to confirm by sensory assessment and GC-MS due to their chemical properties (simple long chain aliphatic hydrocarbon and low volatility) unless the sample is heavily contaminated.

Research and development activities

The taint screening method is being developed for the GWRDC-funded projects of AWR 19 *Chemical analysis of industry technical problems and consulting*. A review of all taint investigations conducted by the Institute in the past 12 years revealed that volatile components of some petroleum products, including paints, epoxy-resins and diesel oil, were frequently the identified contaminants in the very few faulty juices and wine submitted to the Institute. It is important to ensure that Australian wines are free of such contaminants, however, there is no established method to screen for these contaminants and to estimate their concentration. It is necessary to develop a sensitive, reliable and simple analytical method to investigate the presence of the contaminants and to estimate the amount present.

Target compounds for the taint screening method include toluene, styrene, C2 alkylbenzenes (xylene), C3 alkylbenzenes (trimethylbenzene), C4 alkylbenzenes (tetramethylbenzene), naphthalene, C2 alkyl-naphthalenes and C3 alkyl-naphthalenes.

The method developed includes the use of headspace sampling using SPME and GC-MS with selected ion monitoring. The method is rapid, simple and solvent free.

Labelled (deuterated) internal standards were used for quantification: o-xylene-d₁₀ for toluene, styrene and C2 alkylbenzenes; 1,3,5-trimethylbenzene-d₁₂ for C3 and C4 alkylbenzenes; and 1-methylnaphthalene-d₁₀ for naphthalene, C1, C2 and C3 alkyl-naphthalenes.

The detection limits of all target compounds in model wine were as low as 0.10 ppb.

The quantification curves for these compounds in model wine showed excellent linearity (R² > 0.996) through a range from 0.10 ppb to 100 ppb.

Comparative studies between model, red and white wine spiked with target compounds and internal standards revealed there was no significant affect of matrix on quantification.

The method development will be completed by September 2002.

Preliminary investigation of the Stir bar sorptive extraction (SBSE) technique was carried out under the GWRDC-funded projects of AWR 19 *Chemical analysis of industry technical problems* and AWR 6 *Grape composition and wine flavours*.

The sample enrichment prior to the GC-MS analysis is one of most important processes for the identification and quantification of trace compounds in complex matrices. Liquid/liquid and solid-phase extractions or headspace and purge and trap techniques have traditionally been used for sample enrichment (concentration). However, recently solid phase microextraction (SPME) has become more frequently used because of advantages such as simplicity, rapidness and environmental friendliness (solvent free). SBSE has been recently introduced to overcome the problem of headspace SPME technique with the limitation of sensitivity to relatively larger and

polar molecules. The extraction theory of SBSE is the same as SPME but the capacity for sample enrichment is significantly enlarged.

The SBSE coupled with GC-MS technique was evaluated by the analysis of red wine samples for three applications: flavour and compositional analysis, trichloroanisole (TCA) and agrochemicals, with collaboration of Dr Kevin MacNamara of Irish Distillers Limited (Ireland) where the SBSE technique is accessible.

One hundred compounds were identified in the wine, a much greater number than previously possible using SPME and liquid/liquid extraction. The sensitivity of the SBSE was estimated to be 10-100 times better than other sample enrichment techniques.

The concentration of TCA at 1 ng/L was easily detected. It was important to note that such excellent sensitivity (S/N ratio) was achieved without attempting to optimize the SBSE and GC-MS conditions.

Eighteen agrochemicals were easily detected at the concentration of 0.5 µg/L. It was interesting to note that the detection limits by SBSE with GC-MS in scan mode were as low as those (10 µg/L) seen for solid phase extraction with GC-MS in selected ion mode (currently used for residue analysis at the AWRI). The detection limit by SBSE with GC-MS in selected ion mode would be down to low ng/L levels.

SBSE combined with GC-MS is a very promising tool for the elucidation of new wine compounds qualitatively, and following proper validation, quantitatively at levels which are orders of magnitude below those previously obtained by conventional methods.

Electrospray mass spectrometry (ESI-MS), in particular LC-MS, is an indispensable tool for tannin research and has been extensively used for characterization of tannin-related compounds prepared by various members of the tannin project (AWR 96/1 *Wine grape tannin and colour specification* and CRCV1.2). The contribution of the MS facility to the tannin project highlights as follows:

- The tentative characterisation of major wine pigments using nanoESI tandem mass spectrometry was completed.
- The characterisation of seed tannins using ESI-MS was completed.
- The characterisation of the MLCCC fractions of skin and marc using ESI-MS and LC-MS are in progress.
- The analysis and data interpretation for winemaking trial samples and model fermentation trial samples is in progress.
- Analysis and data interpretation of the samples prepared by staff and students of The University of Adelaide was carried out.
- The use of mass spectrometry to differentiate varieties of wine was investigated under the GWRDC-funded project of AWR 8 *Studies on unstable wine proteins involved in haze formation* as described elsewhere in this Report.

Selection and improvement of wine yeast using molecular biology

Staff Dr Miguel de Barros Lopes, Dr Paul Henschke, Jeff Eglinton, Jenny Bellon, Professor Peter Høj

Over the last year, the team has focused on applying the knowledge gained using molecular biology to produce improved wine yeasts using non-molecular methods. These strains are considered safe and not classified as genetically modified organisms.

Jennifer Cartwright, an oenology student from The University of Adelaide, has recently joined the team to study the winemaking attributes of hybrid yeast. The research team also continues productive collaborations with Dr Vladimir Jiranek at The University of Adelaide. Recently, Kate Poole, a PhD student co-supervised by Drs Vladimir Jiranek and Miguel de Barros Lopes, completed writing her PhD thesis and is currently doing post-doctoral research at the Max-Planck Institute in Germany.

Construction of a wine strain producing less ethanol

Strains overexpressing the glycerol synthesis gene (*GPD2-OP*) accumulate more glycerol and produce less ethanol than a non-modified strain during fermentation (Institute publication # 649). These modified strains, however, also increase the acetic acid concentration in wine above 1 g/L, a concentration that can lead to vinegar odour and that is unacceptable in most wine styles. The deletion of a second gene (*ald6Δ*), which encodes for an aldehyde dehydrogenase, provided an effective remedy for reducing acetic acid to acceptable levels while still maintaining the ability to reduce alcohol levels through diversion of metabolic flow into glycerol production.

Preliminary analysis to determine how the modifications to *GPD2* and *ALD6* affect the biosynthesis of secondary metabolites has also been described (see Institute's Annual Report 2001). A more comprehensive analysis has since been done, with the results demonstrating that altering glycerol and acetic acid metabolism can have a dramatic effect on the biosynthesis of important aroma contributors in wine, such that potentially novel flavour diversity is possible (some of these changes are shown in Table 4). Acetaldehyde can impart oxidized, nutty or bruised apple aromas and the substantial variation in the concentration of this metabolite (>10-fold in this study) could strongly influence the sensory character of wine. Isoamyl acetate, which was only detected in the *GPD2 ald6Δ* ferments, has a banana or pear aroma. Acetoin (buttery) and 2,3-butanediol (bitter) concentrations are altered more than 100-fold. Although other important sensory metabolites changed to a lesser extent, the change may still contribute to the sensory property of a wine since, even when they are present in sub-threshold concentrations, variations in the relative amounts of compounds can have a subtle and complexing effect on wine aroma and flavour. For example, ethyl acetate is the main ester occurring in wines and, depending on its concentration, imparts a fruity or solvent (varnish) odour. The aroma of isoamyl alcohol has been described as marzipan-like. The concentration of some phenolic compounds was also shown to change by the modification of glycerol and acetic

Table 4 Concentration of metabolites for wild-type, *GPD2* and *ALD6* modified strains. The amounts are expressed as a % of the strain producing the highest concentration of each compound (rounded to nearest %).

Compound	Sensory properties (where known)	<i>GPD2 ALD6</i>	<i>GPD2-OP ALD6</i>	<i>GPD2 ald6Δ</i>	<i>GPD2-OP ald6Δ</i>
Glycerol	Sweet, mouth-feel	31	82	37	100
Ethanol	Alcohol, hot	100	76	91	80
Acetic acid	Vinegar, pungent	46	100	14	25
Acetaldehyde	Oxidised, nutty, bruised apple	7	95	9	100
Ethyl acetate	Varnish, solvent, fruity	66	100	27	33
IsoButanol	Alcohol	64	15	100	30
IsoAmyl acetate	Banana, pear	0	0	0	100
IsoAmyl alcohol	Marzipan	69	41	100	64
Acetoin	Buttery	1	100	1	48
2,3-Butanediol	Bitter	15	100	0	26
Propanoic acid	Rancid, pungent, goaty	37	100	43	83
4-Hydroxybenzaldehyde	Sweet, woody, balsamic	80	26	100	42
4-Hydroxybenzene ethanol (tyrosol)	Bees wax, honey-like	70	50	94	100

Note: *GPD2 ALD6* is the unmodified natural strain



HARRY HOTTINK
(USA)

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acid metabolism. Some of these compounds (e.g. tyrosol) are believed to have protective effects against several pathologies, which is mainly attributed to their antioxidant properties.

The outcomes of this research were presented at the 11th AWITC. The findings have been published in the international refereed journal, *Yeast* (Institute publication # 684).

The above results indicate that the increased biosynthesis of glycerol is an effective strategy to diminish the amount of ethanol produced by yeast during fermentation. Experiments are underway to isolate wine strains with similar traits using a non-molecular approach.

Hybrid wine yeasts

Increasingly, winemakers are looking for ways to introduce aroma and flavour diversity in order to improve wine style and for product differentiation. While the commercial yeast strains available produce consistently sound fermentations, there are indications that sensory complexity and better palate structure may be obtained when other species of yeasts are active during fermentation. One of the main reasons for the limited use of non-*S. cerevisiae* species in winemaking is that they generally ferment more slowly than *S. cerevisiae* and are often unable to tolerate the high alcohol concentrations encountered during a grape juice fermentation. A potential strategy to take advantage of these yeasts and avoid problems associated with sluggish fermentation is to produce interspecific hybrids between a *S. cerevisiae* wine strain and a second species of interest.

Rare-matings between commercial wine strains and strains of closely related *Saccharomyces* species have been successful. The *Saccharomyces* species used for rare-mating are generally not associated with commercial wine fermentations, and the strains we have trialed to date are unable to grow effectively in grape juice. Their close kinship with *S. cerevisiae*, however, indicate that they could bestow useful winemaking properties, especially in terms of imparting flavours and aromas that are desirable for wine. Whereas the non-*S. cerevisiae* yeast were unable to ferment grape juice, small scale winemaking trials show that the hybrids ferment as efficiently as the winemaking parent (see Figure 5). Both preliminary chemical and sensory analysis demonstrate that the hybrid

made wines were notably different to the wines made using the commercial wine yeast parent. These results demonstrate that producing hybrids between *S. cerevisiae* wine strains and other species is a useful strategy for acquiring yeasts with novel winemaking characteristics from species that are less suited for grape juice fermentation. A paper describing the interspecific hybridisation of wine strains and the characterisation of natural hybrids has been published in *FEMS Yeast Research* (Institute publication # 685).

Three of the hybrid yeasts have been trialed in a commercial winery during the recent vintage. The hybrids completed fermentation at rates comparable to the commercial winemaking strain used. Initial results indicate that most (greater than 80%) of the yeasts present at the end of fermentation were the inoculated hybrid strain. **Most importantly, the wines made with the hybrid yeasts were considered to have distinct and favourable characteristics as compared to wines made with a commercial wine yeast. It should also be mentioned that a wild-ferment in the same study attracted favourable comments. These commercial winemaking trials are of great benefit to the research and development effort and the Institute's scientists acknowledge the important contribution of the winery involved.**

Identifying genes for wine yeast improvement

An extensive amount of information regarding the biology of *Saccharomyces cerevisiae* is available. The strains used in the majority of these studies have been performed with laboratory strains of *S. cerevisiae*, which have important physiological differences when compared to commercial wine strains. The wine habitat is a demanding environment and the 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. Furthermore, the comparison of potential flavour and aroma compounds present in ferments carried out by either laboratory or commercial strains demonstrates that these strains produce secondary metabolites at distinct concentrations, with some metabolites produced at levels ten-fold higher in commercial strains. The gene and proteins that contribute to the desirable attributes of wine strains are generally unknown.

One strategy to identify these key components is to compare the genes and proteins of commercial wine strains with the well-studied laboratory strains. Differences uncovered might explain the different traits of the laboratory and wine strains and explain what makes a wine yeast perform well under different fermentation conditions. During the past year, the research has focused on using two-dimensional gel electrophoresis (2D gels) to compare the protein expression pattern of wine and laboratory strains grown under different conditions. A number of differentially expressed proteins (that is proteins that are present at very different levels in the laboratory strain and the fermentation capable wine strain) have been identified using matrix-assisted laser ionisation/desorption time-of-flight spectrometry (MALDI-TOF) (this work has been done in collaboration with Dr Graeme Currie at the University of Melbourne). One protein, a glycolytic enzyme, is of particular interest as it exists in two forms in the wine strain but only one in the laboratory strain. Experiments are in progress to establish whether this alteration is at the sequence level or generated by post-translational modification. This will allow us to determine whether the change is responsible for a wine-specific trait as well as provide insight into mechanisms that exist for phenotypic diversity or, put differently, the ability to ferment or not ferment grape juice. Identifying proteins that are necessary for the positive traits of wine strains will be beneficial for the prediction of strain attributes, the improvement of yeast fermentation properties, and tailoring strains and fermentation conditions for producing new and improved wine styles.

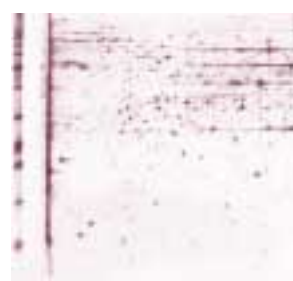


Figure 6 Separation of wine yeast proteins by 2-dimensional gel electrophoresis.

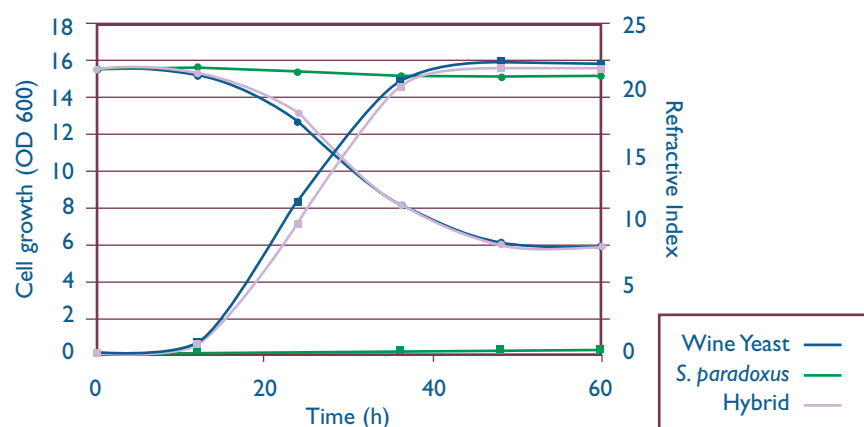


Figure 5 Fermentation characteristics of hybrid yeast

Yeast flavour and fermentation activity

Staff
Dr Paul Henschke, Jeff Eglinton, Dr Eveline Bartowsky, Jane McCarthy, Dr Peter Costello, Kate Howell, Professor Peter Høj, Dr Mark Sefton, Gayle Baldock, Dr Alan Pollnitz, Dimitra Capone, Tracey Siebert

This GWRDC-funded project has three major streams of enquiry related to our investigations into the roles of yeast in the development of wine flavour. These are: development of new isotopic dilution assays for quantifying the key impact compounds produced by yeast, as a tool for determining the importance of yeast strain and fermentation conditions on wine aroma, evaluation of the oenological properties of non-conventional yeasts with an emphasis on correlating chemical composition and organoleptic attributes of wines made under winery conditions, and laboratory studies concerning the use of mixed yeast strain starter cultures, compared with conventional single strain cultures, as a strategy for simulating 'natural ferments'.

Quantification of yeast key impact aroma compounds—methods development

The development of a suite of stable isotope dilution assay methods for quantifying the key impact compounds formed during the yeast alcoholic fermentation has been described elsewhere in this report.

Work can now focus on applying the assays to wines produced through our fermentation trials conducted in the Hickinbotham Roseworthy Wine Science Laboratory with Chardonnay and Cabernet Sauvignon during 2001 and 2002, for which extensive chemical and sensory data has been accumulated. This analytical approach will allow us to relate the chemical and sensory properties of wine so as to enable us, in the longer term, to formulate strategies for more reliably effecting specific changes in wine aroma by manipulating yeast strain and fermentation conditions.

Development of the assays has been carried out largely by Heather Smyth, Tracey Siebert and Kate Howell under the supervision of Drs Mark Sefton and Alan Pollnitz.

After many technical breakdowns in the past of the Gas Chromatograph/Atomic Emission Detector (GC/AED), Tracey Siebert was given the responsibility for the instrument which became fully functional by November 2001. Subsequently, Tracey Siebert has initiated fundamental experiments under the supervision of Drs Alan Pollnitz and Markus Herderich to compare the relative sensitivity and reproducibility of the AED to detection by Mass Spectrometry or Flame Ionisation for over 20 relevant flavour compounds. Additional progress has been made for the analyses of a variety of sulfur compounds, with focus on the selection and optimisation of specialised GC column systems, improvements to the 'signal to noise' ratios and enhanced resolution of critical pairs. To support future sensory studies, the GC/AED has been successfully coupled to a 'Sniff' or olfactory detection device. The acquisition of a cryogenically cooled inlet system in the near future will be of great advantage in increasing sensitivity and resolution of volatile and thermolabile aroma compounds.



Jenny Bellon

Industry evaluation of selected novel flavour yeasts

Winery scale fermentation trials were undertaken during the 2002 vintage with the aim of confirming and extending the results obtained from the 2001 trial. These trials have focused on the special attributes that *Saccharomyces bayanus* appear to induce in relation to wine colour, aroma, flavour and mouth-feel. Because of the effect on wine colour and mouth-feel, the AWR 2 Yeast Flavour Team has collaborated with the Institute's Tannin Group, led by Dr Markus Herderich, to undertake a detailed analysis of the changes to the phenolic compounds throughout the vinification, MLF and maturation stages.

Wines from the 2001 vintage trials have been appraised by several company winemaking teams (Beringer Blass, BRL Hardy, Orlando Wyndham, Southcorp, Yalumba) with generally favourable results, and stimulated more extensive winery evaluations during the 2002 vintage. One company has, after two tastings, decided to conduct trials on the 20 tonnes scale in the 2002 vintage. Another company has indicated that they will use a Chardonnay wine prepared with *S. bayanus*, chosen in preference to three other wines, as a 2002 commercial blending component. In addition, a large wine company (Montana, NZ) had highly favourable results with one *Candida* strain, which led to upgrade of a commercial Semillon wine. These commercial decisions represent an encouraging outcome for this project and indicates the considerable potential for wine attributes modulation that microbiology offers.

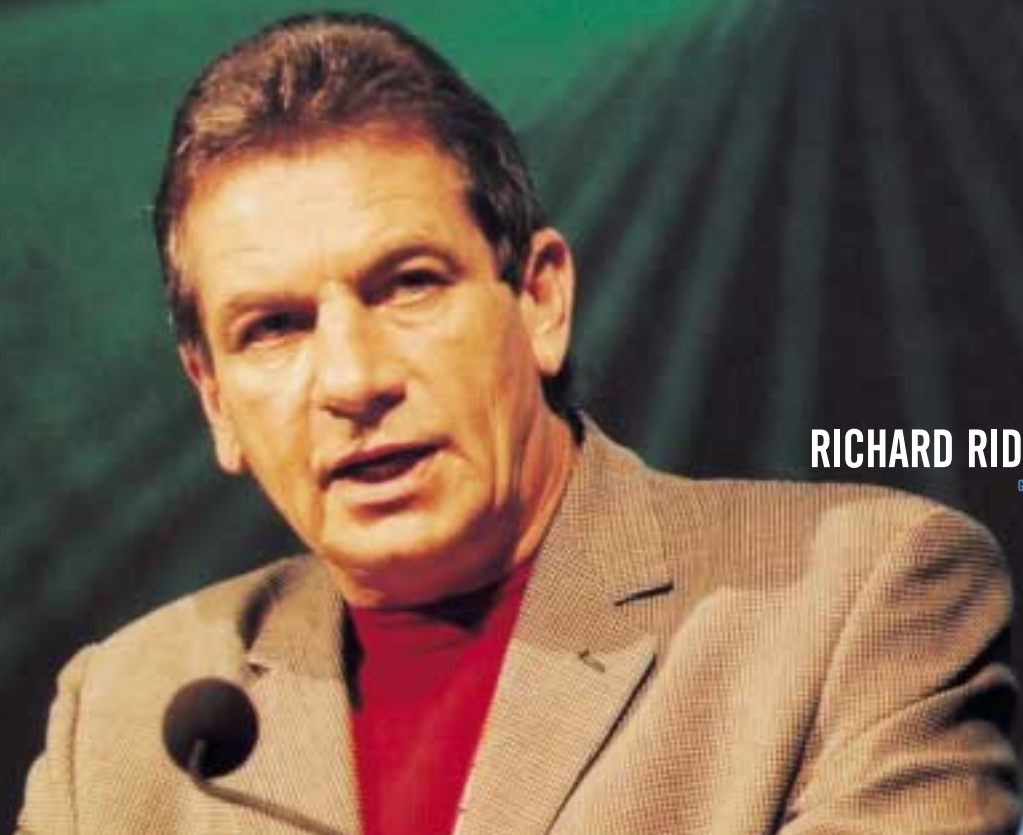
Saccharomyces bayanus trials 2001—Chardonnay

The Chardonnay trial was established to evaluate the performance and to determine the organoleptic properties of *S. bayanus* AWRI 1375 under pilot scale conditions using 260-280L fermentors. *S. cerevisiae* AWRI 838 served as the reference yeast. Because the physiological characteristics of *S. bayanus* yeast are not yet well known, treatments were included to evaluate different methods for ensuring that wine with low residual sugar was achieved after fermentation.

The preliminary results were detailed in the previous annual report. The reference wine yeast, *S. cerevisiae* AWRI 838, completed fermentation at 15°C in 18-24 days whereas *S. bayanus* AWRI 1375 ceased fermentation with a residual sugar of 40 g/L after 35 days fermentation. Sequential inoculation of the *S. bayanus* ferment with *S. cerevisiae*, aerobic pumping over treatment and the use of the *S. bayanus* rescue culture each successfully removed residual sugar from the stuck *S. bayanus* ferment wine.

Chemical analysis of the wines for sugar, alcohol, VA, acetic acid, malic acid, lactic acid, succinic acid, citric acid, tartaric acid, glycerol, total SO₂, TA and pH, revealed no important differences between the *S. bayanus* treatments. The wines were also subjected to quantitative descriptive sensory analysis to establish whether the treatments had any impact on aroma profile. A panel of 18 judges undertook training sessions to agree on the aroma attributes that best described the wines. The attributes, in order of quantitative significance were ester, floral, citrus, pineapple, sweaty, melon, ethyl acetate, cooked apricot, acetic, yeasty/caramel, aldehyde and sulfidic. The *S. bayanus* wines were lower than the reference *S. cerevisiae* wines in floral, ester, pineapple, melon and citrus, equivalent in yeasty/caramel, cooked apricot, ethyl acetate, acetic and acetaldehyde, and higher in sweaty and

(NEW ZEALAND)



RICHARD RIDDIFORD
GUEST SPEAKER II AWTC

sulfidic. No significant differences were identified between the *S. bayanus* treatments. These results with *S. bayanus* were thus not as favourable as those noted in some commercial trials above – or for Cabernet Sauvignon ferments below.

Nevertheless, these results show that the three treatments used to ensure low residual sugar, super inoculation with either *S. cerevisiae* AWRI 838 or *S. bayanus* AWRI 1375, or aerobic pumping over treatment, did not have a significant impact on wine chemical composition or aroma. These results need to be confirmed, but are suggestive that the winemaker has flexibility in terms of choosing a procedure for ensuring the complete fermentation of sugar.

Saccharomyces bayanus trials 2001–Cabernet Sauvignon

Preliminary results for the Cabernet Sauvignon trial performed using 700 kg capacity rotary fermentors located in the HRWSL undertaken during the 2001 vintage was reported in the previous annual report. Triplicated vinifications were undertaken using *S. bayanus* AWRI 1375 with *S. cerevisiae* AWR 838 as the reference yeast. The wines were subjected to MLF, clarified and bottled for analysis.

Only small differences in chemical composition between the *S. bayanus* and *S. cerevisiae* wines were noted. In particular the *S. bayanus* wines had less alcohol (0.4% vol) and more glycerol (2 g/L). The wines also had an apparent colour difference that was confirmed by spectrophotometric analysis. The *S. bayanus* wine exhibited greater colour density (A420 + A520 = 0.75 compared with 0.68) and greater colour hue (A420/A520 = 0.73 compared with 0.64), as shown in the figure below. Analysis of the phenolics by the HPLC method developed by Drs Peng and Waters

(Institute publication # 686) revealed that the anthocyanin content of the *S. bayanus* wines was reduced whereas the chromatographically broad fraction containing unknown pigmented 'polymers' was increased when compared with the *S. cerevisiae* wines. The biochemical basis for this difference is being investigated in model studies with an isolated anthocyanin preparation by Markus Griesser, a visiting student from University of Wurtzburg, under the supervision of Jeff Eglinton and Dr Markus Herderich.



S. cerevisiae
AWRI 838

S. bayanus
AWRI 1375

Under the supervision of Dr Leigh Francis and Ms Kate Lattey, the wines were subjected to sensory descriptive analysis following 12 months storage. The *S. bayanus* wines were rated significantly lower in 'adhesive', 'pucker', 'drying', 'astringent persistence' and 'velvet' attributes than the wine made with *S. cerevisiae*, and higher in 'blackcurrant' aroma. This analysis confirmed earlier casual observations that wines made with *S. bayanus* have a different mouth-feel. A similar trial with the *S. bayanus* yeast has been conducted in 2002 to confirm the results obtained with the 2001 vintage wines.

Saccharomyces bayanus trials 2002–Cabernet Sauvignon

The AWR 2 Yeast Flavour Team has collaborated with the AWR 96/1 Tannin Group to undertake a comprehensive red wine fermentation trial in the Hickinbotham Roseworthy Wine Science Laboratory with Stephen Clarke as winemaker during the 2002 vintage in order to make best use of limited resources and to contain the cost of winery scale work. Only aspects related to AWR 2 will be reported here. The principal aim of the trial is to establish whether the impact of *S. bayanus* AWRI 1375 compared to *S. cerevisiae* AWRI 838 on wine colour, wine and tannin composition, and mouth-feel of red wine that was observed in the 2001 trial holds true using fruit from the 2002 vintage. Regular sampling has been undertaken by the Tannin Group in order to define a detailed picture of the development of colour and changes to the phenolic profile of wine.

The trial was conducted in 700 kg rotary fermentors operated at approx. 20°C. The trial was expanded by performing fermentation with *S. bayanus* in Potter fermentors of 800 kg capacity operated at approx. 20°C or to a maximum of

28°C to determine the effect of fermentation hardware and higher temperature on fermentation, and wine colour, tannin composition and mouth-feel. Due to problems with fruit variability between replicates in the 2001 Tannin Group's trial, extensive planning was undertaken to ensure that uniform must composition across fermentors was achieved. This objective was achieved by randomising the bins during harvest and must preparation in the HRWSL. Orlando (Russell Johnstone, Inca Lee and Nick Bruer) agreed to provide 9 tonnes of premium quality Cabernet Sauvignon fruit from Padthaway for the trial.

The ferments were pressed off on day 6-7 at total soluble solids of 3-5°Be, and fermentation allowed to complete in upright tanks. Much greater yeast biomass was produced with *S. cerevisiae*, with cell counts reaching 2.4 x 10⁸ cells/ml compared with <0.9 x 10⁸ cells/ml for *S. bayanus*, as was observed previously. Fermentation rate for the *S. bayanus* treatments slowed considerably after pressing but completed after several weeks. The wines have now been inoculated Lalvin EQ54 for MLF, and when complete, will be stabilised and bottled for analysis.

An additional trial was undertaken during the vinification stage to relate the colour and phenolic composition between wines made in the rotary fermentors to those made using a 1 kg laboratory scale method developed through the AWRI-Lallemand collaborative project by Simon Dillon. Results to date suggest that under appropriate conditions spectral measurements of the two sets of wines equated closely. HPLC analysis of phenolics will give a further indication of the relativity between the two procedures.

Combined Yeast Flavour Group-Tannin Group 2002 laboratory scale fermentation trials

A project which aims to investigate the importance of viable/actively fermenting yeast for the formation of anthocyanin-tannin conjugates (pigmented 'polymers', or PP) during fermentation of red grape must and a model medium has commenced. A visiting German student, Markus Griesser from the University of Wurtzburg, is undertaking this investigation with supervision by Dr Markus Herderich and Jeff Eglinton. The effect of different yeast species on the changes in phenolic compounds, particularly the so-called pigmented 'polymers', during fermentation is also under investigation. Extraction and/or modification of grape phenols, anthocyanins, tannins, and other compounds that impact on colour and mouth-feel will be studied using a *S. cerevisiae/S. bayanus* model system. The establishment of a good model system will, when coupled with the analytical techniques for the separation and quantification of grape and wine phenolic compounds that are presently under development, aid in the elucidation of the chemical structure(s) and formation of pigmented 'polymers'. Adoption of a model system will allow analysis of the role of individual chemical compounds in the formation of pigmented 'polymers' and other oligomeric pigments. In particular, the model system will be used to investigate the contribution of acetaldehyde (representing a yeast derived compound) and pH to the phenolic changes, especially in pigmented 'polymers', during fermentation. This project will complement the winery scale trial indicated above.

Candida stellata trials–2001

In the previous annual report we summarised the chemical analysis of wines made by sequential inoculation of *Candida* species and *S. cerevisiae* AWRI 838. In brief, the wines made with the *Candida* yeasts showed large and important compositional differences from those made with *S. cerevisiae*. Of special note was the large increase in glycerol (10-14.8 g/L compared with 5.9 g/L) and the low VA (0.16-0.21 g/L compared with 0.41 g/L) for two of the three strains studied.

Quantitative sensory descriptive analysis has also highlighted considerable differences in the aroma profile of these wines. The main aroma characteristics of the *C. stellata* and *C. krusei* wines made by sequential fermentation with *S. cerevisiae* are compared with those of *S. cerevisiae* in Figure 7. Wines made with the non-*Saccharomyces* yeasts were different from the *S. cerevisiae* wine, and from each other. The *C. stellata* AWRI 861 and the *S. cerevisiae* AWRI 838 wines were rated relatively highly in the sensory attributes fruity ester, floral/rose, banana ester, pineapple and citrus, and low in cooked apricot, ethyl acetate and acetic whereas the *C. stellata* AWRI 861 wine had a substantially greater intensity of floral/rose and banana ester attributes. In contrast to the *C. stellata* AWRI 861 and the *S. cerevisiae* AWRI 838 wines, the wine made with *C. stellata* AWRI 1159 was rated highest in cooked apricot, and the volatile acidity attributes (acetic and ethyl acetate aroma) and sulfidic. These data confirm our earlier findings (Soden et al., 2000; Institute publication # 625) that both positive and negative aroma attributes of *Candida* species are maintained

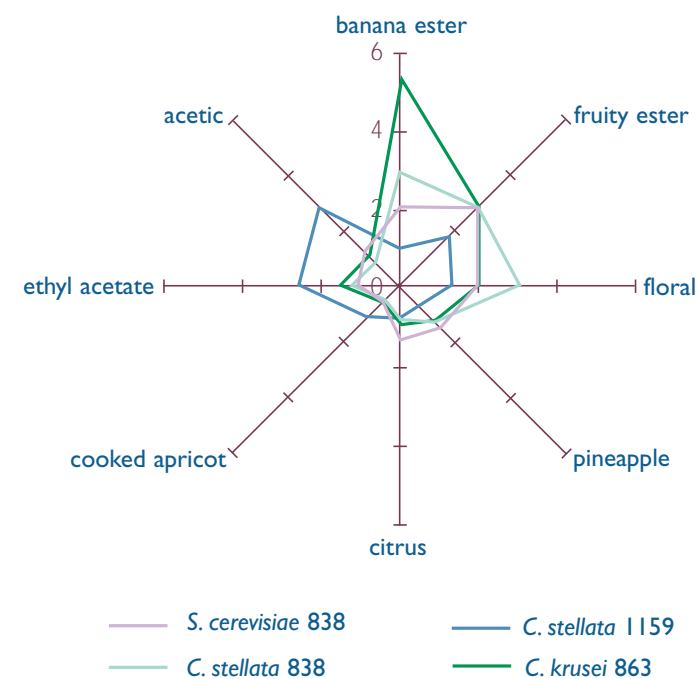


Figure 7 Chardonnay wine aroma profiles (plotted as mean intensity scores) for the reference *S. cerevisiae* and three *Candida* species used in sequential fermentation with *S. cerevisiae*.



Glenn James (BRL Hardy), Paul Henschke and Kate Howell

during cofermentation with *S. cerevisiae*. The enhanced fruity, floral ester aromas are potentially useful in floral styles of wine, and novel attributes, such as cooked apricot, may add a desirable complexity to wine.

The chemical and sensory data confirm the potential of novel yeasts for developing wines with novel attributes.

Winemaking with non-conventional yeasts workshop

The 'Winemaking with non-conventional yeasts' workshop (W05) was organised by Jeff Eglinton and Dr Paul Henschke at the 11th Australian Wine Industry Technical Conference in October 2001 (see Appendix 1). Twenty-five participants attended.

The program consisted of four presentations and two tasting sessions:

- Paul Henschke (AWRI) – *The nature of non-conventional yeasts.*
- Louisa Rose (Yalumba) – *Winemaking benefits and dangers arising from the use of non-conventional yeasts.*
- Jeff Eglinton (AWRI) – *Aroma and flavour modification using S. bayanus.*
- Tasting of wines made with non-conventional yeasts (Chardonnay: indigenous yeast, *S. bayanus* 1176 and 1375, *C. stellata* 861 and 1159, and *C. krusei* 863; Cabernet Sauvignon: *S. bayanus* 1375) by Stephen Clarke, Peter Costello, Louisa Rose and Hylton McLean.
- Alison Soden (E&J Gallo) – *Commercial use of non-conventional yeasts.*
- Hylton McLean (Orlando-Wyndham) – *Blending of wines made with non-conventional yeasts and S. cerevisiae to demonstrate the potential of these wines for generating new styles. Each group prepared their own blends.*

This workshop has helped to bring together trials being undertaken in various wineries and to stimulate interest in non-conventional yeast and the ways in which they can be deployed for increasing flavour diversity and wine quality and most importantly, to receive feed-back from industry on the merits of the use of non-conventional yeasts. Strong interest in this topic

was shown by a quick closure of available places. It is planned to represent this workshop with a greater number of places in the future.

Yeast impact on wine flavour and aroma—mixed culture versus monoculture fermentation

There is anecdotal evidence to suggest that 'natural' ferments produce wine with a different flavour profile to those produced by fermentation induced with a selected yeast, suggesting that a ferment containing multiple yeast strains may produce a wine with characteristics not normally achievable by conventional means. In an attempt to test this concept, experiments have been designed to determine whether wine made by mixed culture fermentation is different from a wine made by blending together wines made by monoculture with the constituent yeast strains. The background to this project and preliminary results were reported in the previous annual report.

In summary, five different strains of *S. cerevisiae* in three different combinations of three strains were tested under nutrient limiting and non-nutrient limiting conditions in a Chardonnay juice. Wines were also made with each of the five strains. These treatments resulted in three wines made by mixed culture ferments and five wines made by monoculture fermentation under the two nutrient conditions. Three blended wines were also prepared by mixing in equal proportion the monoculture wines which corresponded to the yeasts used in the mixed culture ferments. Duo-trio testing of the resultant wines showed that in some cases multiple strains of *S. cerevisiae* interacted to produce wines that were sensorially different to any of the wines produced by monoculture fermentation with the corresponding yeasts, or a wine produced by blending in equal proportion the three wines produced by the corresponding monoculture fermentations.

The results gave an indication that wine with an aroma profile different from wines of single yeast ferments, or their blends, can be produced by mixed culture fermentation only when all of the yeast strains are present in roughly equal proportion to allow their interaction.

In the above experiments, winemaking with five strains of *S. cerevisiae* had been used. In order to establish whether greater aroma differences could be formed by using a yeast with different characteristics from *S. cerevisiae*, a trial was undertaken with *S. bayanus* in combination with two *S. cerevisiae* strains as the mixed culture fermentation. Wines were again made by monoculture fermentation with each of the constituent yeast strains, and a blended wine was prepared from equal proportions of the three monoculture fermentation wines.

Some differences were noted in the chemical composition of the wines. The wines made by monoculture fermentation showed distinct differences in residual sugar, glycerol and acetic acid. The wine made by mixed culture fermentation had lower residual sugar than the blended wine. It is interesting to note that this was the case even though one of the strains in monoculture stuck at 22 g/L residual sugar. We have noted the occurrence of lower residual sugar in other experiments involving a combination of fermentatively impaired and robust yeasts. Glycerol was also higher (7.45 g/L versus 6.88 g/L) in the mixed culture ferment wine, as has been observed previously. Acetic acid also showed a small increase in the mixed culture wine.

Duo-trio testing of the monoculture fermentation wines against the mixed culture fermentation wines showed that for each of the three replicates, the wine made with yeast VL3c, could not be distinguished from the mixed culture fermentation wine, although in one case the blended wine was judged to be different to the mixed culture fermentation wine. Furthermore, that replicate of the mixed culture fermentation wine was also found to differ from the other two replicates. Analysis of yeast strain distribution by microsatellite-PCR in the mixed culture ferments revealed that VL3c quickly dominated, explaining the sensory result that the wines made by mixed culture fermentation could not be distinguished from those made with VL3c alone. Nevertheless, chemical analysis of the wines reveals some interaction between strains that could not be detected by sensory techniques. The results at this time are inconclusive and suggest that although multiple strains of yeast can be inoculated into a must, there is no guarantee that the strains will grow in unison to produce a mixed culture fermentation. The fact that natural ferments are characterised as having multiple strains that persist to the completion of fermentation may result from a large number of strains initially being present in the must.

Ms Kate Howell has performed this work at the Institute as part of her PhD studies undertaken through the University of NSW with supervision by Dr Eveline Bartowsky, Professor Graham Fleet (University of New South Wales) and Dr Paul Henschke.

Malolactic fermentation and wine flavour

Staff
Dr Paul Henschke, Dr Eveline Bartowsky,
Dr Peter Costello, Jane McCarthy

Interaction between wine yeast and lactic acid bacteria – In vitro system for detecting inhibitory and stimulatory effects of commercial wine yeast on wine Oenococcus oeni malolactic bacteria strains

The aim of this ongoing project is to develop a laboratory test system for detecting stimulatory and inhibitory interactions between wine microorganisms. The test system will then be used to characterise the compatibility interaction between pairs of popular wine yeast and malolactic (ML) bacteria. Information generated by this test can then be used to advise winemakers on choosing the most appropriate combination for their winemaking process.

The basic laboratory synthetic test system is free of grape derived components and, therefore, indicates the intrinsic compatibility/incompatibility between the wine yeast – ML bacterium test pair. Validation of the test by substituting the synthetic test medium with several grape juices is nearing completion, and encouraging results were reported in the previous annual report.

Work has also commenced on characterising the compatibility of several ML bacteria with a range of fermentation yeast strains, mainly of commercial origin, using the synthetic test system. To date, four *Oenococcus oeni* strains have been tested with 20 *Saccharomyces cerevisiae* and one *S. bayanus* yeast strains. The compatibility of the ML bacteria with each yeast strain has been gauged by determining the growth response of each ML bacterium in each of the 21 synthetic test wines (Figure 8).

Bacterial growth response in an unfermented chemically defined medium (lacking yeast metabolites: CDWM) has been taken as the reference point.

Preliminary analysis of the results showed that:

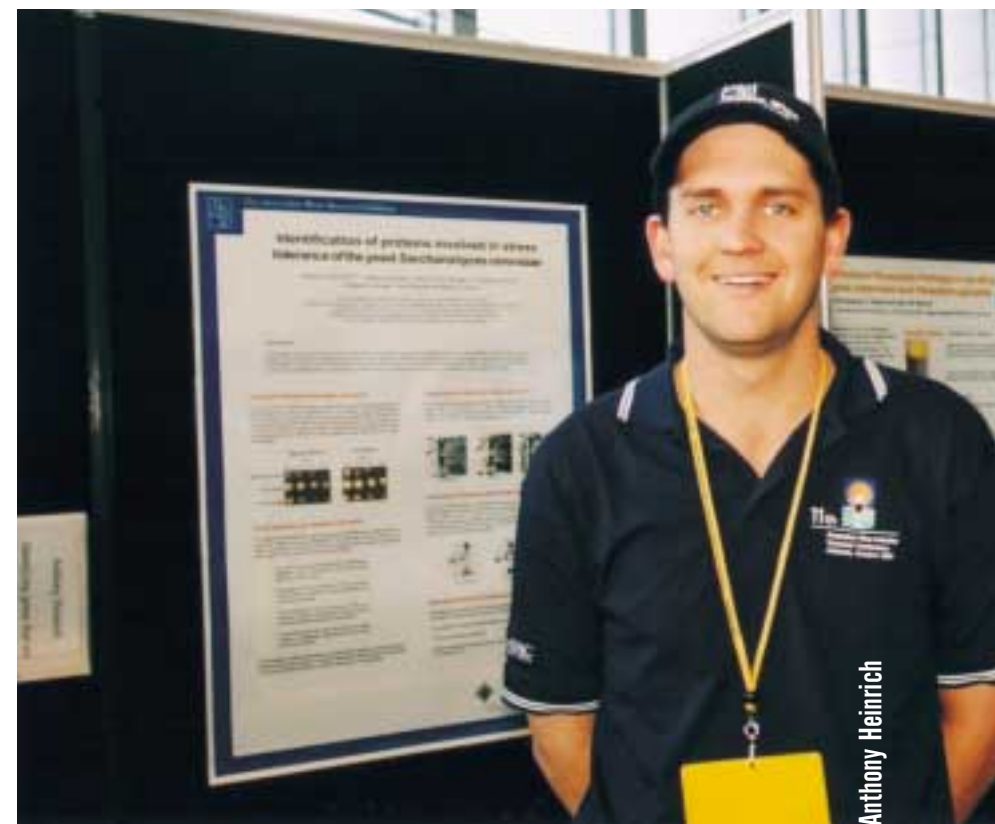
- Compatibility between yeast strain and bacterium strain depended on the combination of strains involved.
- The majority of yeasts (approx. 75%) were compatible with the four bacteria strains, however, one bacteria strain revealed a higher proportion of incompatibility reactions than the other three strains.
- The majority of yeasts were stimulatory but the response depended on the actual combination of strains: in Figure 8 all of the yeasts were stimulatory in bacterium strain 1, whereas only 15 yeasts were stimulatory to bacterium strain 2: this response again depended on the actual combination of strains.
- Depending on the bacteria strain, 6 – 9 yeasts showed a less compatible response, in that bacterial growth rate and/or yield was much reduced. In only one case was a high sulfite concentration (>30 mg/L total SO₂) responsible; most of the wines contained <8 mg/L sulfite. This result suggests that the yeast strain specific inhibitory response was due to that strain either removing a growth limiting nutrient or producing a growth inhibitory substance.

Future work will focus on verifying the inhibitory response in grape juice/wine and determining the nature of the interaction. This test, which is performed in a model system, reveals the intrinsic compatibility reaction between a yeast-bacterium pair. This reaction may or may not be the same reaction as when extrinsic factors, such as those associated with the grape must used to prepare the wine (such as, pesticide residues, pH, nutrient concentration, conditions that stimulates SO₂ production, etc) are tested. Thus, any discrepancy between the two tests would reveal the presence of a modifying extrinsic factor, one that is specifically associated with that particular grape must/juice.

The results suggest that many of the yeasts tested are compatible with the ML bacteria tested but that a number of specific incompatible yeast-bacteria combinations were revealed. Preliminary analysis suggests involvement of SO₂ in one case. These findings need to be verified with grape juice media because under certain conditions some yeast produce inhibitory levels of SO₂. An update of the work was reported to industry through a poster presentation 'Standardised method for testing malolactic bacteria and wine yeast compatibility' by P.J. Costello, P.A. Henschke and A.J. Markides at the recent 11th AWITC held in Adelaide during October 2001. A manuscript describing development of this test is being prepared for publication in a scientific journal.

Changes in volatile compounds and their sensory significance arising from MLF—a preliminary investigation

Malolactic fermentation (MLF) has been reported to modify the concentration of some volatile compounds of white wine (Laurent et al. 1993) but little is known about the changes in red wine. Furthermore, the sensory significance of these



Anthony Heinrich

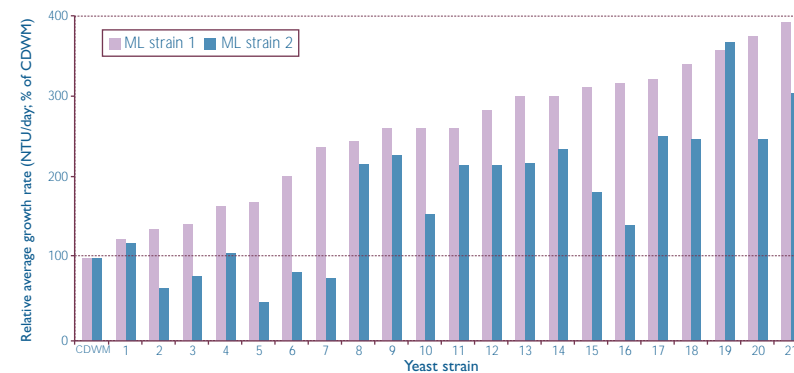


Figure 8 Growth rate of two strains of *Oenococcus oeni* in test wines prepared with 21 different yeast relative to growth rate in a reference unfermented chemically defined wine medium.

changes is largely unknown. A pilot project was undertaken to determine the effect of MLF on the volatile flavour profile of a Shiraz wine using gas chromatography olfactory analysis (GC-O or GC-sniff) coupled with gas chromatography mass spectrometry (GC-MS) analysis for compound identification. The GC-sniff analysis involves introducing a portion of the headspace of a wine sample into a gas chromatographic column which allows the separation of the volatile compounds roughly according to boiling point. As the volatile compounds emerge from the GC column, a portion is directed to the GC detector with the remainder being passed through a port (sniff cup) at which a person sniffs for aromas. The person who is sniffing the port, indicates when he/she is able to detect an aroma and if possible assign a descriptor to it. The GC-sniff and GC-MS chromatograms are then compared to align volatile compound peaks with aroma descriptors. In this way, the chemical identity can be established for many of the sensorially important compounds. This technique also allows changes in wine aroma to be profiled, such as that induced by MLF.

Quantities (2L) of a Shiraz wine (Langhorne Creek 2001; kindly donated by Orlando Wyndham, Rowland Flat), were inoculated for MLF with commercial *Oenococcus oeni* preparations Enoferm alpha (Lallemand) and Viniflora CH35 (Chr Hansen) together with another quantity that was allowed to proceed with the indigenous bacteria. MLF was completed within two weeks for all treatments.

There were numerous aroma differences noted between pre- and post-MLF wines when analysed by GC-sniff. Examples of GC-sniff descriptors (corresponding to individual peaks on the GC chromatogram) which exhibited altered significance following MLF included solvent-like, popcorn, caramel, toffee, waxy, apple, floral, sweet, dusty, burnt, plastic, unpleasant and chemical. We were able to tentatively identify (GC-MS database) 38 of 48 detectable peak of volatile compounds in the wines and the concentration of many of these was altered by MLF. The concentration and odour of some of the volatile compounds was decreased by MLF whereas others were found to increase in sensory significance. As expected, the diacetyl concentration increased as the result of MLF, and this attribute was also noted by the panel in the informal sensory assessment of the wines. The

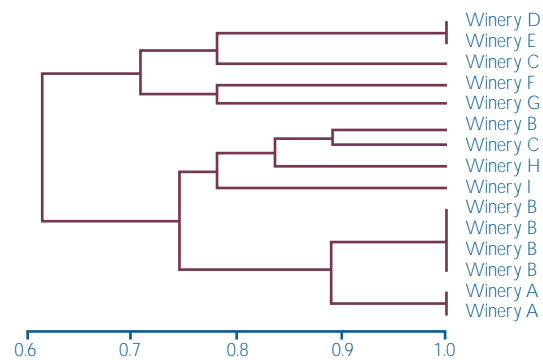


Figure 9 Dendrogram generated by cluster analysis of the similarity matrix of Australian isolates of *Oenococcus oeni*.

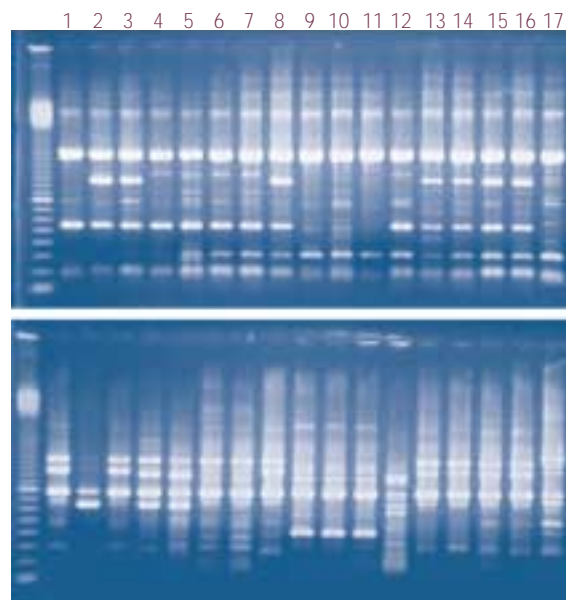


Figure 10 DNA fingerprint using the RAPD technique with two RAPD primers of *Oenococcus oeni* winery isolates. 100 bp ladder molecular weight marker is in the first lane.

Presented by Gideon Rachman



GIDEON RACHMAN (BELGIUM)

GUEST SPEAKER 11 AWITC

diacetyl content of the wines also varied according to the strain of *Oenococcus oeni* used to conduct the MLF. Various volatile compounds which are associated with fruity wine characters were identified in these wines (including ethyl acetate [fruity], isoamyl acetate [banana], ethyl lactate [fruity] and ethyl octanoate [fruity]). From this study, we were also able to show differences between *O. oeni* strains during MLF, particularly variations in the concentration of some 20 different volatile compounds, including alcohols, esters and carbonyl compounds [acetaldehyde, diacetyl and acetoin]. This work needs to be repeated with other wines in order to establish general trends on the impact of MLF on wine aroma.

This project was undertaken by Ms Angela Matthews as an Honours project in the Department of Horticulture, Viticulture and Oenology, with supervision by Drs Eveline Bartowsky and Leigh Francis. Angela was awarded a first class honours degree, and undertook vintage experience with Piramimma Winery in the McLaren Vale. This work was undertaken using the facilities of Dr Graham Jones (DHVO, Adelaide University) in the Wine Science Laboratory. Invaluable assistance with the GC-sniff and GC-MS was provided by Ms Tracey Siebert and Dr Alan Pollnitz.

This project has given us an insight into the potential volatile aroma compounds (other than diacetyl, the buttery note very well characterised and known to be associated with bacterial metabolism during MLF) that can be associated with MLF.

Differentiation and relatedness of *Oenococcus oeni* winery isolates

The differentiation of strains of the homogeneous species *Oenococcus oeni*, which is the main species used for the induction of malolactic fermentation of both red and white wines, can be performed using the well established DNA fingerprinting technique of randomly amplified polymorphic DNA (RAPD). This technique also provides some information regarding the relatedness or similarity of strains. We have previously used the RAPD technique to demonstrate a unique group of wine related strains within the species *Acetobacter pasteurianus* which were causative agents of spoilage of several batches of bottled red wine (*Technical Review* 127, 11-12, 45-47).

The Institute's culture collection contains a series of *O. oeni* strains isolated from Australian wineries, and a number of commercial cultures. Some of these strains were subjected to DNA fingerprinting in order to estimate the relationship between these strains. We were able to demonstrate that *O. oeni* isolates from the same winery often were indistinguishable but grouped separately from other strains isolated from different wineries. A dendrogram showing the degree of similarity between strains isolated from the same and different wineries is given in Figure 9.

In addition to this study, we also examined *O. oeni* strains isolated from alcoholic fermentations from a single fruit source (Cabernet Sauvignon, Padthaway) before and after spontaneous malolactic fermentation. These ferments were from the 2002 vintage *Saccharomyces bayanus* trial conducted in the Hickinbotham Roseworthy Wine

Science Laboratory trial this year and reported on under Project AWR 2 in this report. An example of a RAPD DNA fingerprint of isolates from this study is shown in Figure 10, which reveals some eight different strains amongst the 17 isolates. This analysis highlights the importance of using at least several RAPD primers to distinguish closely related strains. A group of isolates, numbers 13, 14, 15 and 16, were indistinguishable with the two RAPD primers whereas isolates 2 and 3, while being undifferentiated with the first primer, were clearly differentiated with the second primer. This analysis also showed that the *Oenococcus oeni* strains which were isolated following MLF completion were either indistinguishable from or closely related to those isolated prior to MLF. Even though only a limited number of isolates have been examined in this study, this study showed that 3-5 strains dominated the spontaneous fermentation, an observation also noted by other European research groups. More wines require to be investigated to show whether spontaneous fermentations typically involve a group of different strains.

The DNA fingerprinting technique, RAPD can be used to study the relationship of various *Oenococcus oeni* strains. Australian winery isolates of *O. oeni* were examined using this technique and isolates that originated from the same winery were generally found to be indistinguishable. Isolates of *O. oeni* from a red wine pre- and post-malolactic fermentation showed the presence of several different strains which persisted throughout fermentation.

Malolactic fermentation workshop

The workshop (W26) 'Malo cultures – can you taste the difference?' was chaired by Drs Eveline Bartowsky and Peter Costello at the 11th Australian Wine Industry Technical Conference in October 2001. Twenty-six participants attended. The program consisted of four presentations and two tasting sessions:

- *MLF and flavour modification* - Eveline Bartowsky (AWRI)
- *Winemakers' views on MLF* - Glenn James (BRL Hardy)
- *The role played by ML strain on MLF flavour* - Sibylle Krieger (Lallemand)
- A tasting of wines prepared with different ML strains was conducted by Glenn James and Sibylle Krieger.
- Introduction to the second session - Peter Costello (AWRI)
- *Shaping wine style with MLF* - Michael Peterkin (Pierro Wines)
- *Timing of MLF inoculation* - Mai Nygaard (Chr. Hansen)
- A tasting of wines to demonstrate how MLF can be used to guide wine style and the impact of inoculation regimes was conducted by Michael Peterkin and Mai Nygaard (five German wines with two strains pre- or post-fermentation; three WA Chardonnay with/without MLF; five MLF AWRI wines made with Vf CH35, Oenoferm alpha and spontaneous culture).

The workshop informally indicated that:

- MLF with certain strains induces distinctive aroma profiles – this occurrence may in part be due to the recent selection of flavour enhancing strains launched by the suppliers of malolactic bacteria cultures.
- MLF in general induces greater wine mouth-feel
- Timing of inoculation relative to the alcoholic fermentation induced different aroma and palate changes.

Microbiological analysis of industry technical problems

Staff Dr Paul Henschke, Dr Eveline Bartowsky, Dr Peter Costello, Jane McCarthy

This project undertakes to investigate specific winemaking microbiological problems of importance to the wider industry. Recent topics have included causes and control of mousy off-flavour, the suitability of near infrared spectroscopy for measurement of yeast assimilable nitrogen content of grape juice, spoilage of bottled red wine by acetic acid bacteria and the role of acetic acid in restarting stuck fermentation as summarised in previous annual reports. A new project to investigate the causes and control of 'Brett' spoilage in wine has been initiated. This project is part of a GWRDC-funded collaborative project involving four teams within the Institute. Project AWR 5 also provides resources for maintaining the Institute's culture collection which provides yeast and bacteria for teaching and research, performing advanced wine microbiological analysis, undertaking dried yeast quality testing, and providing microbiological consultation to industry personnel. A highlight for the year was the highly successful undertaking of a workshop on the identification and oenological characteristics of wine spoilage microorganisms during the 11th Australian Wine Industry Technical Conference in October 2001.

Mousy off-flavour occurrence and formation in wine

Two manuscripts communicating the proposed mechanism for the biosynthesis of the mousy off-flavoured N-heterocycles by a *Lactobacillus* species and *Dekkera (Brettanomyces) anomala* are nearing completion:

- Costello, P.J.; Lee, T.H.; Henschke, P.A. (2001) Ability of lactic acid bacteria to produce N-heterocycles causing mousy off-flavour in wine. *Aust. J. Grape Wine Res.* 7: 160-167 (Institute publication # 680).
- Costello and Henschke. Mousy off-flavour of wine: precursors and biosynthesis of the causative N-heterocycles 2-ethyltetrahydropyridine, 2-acetyl-1-pyrroline by *Lactobacillus hilgardii* DSM 20176. *J. Agric. Food Chem.* (accepted for publication).
- Grbin and Henschke. The role of lysine amino nitrogen in the biosynthesis of mousy off-flavour N-heterocycle compounds by *Dekkera anomala*. (in preparation).

Based on an analysis of precursor compounds required for the formation of mousy N-heterocycle compounds using chemically defined minimal media these papers propose a mechanism for the biosynthesis of N-heterocycles by a *Dekkera (Brettanomyces)* yeast and a *Lactobacillus* species. In brief, our data showed that 2-acetyl-1-pyrroline was derived from L-lysine and 2-acetyl-1-pyrroline was derived from L-ornithine. In the case of *Dekkera anomala*, using mass labelled ϵ -N of lysine, it was shown that the ϵ -N, and not the α -N, was incorporated into the N-heterocycle. A fermentable sugar was necessary and by using mass labelled ethanol, and an analogue (propanol) it could be shown that the acetyl group was derived from ethanol. When ethanol was substituted with propanol, the propyl N-heterocycle was formed, verifying the source of the acetyl group. Iron (Fe²⁺) was also necessary for N-heterocycle formation by *Lactobacillus hilgardii*. This work has improved our understanding of the origin of mousy off-flavour compounds by wine spoilage yeast and bacteria and complements the previously published work on the scope of wine microorganisms capable of forming mousy off-flavour. The practical implications of this work are that relatively high populations of *Dekkera/Brettanomyces* and lactic acid bacteria are needed to cause a detectable level of mousy off-flavour. The main tools for controlling unwanted microbial growth are good general winery hygiene, low residual sugar and adequate molecular SO₂. Decreasing lysine and ornithine to negligible levels is probably unpractical.

Brettanomyces spoilage of wine

Through a recent survey of Australian wines for 4-ethylphenol conducted by the Institute under projects AWR 19 and 11 (see also Institute publications # 623, 627, 638), it has become clear that the incidence of *Dekkera/Brettanomyces* yeast is much wider spread in Australian wineries than thought. A collaborative project to investigate the physiological properties of wine isolates of *Dekkera/Brettanomyces* with the aim to formulate practical control measures has commenced. This project, which will involve industry services, chemistry, molecular biology and microbiology teams, is coordinated by Peter Godden through GWRDC-funded project AWR 11.

This project aims to:

- Review current knowledge on the wine spoilage characteristics of *Dekkera/Brettanomyces*;
- Isolate *Dekkera/Brettanomyces* strains isolated from winery samples for genetic, physiological and biochemical characterisation; and
- Define conditions that limit the growth of *Dekkera/Brettanomyces* yeast in wine.

Wine microbiological spoilage workshop

The workshop (W21) 'What bug is that? Bretts, bacilli and other nasties – How to spot them in your wine' was presented by Holger Gockowiak at the 11th Australian Wine Industry Technical Conference in October 2001. Twenty participants attended. The program consisted of two presentations and four demonstrations/practical exercises:

- *Selection, use and care of microscopes in the winery* – Peter Hunt (Olympus Australia)
- *Isolation and identification of wine related yeast and bacteria* – Holger Gockowiak (AWRI)
- *Microscopic examination of wine related yeast and bacteria* – demonstration and practical exercise.
- Gram staining and catalase test – demonstration and practical exercise.
- Isolation and purification of wine microorganisms – demonstration and practical exercise.
- Use of selective media for identification of wine microorganisms.

Participants gained a better understanding of how to quickly recognise various genera of yeast and bacteria with a microscope and using various simple microbiological techniques which can be applied in most winery laboratories.

Yeast interaction with red grape phenolics and effect on wine sensory properties

Staff Dr Paul Henschke, Dr Eveline Bartowsky, Simon Dillon

A collaboration between the Institute and Lallemand has continued to investigate the interactions between yeast and grape phenolics during red wine fermentations. Lallemand is contributing a substantial sum of money for our investigations, for which we are most appreciative. The donation of Shiraz grapes by BRL Hardy and Orlando Wyndham for these studies is also appreciated.

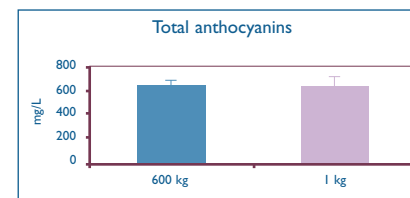
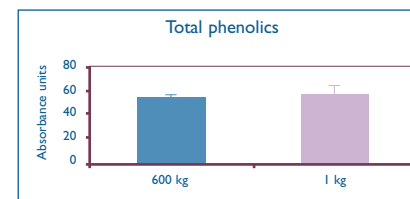
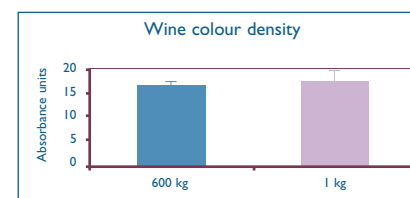


Figure 11 Comparison of wine colour density, total phenolics and total anthocyanins of young wines made from Cabernet Sauvignon grape must (Padthaway, 2002) by fermentation with *S. cerevisiae* strain AWRI 838 on a winery scale (600 kg) and laboratory scale (1 kg).

The background to this project was described in the 2001 Annual Report. In this report we also reported on the development of a reliable laboratory scale (1 kg) red fermentation method, and its application to comparing 17 different yeast strains for their ability to effect extraction and/or retention of various grape phenolic compounds into wine. In this work, we found evidence for the importance of the strain of fermentation yeast on the development of wine colour and phenolic composition. Furthermore, we showed that the performance of the yeast strain was relatively consistent for Shiraz fruit from two different viticultural regions (Adelaide Hills and Langhorne Creek, vintage 2001). This result suggests that the yeast strain has an intrinsic ability to influence the colour and phenolic profile of wine to a significant extent, and supports anecdotal observations by various winemakers and Lallemand's experience.

In addition to these trials, we have examined the performance of the same six yeast strains in fresh and frozen (-20°C stored) fruit. The wine colour density in frozen fruit was significantly higher than that of fresh fruit (P<0.0001), however, the relativity between yeast strains was similar, that is they ranked in a similar order with respect to wine colour density. It could be expected that the freeze-thaw process will assist in the release of phenolics into the wine. This result is important in that it allows us to conduct trials throughout the year with frozen fruit, knowing that the yeast will perform in a relative manner to that observed in fresh fruit, at least in respect of wine colour.

In conjunction with the 2002 Cabernet Sauvignon trial conducted in the Hickenbotham Roseworthy Wine Science Laboratory, reported under *Yeast flavour and fermentation activity*, we conducted a trial to compare wine colour density and phenolic profiles of wines made on the 1 kg scale in the laboratory with those made on a winery scale with 600 kg of fruit. Premium quality Cabernet Sauvignon fruit from Padthaway was inoculated with *Saccharomyces cerevisiae* strain AWRI 838 and fermented in triplicate 700 kg capacity rotary vessels operated at 20°C. Samples of the red must were taken from the rotary vessels prior to inoculation and divided into triplicate 1 kg lots, inoculated from the same starter culture and fermented under similar conditions of temperature and protection from air. The standard laboratory procedure of cap plunging four times daily was used; the rotary tank was given four rotations twice daily. Spectral analysis of the wine samples taken at the end of alcoholic fermentation has shown that the wine colour density, total phenolics and total anthocyanins were equivalent (Figure 11).

These results indicate that the laboratory scale red wine fermentation procedure is reliable and can produce wines which resemble those made on a pilot winery scale. Such a method will facilitate the study of yeast interaction with red wine phenolics and provide data suggestive of industrial relevance.

Links between viticultural and oenological research

Staff Dr Sally-Jean Bell

A meeting was conducted with approximately 16 industry representatives in May to review recommendations on the use of agrochemicals. Thereafter, nine thousand copies of the Institute's annual publication, *Agrochemicals registered for use in Australian viticulture 2002/2003* were produced and the information duplicated on the Institute's website. The booklet was distributed with the *Australian New Zealand Grapegrower and Winemaker, Technical Review* and in the *Research to Practice™* IPM and Spray Application manuals. The tables are also featured in *Australian Viticulture*. The 2002/2003 MRLs for Australian export markets will be placed on the Institute's website.

As a direct result of the Institute's agrochemical publication and its Residue Analysis Service, Dr Sally Bell and Greg Ruediger devote a significant amount of time in liaising with major chemical companies. Companies are increasingly aware of the importance the wine industry places on meeting export market specifications. Thus, they are keen to work more closely with the wine industry and the Institute to ensure that their products can be used in viticultural pest and disease programs in such a way that the maximum residue levels set by Australian export markets will not be exceeded.

During 2001/2002, Dr Sally Bell responded to 566 enquiries, twenty-four less than in the previous 12 months. Approximately 75% of these enquiries related to agrochemicals.

Dr Sally Bell with Mr Alex Sas (BRL Hardy) coordinated a workshop held at the Eleventh Australian Wine Industry Technical Conference on two occasions entitled *Future trends in agrochemical use*. Dr Sally Bell delivered two presentations at the Institute's Roadshow in Stanthorpe, QLD (see Appendix 1).

Dr Sally Bell participated in four *Research to Practice™* Grapevine Nutrition Workshops at the Clare Valley and at McLaren Vale (see Appendix 1). A lecture was presented on *Herbicides in viticulture* to viticulture and oenology students at The University of Adelaide (see Appendix 2). While on leave, she gave a presentation entitled *Effects of water stress on the phenolic composition of Shiraz grapes* at the University of Oulu, Sotkomo, Finland.

The investigation of the effect of plant water status and canopy management on the phenolic profiles of grapes and wine in collaboration with Dr Patrick Iland and Dr Peter Dry (The University of Adelaide), Orlando Wyndham (Lyndoch) and Professor Enrico Peterlunger (Universita di Udine, Italy), commenced in October 2000. To date, it has been confirmed that low vigour water stressed vines produce higher concentrations of seed catechin and epicatechin and skin anthocyanins, than high vigour well watered vines throughout ripening, regardless of trellis type. The smaller berry weight from stressed vines was a contributing factor. Vertical Shoot Positioned (VSP) vines

produced higher concentrations of seed catechin and epicatechin and skin anthocyanin than Scott Henry vines, throughout ripening. The higher canopy density and lower bunch exposure exhibited by VSP in a hot season appears to be beneficial. It remains to be seen if this was the case in the much milder 2001/2002 season, where canopy responses could be markedly different.

The sensory analysis has been completed and a brief summary has been prepared by Dr Leigh Francis: A viticultural experiment in the Barossa Valley involving an investigation of two trellis systems, Scott Henry and VSP, together with an irrigation regime of low, moderate and high water was carried out at a Lyndoch site during the 2000/2001 season. Wines from the 2000/2001 season were made under replicated, identical conditions at the Hickenbotham Roseworthy Wine Science Laboratory, and a formal sensory analysis was carried out, with the main aim of assessing mouth-feel characteristics and aroma differences among the wines. With this study, there were some fermentation replicate differences observed for some sensory attributes, which complicates the interpretation of the treatment effects, but there were, nonetheless, consistent differences among the treatments in mouth-feel attributes rated by the panel. For example, for the VSP treatment, the wines made from fruit with a higher level of water had a significantly higher 'silky' mouth-feel, together with a lower rating for 'drying'. The Scott Henry canopy wines in contrast, displayed higher 'silky' sensation perceived 'in mouth', in the low irrigation treatment, with the higher irrigation treatment being more 'drying'. One of the fermentation replicates of the Scott Henry low irrigation regime treatments showed a remarkably strong, simple 'raspberry' aroma which was of great interest, but as the other two replicates did not have such a high rating, this result will require further investigation with another season's data. Due to the large meteorological differences between 2001 and 2002 vintages, it is important to await the outcome of our 2002 trials prior to drawing any global conclusions about the links between viticultural practice and phenolic composition of wine.

Preparation of information on wine and health issues

Staff Creina Stockley

Over the past 12 years, Ms Creina Stockley, in her position as the Health and Regulatory Information Manager, has overseen the development of a database of research on the beneficial and detrimental health effects of alcohol and in particular, wine, located on the internal database of the John Fornachon Memorial Library. This was 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. From 31 October 2001–30 June 2002, 64 independent information requests were received on wine and health issues from industry, government and the general public by the Health and Regulatory Information Manager. Subscription

to relevant medical and other journals has continued. 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 Institute'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 quarterly newsletter of the Australian Society of Wine Education, in addition to three papers for the *Australian and New Zealand Grapegrower and Winemaker* and one paper for the *Bulletin de l'OIV*. A video taped presentation for a subsequent CD-ROM entitled *Your invitation to a cardiovascular journey: potential cardiovascular benefits of moderate wine consumption* was also undertaken which related to the GWRDC-funded research projects coordinated by the Health and Regulatory Information Manager.

Submissions prepared or commented on for the Australian wine industry include:

- National Alcohol Research Agenda of the Tobacco and Alcohol Strategies Section, Commonwealth Department of Health and Aged Care;
- draft resolutions OENO/ASP/99/129/Step 7 Health warning: OENO/VINSAN/01/191/Step 7 Specificity of wine and scientific research: ECO/REGL/96/23/Step 5 International OIV standard for labelling wines: OENO/ASP/01/190/Step 3 Wine consumption and young adults; and OENO/ASP/01/191/Step 3 Effects of wine and scientific research for the Nutrition and Wine Expert Group, and questionnaire on the *Responsibilities of the Nutrition and Health Sub-commission and Expert Groups*, of the Office International de la Vigne et du Vin (OIV)—the Health and Regulatory Information Manager is vice-president of the Nutrition and Wine Expert Group; and
- GWRDC new project application entitled *The identification and measurement of potential allergens in wine* in conjunction with Professor Robyn O'Hehir, Director of the Department of Allergy, Asthma and Clinical Immunology, The Alfred and Monash University. This funded application will address an important change in the wine industry's national and international regulatory environment.

A four-hour workshop entitled *Do wine-derived phenolic compounds have a role in improving human health?* was designed and developed, as well as the second two-hour session entitled *Research efforts in Australia on a potential beneficial role of wine in human health*, for the 11th Australian Wine Industry Technical Conference. Also organized was the wine and health symposium entitled *Current wine and health information: the issues in perspective* for the 26th World Congress and 81st General Assembly of the Office International de la Vigne et du Vin which was held on 15-17 October 2001—the Health and Regulatory Information Manager was the Wine and Health Section President (see Appendix 1).

Project coordination

Through Creina Stockley, the Institute has played a coordinating and a participating role in three GWRDC-funded research projects on medical aspects of wine consumption entitled *Grape antioxidant phenolics: absorption and inhibition of lipid peroxidation in humans; Reduction of damage to LDL and DNA from oxidative free radicals by the regular and moderate consumption of wine, and Potential cardioprotective activities of wine components based on synergistic interaction with vitamin E*. The first project has been completed and the latter two projects are currently being completed.

Technical problem solving and consulting

Staff Peter Godden, Adrian Coulter, Peter Valente, Ella Robinson, Mark Gishen, Greg Ruediger, Professor Peter Hoj, Yoji Hayasaka, Trudy Weber, Heather Donnell

The Industry Services team provides a technical support service to the Australian wine industry, primarily in the form of an advisory service which 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 also manages a large research trial that is examining the technical performance of various types of wine closure, and a project which is developing a web-based technical reference manual for the Australian wine industry, with associated workshops which are presented in conjunction with Institute seminars. In addition, during the year, work commenced on a new Institute-wide project to be managed by Industry Services, which will investigate the relationships between the spoilage yeast *Dekkera/Brettanomyces* and wine in Australia. The team played a substantial role in the planning and staging of the 11th Australian Wine Industry Technical Conference, in the coordination of 72 workshops which were presented as part of the Conference program. In addition, the team presented two formal papers at the conference, jointly authored a third, and organised six of the workshops, two of which were entirely presented by members of the team. The team Manager and Mark Gishen also provided formal presentations for the 26th General Assembly of the OIV, which was held in Adelaide in October 2001, and members of the team, in conjunction with other Institute staff, also co-authored a third paper for this conference. In addition, the Industry Services team provided eleven hours of lectures to Oenology students at the University of Adelaide, and other lectures to students from the University of Western Australia and La Trobe University.

The Industry Services Laboratory analyses several hundred samples each year, using a wide range of routine and unique analytical techniques. The majority of samples are wine, the analysis of which is supplemented by detailed sensory evaluation by an Institute panel of experienced tasters. Of the remaining samples analysed, the majority consist of wine additives, closures, or compounds which are suspected to have caused taints and or deposits in wine. The sensory analysis capability of the Institute was enhanced during the year by

the recruitment of Kate Lattey in the newly created, GWRDC-funded position of Chemist/Sensory Analyst, a position reporting to Dr Leigh Francis.

The aim of the service is to offer remedial and preventative advice based on the cumulative problem solving experience of the staff, and the practical winemaking experience of the team Manager, Oenologist and other staff members, rather than providing a simple diagnosis of the cause of the problem. Increasingly, staff regard their role as educational, seeking to disseminate information in a variety of ways in order to prevent the recurrence of particular types of problem. The Industry Services team also provides technical support to the Institute'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 Institute's problem solving 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 Institute and its clients, which in turn allows the maximisation of the knowledge gained from the provision of these services. When a particular problem is considered to be of interest to the wider industry, the results of investigative work are made available through relevant publications, and the Industry Services team has contributed six Technical Notes and three other articles to *Technical Review* during the past year. When preparing material for publication, great care is taken to ensure that, under no circumstances is the name of the client, or any possible identifying reference, revealed.

It is becoming clear that an increasing proportion of the investigations conducted relate to disputes arising between either grapegrowers or wineries, and suppliers of either materials or contract services. Consequently, Industry Services staff, with the involvement of the Director and Company Secretary increasingly find themselves in a mediation role in these disputes, and spend a considerable amount of time providing technical information to legal professionals representing grapegrowers and wine companies. It is, therefore, clear that the support offered to the Australian wine industry by the provision of these services is increasing in scope, and of great benefit to clients, as the majority of disputes are settled before formal court proceedings are instigated. Equally, it is also clear that the Institute's ability to continue to serve in such a demanding role requires an enhancement of resources.

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 total number of samples analysed increased by 17% from the previous year. The number of investigations conducted also increased by 20% on the previous year. Whilst the number of samples which were found to exhibit the related problems of hazes and deposits and microbiological instability remains high, the fact that there has been a slight decrease over the two year period



Peter Godden

Table 5 Summary of the number and type of problem solving investigations conducted by Industry Services during the past three years

	Samples received (99/00) and investigations conducted (00/01 and 01/02) ^a		
	1999/00	2000/01	2001/02
Identification of hazes and deposits	114	135	85
Microbiological investigations	85	50	89
Sensory assessments	29	51	98
Taint problems	186	66 ^b	43
Other investigative analyses	252	128	209
Closure-related investigations	122	24 ^b	22
Total	788	454	546 ^c

^athe manner in which sample numbers are calculated for Table 5 was changed for the 2000/2001 Annual Report. Some investigations require the analysis of multiple samples, while, in some situations, one investigation is conducted on several different wines from the same winery which all exhibit the same problem, and all become the subject of a single report. Therefore, from 2001 onwards, the actual number of investigations conducted will be listed in the body of the Table, while the number of samples analysed will be provided as a footnote.

^bmuch of the decrease in the number of 'taint problems' and 'closure related investigations', many of which involve the analysis of multiple samples, can be attributed to a portion of this work now being conducted by the Institute's Analytical Service on a commercial contractual basis.

^ca total of 1223 separate wine samples were analysed as part of these investigations

is encouraging, especially considering that the 2000 and 2001 vintage conditions were generally considered difficult throughout much of Australia. The issues related to these wine instability problems are being addressed by the GWRDC-funded Project 99-1 *Targeted training of wine industry personnel: Compilation of a technical reference manual and development of associated workshops*, which is discussed in more detail later in this report. The steady increase in the number of sensory assessments conducted can be partly explained by the Institute's ever increasing capabilities in this area.

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

- The number of 'problem' wines submitted to the Institute and found to be contaminated with substances that are not permitted additives to wine, is of continuing concern. The most common causes of contamination continue to be refrigerant brine and hydraulic oil. Issues related to the minimisation of the risks of this type of contamination continue to be addressed in the workshops presented during Institute Roadshows, and it is apparent that in many of the cases investigated, the contamination was easily avoidable if the advice presented in these workshops had been heeded.

The Institute believes that it has performed an important role for the wine industry by encouraging a culture of openness from industry practitioners in regard to matters concerning this type of contamination, and to the best of its knowledge, all the cases of which the Institute has been notified over recent years have been settled through insurance claims. The possibly huge negative consequences of a contamination incident being 'covered up', only to be later identified during testing conducted by a third party, are obvious. The Institute is, therefore, concerned about recent events with regard to two insurance claims resulting from known hydraulic oil contamination of grapes during harvesting, which were apparently initially refused by the respective insurers. In addition, the growers and wineries concerned were apparently advised by insurance assessors to continue processing the grapes in order to 'recover' some value. In these cases, the implication could be taken that the insurance assessors, and the companies they represented, deemed the grapes to be fit to re-enter the production chain, even though contamination was known to have taken place. The Institute would find this position unacceptable, and under these circumstances would in contrast, strongly recommend that the grapes not re-enter the production chain. The Institute is pleased that following its intervention, both of the claims were subsequently settled. However, serious issues are raised by these cases, and the Institute wrote to the Insurance Council of Australia to voice its concerns. The Insurance Council responded by circulating the letter to all of its members. The Institute commends the honesty and integrity of those industry practitioners involved in these cases, and strongly encourages the industry-wide continuation of the culture

of openness regarding issues of this type, as the possible consequences of the diminution of this culture could be disastrous for the entire industry.

- Several apparently fraudulent consumer complaints to Australian wine companies have been investigated during the year. With a number of these complaints, consumers have apparently claimed that the bottles they have purchased have in fact been full of water instead of wine. In all cases, with assistance from the Australian Water Quality Centre Laboratory in South Australia, Industry Services staff have been able to demonstrate that the water in the returned bottles did not originate from the water supply at the place where the bottles were originally filled. In the case of a bottle returned in the UK, comparison of water composition data held by the Australian Water Quality Centre demonstrated that the composition was consistent with that found in a relatively small region of Southern England, and that the water certainly did not originate from the place in Australia where the bottle was originally filled.
- Five particular investigations are of concern, as the problems appeared have their origins in flawed bottling procedures. Two of these cases involved obvious damage to closures from corking jaws, that appears to be present throughout each of the bottling runs concerned, and which was apparently not identified during quality control checks. In addition, three separate cases of sporadic re-fermentation of white wines in bottle were investigated. The absence of yeast or bacteria in 'unaffected' bottles of these wines, all of which had been 'sterile filtered', suggests that contamination is most likely to have occurred due to poorly sanitised bottling equipment.
- During the year, the Institute was approached by both winemakers and grapegrowers, regarding the use of chlorine dioxide as a disinfectant for ground water to be used in wineries, and as a vineyard spray. Industry Services' staff were immediately concerned about the possibility of the formation of chlorophenols by the reaction of chlorine dioxide with phenolic compounds in both grapes and wine. However, in each case the suppliers of equipment to generate chlorine dioxide had assured callers that chlorophenol production was not a problem, and the literature supplied by a distributor of chlorine dioxide generating equipment to one winemaker stated 'chlorine dioxide oxidises phenols so that chlorophenols can't be produced'. To test this claim a trial was conducted. A solution of approximately 10 mg/L of phenol in water was treated with chlorine dioxide at two rates: 0.5 mg/L and 5 mg/L, which covered the concentration range being recommended to

winemakers for the treatment of water. The solutions were allowed to stand overnight at room temperature, and were then extracted with dichloromethane and analysed by GC-MS.

No chlorophenols were detected in the phenol solution treated with 0.5 mg/L chlorine dioxide. However, 4 chlorophenol was detected in the phenol solution treated with 5 mg/L chlorine dioxide. From the semi-quantitative data available, the concentration of 4-chlorophenol in the sample was considered likely to be between 500 and 700 micrograms per litre, and corresponded with a reduction in the concentration of phenol in the solution.

Winemaking consultation

The Industry Services team provides a winemaking consultancy service principally through the Manager, Peter Godden, a qualified and experienced winemaker; Adrian Coulter, holder of a Graduate of the Diploma in Oenology from The University of Adelaide; and Mark Gishen, a qualified engineer and former Q/L Manager in the Hunter Valley for five years. Greg Ruediger, the Trace Analysis Laboratory Supervisor, works with the Industry Services team on a 0.25 basis, and also holds a Graduate of the Diploma in Oenology from The University of Adelaide. Both Adrian Coulter and Greg Ruediger have gained vintage experience with local wineries during recent years.

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 number of calls received from journalists has increased substantially over a number of years, and this is considered to reflect both increasing public awareness and interest in the wine industry, and also the nature of the projects being pursued by the Industry Services team, particularly the ongoing investigation into the technical performance of wine closures. The majority of queries are answered either by telephone, e-mail or facsimile, and Industry Services staff supply approximately five hundred technical papers or other pieces of relevant literature to callers each year. Increasingly, Industry Services staff are also able to direct callers to web-based information, both on the Institute's own, and other, web sites. In addition, the support facilities provided by research and library staff are important in supplying relevant information to callers, and the analytical capacity of the Industry Services Laboratory plays an important role in responding to many of these enquiries. For approximately 45% of the investigations recorded in Table 5, the results are reported to the client in the form of a detailed



Mark Gishen

discussion and interpretation of tabulated data that has been generated by laboratory analysis. For the remainder, a full written report is 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 them re-occurring. The reports are often accompanied by a number of technical references relating to the area of investigation.

The Institute also often acts as a referral service, increasingly for Government bodies, wine industry suppliers and wine journalists, having links to Australian and international wine research and political bodies. The vast store of information, both formal (in the John Fornachon Memorial Library) and informal, is a valuable resource to the wider industry.

During this reporting period, the Institute, particularly Greg Ruediger, Dr Leigh Francis, and the team Manager, continued to provide technical assistance to the developers of public displays for the National Wine Centre, which opened in October 2001.

The team Manager acted as an Associate Judge at the 2001 Royal Adelaide Wine Show between the 25th to 28th September 2001, and as a judge at the Australian Alternative Varieties Wine Show, which was held in Mildura on the 19th of November 2001 (see Appendix 1).

A summary of the enquiries received by Peter Godden, Adrian Coulter, Mark Gishen and Greg Ruediger during 2001/2002 is presented in Table 6. The figures show a 2.9% decrease in the number of enquiries received compared to the previous year, and the proportion of calls received from wineries has decreased for the first time in five years. The increase in the number of calls recorded in the 'other' category in Table 6 is largely

accounted for by the increase in requests for technical information received from journalists, as discussed above.

The Consultation and Investigative and Advisory Services provide vineyard and winery visits and seminar tours to all major wine growing regions, generally organised in conjunction with the local vignerons' associations. Due to the large involvement of Institute staff in the conduct of the AWITC, Roadshow activity was reduced during 2001/2002. However, a formal 'Roadshow' visit was made to Queensland in November 2001. Six senior Institute staff presented a full-day seminar; the twelve presentations focussing on current areas of Institute research, or topical issues in the wine industry. The regional winemaker's association was asked to select the presentations to be made from a list of approximately forty areas of current Institute activity, in order that the seminar was closely tailored to the interests and needs of the audience. In addition, two workshops developed under GWRDC project 99/1 *Targeted training of industry personnel: compilation of a technical reference manual and delivery of complementary workshops*, were delivered concurrently on each of the two days following the seminar, allowing all attendees to participate in two workshops and the seminar over a three day period.

The fifteenth Advanced Wine Assessment Course was held in July 2001, giving another thirty participants the opportunity to develop and test their sensory evaluation performance (approximately 450 participants have undertaken the course since its inception). This was the third Course presented under a four-day format, which includes over 40 hours of activities over the four days, and fifteen leading wine show judges, journalists and winemakers assisted in the presentation of the Course. The four-day course has proved to be very successful, and demand for places in the course has increased since the format was changed from the previous three-

days. However, the costs of staging the course have also increased dramatically, especially over the last two years, such that the course fees are now substantial. Should a point be reached that continually rising costs are considered to make the course unaffordable for many potential participants, then a reversion to the three-day format may be considered. As in the past, Associate Judges for the 2001 Adelaide Wine Show were selected from the most successful recent participants in the Course. In addition, other shows have expressed interest using Course results as part of their selection criteria for new Associate Judges, especially since the selection of new wine show judges was discussed in detail at the ASVO *Who's running this show?* seminar, which was held in Melbourne in August 2001. Similar approaches from other wine shows are encouraged.

Evaluation of new analytical techniques and of processing aids for winemaking

Peter Godden, Mark Gishen, Dr Mark Sefton, Adrian Coulter, Dr Alan Pollnitz, Peter Valente, Ella Robinson, Greg Ruediger, Dimitra Capone

The Industry Services Laboratory maintains a GWRDC funded project for the improvement, development and evaluation of methods of wine analysis, and the evaluation of winemaking processing aids and additives. The evaluations take one of two forms: the relative performance of commercially available products, and the evaluation of new materials marketed to the industry. Industry Services staff also provide advice to the Institute's Analytical Service on the development of protocols which relate to trials being conducted on a fee for service basis for various wine companies and industry suppliers.

During the year, the vast majority of resources allocated to this project were once again used for the Closure Trial, a wide-ranging trial that is examining the technical performance of 14 different closure types.

Note: This study was not designed to be a survey of closures available in the market place, and not all of the closures available at the time the trial commenced were included. Readers should also note that the currently available stocks of some of the closures examined might differ from those available when the trial was implemented, and that the results reported represent the performance of each closure when used to seal only one wine, under the conditions defined. Care should, therefore, be exercised in relating the results to other wine types, or to wines bottled and stored under different conditions. It should also be noted that some closures may be suitable for use in products with a short shelf life, and may perform well over a particular time span, but less well over a longer term. Winemakers, therefore, need to assess the anticipated shelf life of the wine to be bottled when making decisions on closure use.

The closures that are being examined in this study comprise a roll-on tamper-evident (ROTE) screw-cap closure, two grades of natural conventional cork (Reference 2, 44 mm length

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

	1999/00	2000/01	2001/02
Wineries	1152	1155	1008
Government organisations	99	117	101
Other	413	359	469
Students	36	26	31
Total	1700	1657	1609

and Reference 3, 38 mm length), two 'technical cork' closures (cork-based closures that also contain a synthetic component: Sabaté 'Altec' and Amorim One plus One 'Twintop'), and nine closures manufactured from synthetic materials, three of which are produced by extrusion processes (ECORC, Nomacor and NuKorc) and six by moulding (Aegis, Auscork, Betacorque, Supremecorq, Californian 'Tage' and Integra). Various aspects of the performance of the closures are being examined, including those relating to each closure's physical characteristics and extraction from the bottle, chemical analysis of the wine in order to examine apparent sealing performance, and sensory analysis. In addition, Dr Mark Sefton's team will perform chemical analysis using GC/MS, to examine any effect the closures may have on wine aroma and flavour.

The first results from the trial were published in the *Australian Journal of Grape and Wine Research*, Volume 7 issue number 2, in July 2001 (Institute publication # 666). This publication dealt with the results obtained in the first 20 months following bottling of the trial. Subsequent updates of SO₂ concentrations in wine sealed with each of the closures after 21, 24, and 30 months following bottling have been published in *Technical Review* issues 133 (August 2001) and 137 (April 2002). In addition, more details of the results generated at 24 months post bottling have been published in the *Proceedings of the 26th World Congress and 81st General Assembly of the Office International de la Vigne et du Vin*.

The retention of SO₂ in wine sealed with each of the closures has proved to be a pivotal measurement in this investigation. Loss of SO₂, presumably due to the degree of oxygen ingress allowed by the closure, has been found to correlate well with the development of brown colour in the wine, and also the rating of oxidised character during sensory evaluation, at all testing intervals. The concentrations of free SO₂ recorded in wine sealed with each of the closures at six months, when compared with the SO₂ concentrations at bottling, were also found to be excellent predictors of free SO₂ concentration and browning of the wine after 24 months, and to a lesser extent the rating of oxidised aroma in wine sealed with the different closures at 24 months.

Analysis of the wine at the three-year post bottling time point was concluded late in the reporting period, and full analysis of all the data generated was not available at the time of writing. However, initial results indicate that the important trends observed during earlier testing have continued up to the three-year post bottling time point. In particular, wine sealed with the ROTÉ closure continues to retain the greatest concentration of SO₂, but the concentration was not significantly different to that in wine sealed with the Altec closure. Wine sealed with the ROTÉ closure was also found to have retained greater overall freshness and positive fruit-derived sensory characters than wine sealed with any other closure, and also demonstrated the lowest level of brown colour development. However, wine sealed with this closure continued to display a low-level *reductive* or *rubbery* character during sensory evaluation, and all bottles sealed with the Altec

closure were again found to be tainted with TCA at concentrations typically between 1 and 2 ng/L. However, data from this trial clearly demonstrates that even at concentrations as low as 1 ng/L, the presence of TCA suppressed the ratings for overall aroma intensity, and of positive fruit-derived characters in wine, during sensory evaluation conducted by a panel with extensive experience in the identification of TCA-taint in wine. In addition, a greater proportion of bottles sealed with the other three cork-based closures which have been opened for sensory evaluation after 24 and 36-months of storage, have been found to be tainted with TCA, compared to those assessed during the first eighteen months of the trial and reported in the *Australian Journal of Grape and Wine Research*, Volume 7, issue number 2. However, the relatively low number of bottles assessed during sensory evaluation makes comment on the overall incidence of TCA taint in the batches of these closures that are being assessed, unsafe.

The results of this trial have been actively and widely disseminated by the Institute, and have attracted considerable media interest, both from wine industry publications, and mainstream television, radio, and state-wide and national newspapers, both in Australia and overseas (see

Communication and Publicity report). A large proportion of the media attention has centred on the performance of the screw cap closure, which appears to have given impetus to the pre-existing move by some wine producers to bottle a portion of their mainly white wines with this type of closure. Late in the reporting period, certain wine producers in both Australia and New Zealand also bottled some of their premium red wines with screw cap closures. Whilst the Institute would emphasise that care should be exercised when extrapolating the results of its trial to wine types other than the one Semillon wine being examined, it will follow with interest the evolution of the commercially bottled red wines under screw cap closures.

*The Institute wishes to make it clear that any reference to 'Tage' closures in these publications, and also in this publication, are references to closures that were supplied by Esvin Wine Resources, Auckland, New Zealand in May 1999, and not to closures supplied by Novemba. The Institute has been informed that the closures being examined were made by a Californian manufacturer, and not by Novemba, and Novemba has informed the Institute that these closures are not the same as Tage closures now being made by Novemba. The Institute takes no position on Esvin's right, or that of the Californian manufacturer, to make or supply closures under the name 'Tage'.



HELMUT GUTH (GERMANY) GUEST SPEAKER I1 AWITC

Flavour scalping sub-project

Staff Dr Mark Sefton, Dimitra Capone, Dr Alan Pollnitz

A study on the effect of closures on wine flavour composition is well advanced. All samples of wine taken after two years in bottle have been analysed and the data are now being evaluated. Significant absorption of flavour compounds from the wine by some of the closures appears to have taken place over the storage period. One set of data has indicated that while Stelvin closures 'remove' no TDN ('kerosene' flavour in aged Riesling wines) from wine after two years in bottle, natural closures and 'twin tops' closures remove around 50% and three of the synthetic closures 'removed' between 95 and 98% of this compound over the same period. The taint compound naphthalene was also strongly absorbed by some of the synthetic closures. The concentrations of the oak-derived components, cis- and trans-oak lactone, vanillin, guaiacol, 4-methylguaiacol, 4-ethylphenol and 4-ethylguaiacol were unaffected during bottle storage by two of the closures examined so far, and were relatively stable during bottle storage. Similarly, the concentration of isobutylmethoxypyrazine, a compound generally known to impart capsicum characters to Sauvignon Blanc and Cabernet Sauvignon wines in particular, was unchanged during

storage. The monoterpene alcohols geraniol, linalool and nerol on the other hand were almost entirely degraded over the two years regardless of whether the wines were stored in bottles or in glass ampoules.

The concentration of other components also changed during storage. *trans*-Rose oxide was converted to the more potent *cis*-isomer while the concentration of β-damascenone and β-ionone both decreased substantially during storage. These changes appear to have resulted mainly from reactions with other wine components rather than interactions with the closures.

Among the simple fermentation ethyl esters, ethanol decanoate and ethyl octanoate were the most strongly absorbed, while the shorter chain esters were largely unaffected.

These data are from a small portion of the samples only. A more complete picture of the affect of all of the closures on the composition of the wines will be forthcoming.

Informal sensory analysis of the wines from the Institute cork permeation/ascorbic acid trial (see report under *Studies on random oxidation of bottled wines*) indicates that these wines are also affected by flavour absorption. Instrumental analysis of these wines will be carried out to confirm the basis for these sensory observations, and also to determine whether bottle storage position also affects the degree of flavour absorption.

The data so far show that closures have the capacity to influence wine flavour by absorption, as well as by imparting volatiles derived from the closures themselves, or by allowing the ingress of oxygen. There continues to be a very strong argument for paying due regard to the 'fate' of wines following vinification, as post-vinification choices have the potential to modulate all prior inputs into the winemaking process.

Analytical method development and evaluation

Methods development was also performed during the year, although the majority of the resources available under this project once again have been devoted to the closure trial. This work has been facilitated by the acquisition of new instrumentation over the previous two years, which has been supported by GWRDC funding.

Much of the methods development work performed under this project is done on an 'as needs' basis, based on the types of samples and calls received from industry as part of the problem solving and advisory service, as well as project work being performed by the Industry Services team. As mentioned elsewhere in this report, the team will manage a large Institute-wide investigation into the relationship between *Dekkera/Brettanomyces* yeast and wine. As a prelude to these investigations, the development of a GC-MS method for the quantification of isovaleric acid in wine was reported in the previous Annual Report. Isovaleric acid is reported in the literature as an important spoilage compound produced by *Dekkera/Brettanomyces* yeast, and the method was finalised in time to analyse wine samples used in two workshops based on microbiological spoilage, which were staged as part of the 11th Australian Wine Industry Technical Conference, held in October 2001. Later in the year, the method was further enhanced and validated, using a deuterated standard of isovaleric acid that was produced by another team at the Institute, under the GWRDC-funded project AWR 6 *Grape composition and wine flavour*.

Also in response to the requirements of the *Dekkera/Brettanomyces* investigations, HPLC methods for the quantification of coumaric and ferulic acids in both red and white wines have been developed and validated during the year. Coumaric and ferulic acids are precursors for the production of the spoilage compounds 4-ethylphenol and 4-ethylguaicol, by *Dekkera/Brettanomyces* yeast. Work has also commenced on a method to quantify coumaric and ferulic acids in red grapes.

In response to the increasing number of wines received as part of the problem-solving service that the Institute's sensory panel consider to be tainted with chlorophenols, a GC-MS method for the quantification of this group of compounds in wine was also developed during the year. **As discussed elsewhere in this report, this analysis has already been used in two investigations, which are considered to have been of great benefit to the wine companies involved. The first of these investigations disproved a claim by certain suppliers of equipment to generate chlorine dioxide, that the use of this chemical to disinfect water for use in wineries, or as a vineyard spray, could not result in the production of chlorophenols. In the second case, a 2,4-dichlorophenol taint observed and quantified in wine was apparently attributable to the use of a registered vineyard spray that is reported in the literature to form 2,4-dichlorophenol upon degradation.**

In conjunction with the Institute's Analytical Service, the following analytical methods have been validated during the year, to the standards required by the Institute's internal Quality System, which is based on NATA requirements and ISO Guide 25. This work is conducted by the Institute's Quality Liaison Manager, Mr Mark Gishen, who is part of the Industry Services team:

- Tetraconazole and cyproconazole in wine, juice, grapes and marc by GC-MS;
- Quinoxifen in wine, juice, grapes and marc by GC-MS;
- Histamine and tyramine in red and white wine by HPLC;
- Fludioxonil, pyrimethanil, fenhexamid, tebufenozide and cyprodinil in juice wine and grapes by HPLC;
- Isovaleric acid in red and white wine by GC-MS; and
- Emamectin in juice, wine, grapes and marc by HPLC.

In addition, NATA accreditation was obtained for the methods: Malathion and Fenitrothion in wine and juice.

Targeted training of wine industry personnel: compilation of a technical reference manual and delivery of complementary workshops.

Staff Peter Godden, Mark Gishen, Dr Paul Henschke, Adrian Coulter, Peter Valente, Ella Robinson

The primary aim of this project is to produce flexible and updateable information packages on selected technical subjects, which will be delivered to wine grape levy payers via the world-wide-web, and workshops to be held in all major grape growing areas of Australia in conjunction with the existing Roadshow seminars.

The Institute 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 Institute, 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. It is envisaged that the project will be ongoing, and that many areas of interest to winery technical personnel will be addressed in due course.

The information collated to date has been posted to the Institute's web site over the past two years by Industry Services staff. Australian wine industry personnel can access the information at <http://www.awri.com.au> At the AWRI Home Page they should select 'Services' followed by 'Solutions' and 'Hazards and Deposits'. This section of the site is password protected, and the password has been supplied to Australian wine levy payers by mail. If winery personnel do not have the password then it can be obtained from the Institute's Librarian or Communication and Publicity Manager.

However, the 'Services' section of the Institute's web site also contains a great deal of other technical information, which is readily accessible by the general public.

New material is periodically added to the site, and the existing information is enhanced. For instance during the year new sections on wine microbiology have been developed, and video clips demonstrating certain laboratory techniques have been added. 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 sample investigations conducted by the Industry Services team. 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 have addressed these issues, providing practical trouble-shooting information and simple diagnostic tests, with which to isolate and identify a wide range of hazes and deposits in wine. In spite of the fact that the number of problem wines presented to the Institute, as a proportion of all wine manufactured in Australia, has declined steadily over a number of years, the continued growth of the industry clearly puts pressure on the ongoing provision of problem solving services. Therefore, in future, the Institute will increasingly expect wineries to have performed routine trouble-shooting investigations for themselves, using the information provided on the web site, and techniques demonstrated in workshops, before they contact the Institute. This will allow Industry Services staff to spend less time on common problems, and more time on the many non-routine investigations that can potentially yield the most useful new knowledge, which has the potential to be of greatest long-term benefit to the whole industry. However, the success of this strategy clearly depends on industry awareness and acceptance of the web site and its applications, and this will be further addressed in a series of ten Roadshow seminars, and six workshops, to be held in four states during the second half of 2002. In addition, information disseminated by Industry Services staff, both in workshops and on the web site, increasingly has a quality management component, in order to help winemakers avoid many common problems through the adoption of quality-management-focussed work practices. On two occasions in Queensland in November 2001, the team Manager and Mark Gishen presented full-day workshops that addressed these issues. In addition to discussing formal quality management systems and accreditation, the workshops contained practical advice on the most efficient way to incorporate routine analytical testing into quality management programs, and suggestions on the most efficient way to use the information generated to identify potential problems before they occur.

Technical and regulatory support to the Australian wine industry

Staff Professor Peter Høj and Creina Stockley

Information requests

One of the activities of the Institute has been to provide legal/regulatory and technical advice and assistance to the Australian wine industry, through the Health and Regulatory Information Manager, the Industry Services team and the Director. From 31 October 2001 until 30 June 2002, 118 independent information requests on technical and regulatory issues from the government and industry were fielded by the Health and Regulatory Information Manager (activities of the Industry Services team are reported elsewhere in this Report).

Industry committee membership

During the year, additional support to the industry has been derived from the Director's membership on the AWBC Compliance and Technical Committee and the WFA Technical Committee. One of the important aspects of the Institute'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 (see page 12 and Appendix I). Professor Peter Høj and Ms Rae Blair were members of the Australian Organising Committee of the 26th World Congress and 81st General Assembly of the Office International de la Vigne et du Vin which was held on 11-18 October 2001. Professor Peter Høj served as Section President Oenology during that Congress which involved providing an oral summary of the proceedings of that Section; Creina Stockley performing as Chair of the Wine & Health Section 4; and Institute staff Mark Gishen, Peter Godden and Dr Paul Henschke providing presentations (see Appendix 1).

During the year, the Health and Regulatory Information Manager was a member of the following committees: the AWBC Legislation Review Committee; the WFA Technical Committee; the National Reference Committee–Environment Strategy Development; and Australian delegate for the Office International de la Vigne et du Vin

Nutrition and Health Sub-commission. The Director and Health and Regulatory Information Manager also coordinate Course 3005WT *Grape industry practice, policy and communication* for the Department of Horticulture, Viticulture and Oenology at The University of Adelaide, a subject in which more than 250 graduating viticulture and oenology students have been exposed to organisational, political and commercial issues relating to the wine industry's operating environment.

Reviews and publications

During 2001/2002, significant efforts, energies and resources have continued to be directed towards the *Review of alcoholic beverages in the [Australian] Food Standards Code*. Complimentary to Standard 2.7.4 for wine and wine product of the Food Standards Code of Australia and New Zealand, draft Standard 4.1.1 Wine production requirements (Australia only) of Volume 2 have been prepared and published for public comment, and amendments have been submitted in consultation with the Australian wine industry. An update of the Institute's booklet/guide *Analytical specifications for export of Australian wine* was published. 56 countries were contacted compared with 35 countries contacted in 1995 when the booklet was first published. An update was also prepared for the second edition of the chapter *Reduced additive brewing and winemaking* in the book *Technology of reduced additive foods* (Blackwell Science). During 2001/2002, technical information and/or issues that have been reviewed, and discussion or position

papers prepared include the establishment of maximum limits for additives used in winemaking for the Agriculture, Fisheries and Forestry Australia (AFFA) delegation to the Codex Alimentarius Commission; cation exchange; contamination of wine with hydraulic oil sources of arsenic, copper and lead in grapes and wine; discrete pieces of oak versus barrels and staves; health and safety issues related to the dietary intake of sodium in wine and the allergenic potential of processing aids used in winemaking. Technical information is also disseminated to the Australian wine industry, including all grape and levy payers, by the Institute's bimonthly publication, *Technical Review*, of which the Health and Regulatory Information Manager is editor; by the Institute's Annual Report edited by the Director and Communication and Publicity Manager; through Institute Roadshows in various winemaking regions (see report elsewhere in this Annual Report); through the remote web-accessible database of the John Fornachon Memorial Library (available to Australian winemakers and grapegrowers only); through the Institute's *Email Advice Bulletin on Technical Issues*; through the Institute's website (www.awri.com.au); and through the many workshops and presentations given by Institute staff (see Appendix 1).

This project of technical and regulatory support to the Australian wine industry is ongoing, as technical and regulatory issues are regularly raised by the government or by industry, both in Australia and internationally. Furthermore, these issues often span several years.

Library catalogue databases	
AWRI_Database: Books, conference proceedings, Theses, scientific and medical papers	44,314
JOURNALS: journals, newsletters, statistics and annual reports	424
Library information databases	
REGS: European Community wine legislation	382
ISYS – full text retrieval database covering	
United States of America Federal Register	877
Web accessible database (with searchable abstracts)	20,000

	Wine industry	Staff	Other ^a	Total	% inc/(dec) over 2000/2001
Information requests	869	792	1702	3363	56%
Interlibrary loans					
• requests sent ¹	182	345		527	(20%)
• requests received ²				53	(67%)
<i>Technical Review</i> requests ³				193	16%
<i>Technical Review</i> articles forwarded ⁴				970	7%
Articles forwarded ⁵				1031	56%
Institute publications forwarded				585	33%
Articles photocopied				3740	1%

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 from *Technical Review* (usually more than one article is requested).
⁵ Number of other articles forwarded, excluding staff publications.
⁶ 90% of 'other' requests come from students and Government sources.

Provision of technical information	
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 Institute. The Library is also used extensively by

Table 9 Email bulletins sent during 2001/2002	
11 Jul	Wine bottle closure trial results
13 Jul	Agrochemicals booklet available
27 Jul	Sale of Benlate to be phased out
9 Aug	Teldor is registered and Shirilan Permit renewed
20 Aug	SWITCH for export wine grapes
21 Aug	Press release regarding MNRF grant
7 Sept	11 AWITC – available workshops
16 Nov	AVATAR Registered
21 Nov	Announcement of new Chairman for AWRI
17 Nov	RIDOMIL GOLD PLUS - Change in formulation

other groups such as students, government bodies and private companies (see Table 7).

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.

Following a 108% increase in requests to Library staff for information in 2000/2001, the Library staff responded to a further 56% increase in requests for 2001/2002 (Table 7). The number of Institute publications forwarded rose by 33% over the previous year, and the number of other articles forwarded also rose by 56%. Industry's increased awareness and use of the Institute's web-accessible database has contributed to an increase in enquiries, and this important service continues to be a valuable way for industry to access its technical literature.

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 *Technical Review*, and several in-house technical information databases.

Library collection
A total of 133 monographs and 18 conference proceedings were added to the library collection in 2001/2002. 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 20,000 reprints.

Library databases
A single search screen provides access to the Library's collection of over 44,000 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 8.

The Library provides access to its web-accessible databases, 24 hours a day to Australian winemakers and grapegrowers paying the Primary Industries Excise Levy Tax 1999. The restriction in access is enforced to comply with copyright approvals obtained from the various publishers. Library staff 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 twelfth 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 (accessible on www.awri.com.au) is updated on a regular basis.

Technical Review
Technical Review is received by all Wine Levy paying wineries 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 Institute'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 access to *Technical Review* is also available on the Institute's website. Dr Barbara Hardy AO continues to support the publication of *Technical Review* through regular financial contribution to the Thomas Walter Hardy Memorial Trust.

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 approximately 700 email addresses recorded to receive the email bulletins to date. Ten email bulletins were issued during the year and are shown in Table 9.

The John Fornachon Memorial Library Endowment Fund
The Institute 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.

Acknowledgements
The Institute 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, Yalumba Winery.

method for the determination of malathion and fenitrothion was successful in gaining accreditation, and several changes to the signatory status of staff were approved. 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. It continues to manage its quality management system with the aid of the Paradigm Quality software package and is still in the transition of implementing an upgraded version released this year. Following successful implementation, the software will be introduced throughout the Institute, primarily as a means of assisting control and availability of policy and procedural documentation, as part of a general move toward quality management systems implementation.

Mark Gishen remains heavily involved in the collaborative research project evaluating the use of near infrared spectroscopy (NIRS) 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 elsewhere in the Annual Report.

Table 10 Staff interviews by media 2001/2002			
Date	Staff	Discussed	Media
4 July 2001	Peter Høj	MNRF Bid	Kevin Norton and David Bland - ABC Radio 5AN Drivetime
18 July 2001	Mark Gishen	Wine bottle closures	Chris Snow – ABC
31 July 2001	Dr Markus Herderich	Additions to wine	Alice Feiring – New York Times
30 July 2001	Professor Peter Høj	Innovation in the Australian wine industry	Radio National by Natasha Mitchell on the Robin Williams Science Show
21 August 2001	Professor Peter Høj	MNRF Grant press release	Martina - Wagga Wagga Advertiser
21 August 2001			Natalie Stallenberg - ABC Radio News
22 August 2001			Scott Tucker – Newcastle Herald
22 August 2001			Kevin Norton and David Bland - ABC Radio 5AN Drivetime
22 August 2001			Kim Lyle – ABC Radio Mildura
30 August 2001			Adele - WM Horsham
31 August 2001			Kate Dowler – National Grapegrower
18 September 2001	Professor Peter Høj	Institute's activities	Melanie Cooper, journalist with New Scientist
7 October 2001	Professor Peter Høj	Institute's activities	ABC News
20 November 2001	Peter Høj	Institute Roadshow activities	Win Television (Regional Channel 9 in Toowoomba)
21 November 2001	Peter Høj	Institute Roadshow activities	Win Television (Regional Channel 9 in Toowoomba)
23 November 2001	Adrian Coulter	Analysis of alcohol in wine	Daniel Daisy – Sydney Morning Herald
5 December 2001	Several staff	Institute's activities	ABC Asia film crew for broadcast around Asian cities.
21 December 2001	Mark Sefton	Effects on wine flavour relevant to bag-in-the-box	Miriam Chastaingt from La Journée Vinicole
1 February 2002	Mark Gishen	Wine bottle closures	Huon Hooke
4 February 2002	Peter Høj	Wine Colour	Sandra – Weekly Times
22 February 2002	Leigh Francis	Wine bottle closures	Ian Walsh
7 May 2002	Peter Godden	Wine industry careers	Hannibal Rossi, Sunday Mail
3 June 2002	Peter Godden	Wine bottle closures	Bourke Owens, Associate Curator of Wine Copia: The American Centre for Wine, Food and the Arts and journalist for the US Wine & Spirits magazine
13 June 2002	Peter Høj	Technologies of micro-oxygenation and reverse osmosis/spinning cones.	Jamie Goode (Harpers Wine & Spirits Weekly,UK)
17 June 2002	Peter Godden	Arsenic in treated pine posts	David Cooper from GW News (WA)

Quality Liaison Manager	
Staff	Mark Gishen

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 (hazard analysis and critical control point) module in 1999. Industry interest remains greatest in the HACCP module—a simple program delivered in a one-day course that incorporates a HACCP-type food safety plan. This module was designed to satisfy the requirements of the proposed (now delayed) changes to the food hygiene regulations, and meet the needs of the smaller scale businesses in the industry. Two courses for the HACCP module were conducted throughout the year, adding 15 more to the growing list of companies having attended (now 88 in total). 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 assisted in the staging of events of interest to the wine industry including convening and presenting in workshops on objective grape quality measurement, quality management system options for grapegrowers and winemakers, HACCP food safety plan development, and NIR spectroscopy calibration for the 11th Australian Wine Industry Technical Conference (Adelaide, October 2001), and contributed as part of the speaker program for the same conference (see Appendix 1).

Mark Gishen takes primary responsibility for the internal quality management systems of the Analytical Service, overseeing management reviews, documentation, auditing, and corrective actions. The

Communication and publicity	
Staff	Rae Blair

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. 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 Institute. This positioning is developed in line with the Institute's mission statement and business plan objectives. Part of her role is to coordinate the Institute'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.

Media contacts and interviews
Institute staff were interviewed by representatives from various forms of media over the year (Table 10). During the year, the Manager – Industry Services, the Communication and Publicity Manager and the Director constructed a press release to announce the publication of the preliminary results of the Institute's *Closure Trial*, which was distributed via facsimile and email on 12 July 2001. Excellent media interest and exposure was achieved nationally and internationally, and Table 11 shows the media results over the past 12 months. A further press release was issued on 21 August 2001 regarding the successful bid for the *Major National Research Facilities* grant. Media requests generated from this press release and fielded by the Director are shown in Table 10. Several media enquiries were fielded by other partners in the successful bid, however these have not been captured.

Table 11 Media enquiries resulting from closure trial press release: 6 July 2001 - 31 July 2002 *PVG = Peter Godden, PBH = Peter Hoj, MG = Mark Gishen (cont. next page)

Date	Journalist	Organisation	Staff member*	Format in which information was provided	Comments/notes
6-Jul-01	Cyril Penn	<i>Wine Business Monthly</i> (USA)	PVG	Email interview	Future article on website and magazine
10-Jul-01	Tim White	Freelance journalist	PVG	Interview	Article <i>Australian Financial Review</i> 14/07/2001
10-Jul-01	Steven Brook	<i>The Australian</i>	PVG	Interview	Article proposed for 12/07/2001 did not appear
11-Jul-01	Steven Brook (2)	<i>The Australian</i>	PVG	Interview	Article proposed for 12/07/2001 did not appear
12-Jul-01	Jo Burzynska	<i>Harpers Magazine</i> (UK)	PVG		PVG offered telephone interview
12-Jul-01	Cathy Parker	ABC <i>Country Hour</i>	PVG	Recorded radio interview	Broadcast on <i>National Country Hour</i> 13/07/2001
12-Jul-01	Nigel Austin	The (Adelaide) <i>Advertiser</i>	PVG	Interview	Article 13/07/2001
12-Jul-01	Simon Evan	<i>Australian Financial Review</i>	PVG	Interview	Article 13/07/2001
12-Jul-01	Kevin Norton and David Bland	ABC Adelaide <i>Drivetime</i>	PVG	Live radio interview	Broadcast ~ 17:40 - 17:45 12/07/2001
12-Jul-01	Robert Joseph	<i>Wine Magazine</i> (UK)	PVG	Email interview	Future article on website and magazine
12-Jul-01	Cyril Penn (2)	Winebusiness.com and Mag	PVG	Email interview	Future article on website and magazine
13-Jul-01	Doug Drew	ABC Sydney and NSW	PVG	Live radio interview	<i>Sally Loane</i> show, live Sydney and NSW ~ 10:30 13/07/2001
13-Jul-01	Stephanie Bared	Radio New Zealand	PVG	Recorded radio interview	Various news bulletins 13/07/2001
13-Jul-01	Bronwyn Martin	Radio 2UE News, Sydney	PVG	Recorded radio interview	Various news bulletins 13/07/2001
13-Jul-01	Lindell Hawk	ABC Radio Adelaide	PVG	Recorded radio interview	Various news bulletins 13/07/2001
13-Jul-01	Katrina Bolton	ABC National Radio News	PBH	Recorded radio interview	Various news bulletins 13/07/2001
13-Jul-01	Steve Larkin	AAP Information Services	PBH	Interview	Article for international wire service
13-Jul-01	Rob Hicks	Radio 3AK (Melbourne)	MG	Recorded radio interview	Recorded interview for future <i>Grape Expectations</i> program
13-Jul-01	Tom Stevenson	Wine writer	PVG	Email interview	Future freelance articles
13-Jul-01	Anthony Denis	<i>Sydney Morning Herald Good Living</i>	PVG	Interview	Interview for future article in <i>Good Living</i>
13-Jul-01	Andrew Coorey	ABC Radio in Sydney	PVG	Live radio interview	<i>James O'Loughlin</i> show ~19:40 - 19:50 13/07/2001
13-Jul-01	Mike Christie	Reuters	PVG	Interview	Article for international wire service
15-Jul-01	Rob Hicks	Radio 3AK (Melbourne)	PVG	Live radio interview	Recorded interview for future <i>Grape Expectations</i> program 10:40 - 10:50 15/07/2001
19-Jul-01		BBC (UK)	PVG	Live radio interview	Broadcast simultaneously on 36 local BBC stations
23-Jul-01	David Robertson	<i>Wall St Journal</i> wire (USA)	PVG	Article published on wire service	
25-Jul-01	Rose Murray Brown	<i>Decanter</i> (UK)	PVG	Article in September issue	
25-Jul-01	David Bird MW,	Freelance journalist, UK	PVG	Supplied info for article	
25-Jul-01	Isabella Chaney	<i>Journal of Wine Research</i> (Institute of Masters of Wine, UK)	PVG	Supplied info for article	
3-Aug-01	Audrey Farolino	USA consumer organisation	PVG	Interview for article in organisation's magazine <i>Consumer Reports</i> (equivalent of <i>Choice</i>)	
3-Aug-01	Ken Gargett	<i>Brisbane Courier Mail</i>	PVG	Interview for article	
9-Aug-01	James Halliday	<i>Weekend Australian</i>	PVG	Discussion at ASVO seminar, quoted in subsequent article	
9-Aug-01	Phillip Rich	Melbourne <i>Age</i>	PVG	Interview	
15-Aug-01	Tim White	<i>Australian Financial Review</i>	PVG	Interview - extensive subsequent article	
24-Oct-01	Finlay McLaren	<i>Which</i> magazine (USA)	PVG	Interview for article	
5-Dec-01	Cyril Penn	<i>Wine Business Monthly</i> (USA)	PVG	Interview for second article in WBM	
10-Jan-02	Justin Ross	Radio 2SM, Sydney	PVG	Live to air interview	
17-Apr-02	Christopher Zinn	Channel 9 <i>Today</i> program	PVG	Taped interview for national TV	
24-Apr-02	Jo Burzynska	Crier Media Group (UK)	PVG	Supplied data for article in <i>Drinks Buyer Europe</i> and other Crier Group publications	
17-May-02	Cherry Ripe	<i>The Australian</i>	PVG	Article on trial	Large photo of PVG published with article
17-May-02	Cathy Bolt	<i>The Australian Financial Review</i>	PVG	Prominent article on page 3 about red wines being sealed with screw caps	
18-May-02	Tim White	<i>The Australian Financial Review</i>	PVG	Full-page article quoting PVG about screw caps and APCOR advertising campaign	
22-May-02		<i>La Rioja</i> (the leading newspaper of the Rioja province in northern Spain)	PVG	Full page interview with PVG with references to use of screw caps to build product image	Large photo of PVG published with article

Table 11 Media enquiries resulting from closure trial press release: 6 July 2001 - 31 July 2002 *PVG = Peter Godden, PBH = Peter Hoj, MG = Mark Gishen

Date	Journalist	Organisation	Staff member*	Format in which information was provided	Comments/notes
03-Jun-02	Tom Skeen	Blethen Family Newspapers (USA)	PVG	Supplied information on trial for use in article	Article to be published in Blethen Family Newspapers including the <i>Seattle Post</i> , <i>The Seattle Times</i> , the <i>Yakima Herald Republic</i> and the <i>Walla-Walla Union Bulletin</i>
Jun 2002	Huon Hooke	<i>Australian Gourmet Traveller</i> , <i>WINE Magazine</i>	PVG	Supplied information and discussed issues on two occasions for major article on closures debate, published with extensive reference to the trial	The same issue also contains two other articles on the use of screw caps
30-Jul-02	Kevin Norton and David Bland	ABC Adelaide <i>Drivetime</i>	PVG	Live radio interview	Broadcast ~ 17:45 - 17:50
Jul 2002	Susie Barrie	<i>Decanter</i> (UK)	PVG	Supplied information for article	This major article <i>Cork debate rages on</i> contains a long commentary on the closures issue, extensive opinions from leading wine industry personnel from around the world, and reports the findings of the study under a sub-heading <i>Findings form Oz</i>
25-Jul-02	Michael James	ABC (USA) News, New York	PVG	Supplied information for use in report on closure issues	
Additional Media coverage					
13-Jul-01		<i>The Cordeaux Show</i> 5DN		Discussion of the trial's findings	PVG spoke with Paul Boulden beforehand
13-Jul-01		<i>6WF Morning Show</i>		Discussion of the research findings with Paul Boulden (Sandalford) and Geoff Grosset (Grosset Wines)	to discuss findings
13-Jul-01		ABC Triple J <i>Breakfast</i> show		Discussion of research findings	
13-Jul-01		ABC AM (Local stations and Radio National)		Discussion of research findings with Bruce Tyrrell	
13-Jul-01		<i>Herald Sun</i> Melbourne		Article based on press release	
Oct & Nov 01		<i>La Vigne</i> , France		Articles summarising results (x2)	
Jan & Apr 02		<i>Revue des Oenologues</i> , France		Articles summarising results (x2)	
Apr 02		<i>Revue Francaise d'Onologie</i> , France		Article summarising results	
Winter 2001, Spring 2002, (Summer 2002 in print) issues 127, 128 and 129		<i>The Grape Press</i> , Journal of the UK Vineyards Association		Reproduction of articles previously published in the <i>Australian & New Zealand Grapegrower & Winemaker</i>	
May 2002	James Laube	<i>Wine Spectator</i> (USA)		Full page opinion piece under the heading 'A screwy Solution' by one of this magazine's most high-profile contributors	This article strongly advocates a move from cork to screw caps. While not mentioning the trial, it acknowledges the actions being taken by Australian and New Zealand producers
General	The number of advertisements by various closure companies that refer to the trial have become too numerous to count. These advertisements, often full-pages in prominent positions, have become common in issues of many wine trade and mass market wine publications from around the world, including all those named above. In addition, the two most prominent advertisements in the Abstracts book for the 26th World Congress and 81st General Assembly of the Office International de la Vigne et du Vin (OIV), and the only advertisement in the 'Congress Proceedings - Section Oenology' were devoted to the results of the trial.				

Analytical Service

Staff Don Buick, John Hughes, Matthew Holdstock, Greg Ruediger, Amanda Cook, Matthew Cream, Kevin Pardon, Randell Taylor, Sandra Lloyd-Davies, David Boehm, Athina Massis, Anna Catalano, Danielle Leedham, Maria Mills, Heather Brooks and Kate Lattey.

The Analytical Service (AS) is a commercial facility operating independently within the Institute with the Manager, Don Buick, reporting to the Director. It is funded by the income generated from fee-earning work and contributes significantly to the financial well being of the Institute. The Analytical Service provides services to the Australian wine industry including use of the practical outcomes of the Institute's published research. Due to the nature of some of its work the Analytical Service, on a commercial basis, makes use of the expertise

existing within the Industry Services team and within the Research groups to interpret or advise on results or project design. Our aim is to provide a range of quality analytical services that are important to industry which can be provided in a reliable, price competitive, accurate and timely manner. The AS is divided into three sections namely the Analytical laboratory supervised by Matt Holdstock, the Trace analysis laboratory supervised by Greg Ruediger and the Administration section supervised by John Hughes.

Early in the new financial year, a revised fee schedule will be mailed to all clients providing details and pricing of the services offered. Procedures for establishing contractual arrangements with clients are being progressively improved with new accounts being established, purchase orders being utilised more extensively and the terms and conditions of service provision being promulgated.

Table 12 Comparison of selected tests performed by the Analytical Service by period.

Test or certificate	98/99	99/00	00/01	01/02
Total number of data points	60305	64193	68543	62354
Total number of tests	39087	44846	46037	48314

The Analytical Service conducted over 48,000 individual analyses on wine and grapes in 2001/2002. These services included analyses consistent with the requirements of export certification and support for the quality control activities of winemaking and viticulture often requiring more specialised skills than those commonly found in winery laboratories. The growing interest in grape quality has led to increasing numbers of grape samples being tested for colour, glycosyl-glucose, brix, pH and titratable acidity over the last two vintages.

During the year, the commercial viability of the DNA typing service was assessed and found to be unprofitable due to limited demand for the service and on-going technical difficulties with the methodology. Coinciding with the resignation of our two analysts proficient in the DNA typing service, the service was suspended in March pending an assessment of alternative suppliers or improved methodology.

The number of tests performed by the Analytical Service increased by 5% over the previous year. Significant increases occurred in the number of AWBC certificates and EU certificates but the number of Japanese certificates declined as some of the major exporters became approved to issue their own certificates. Testing for chloroanisoles and 4-ethylphenol increased with the latter being related to growing concern with *Brettanomyces* spoilage of wine and the associated Industry Services investigation. The number of data points produced decreased by 9% reflecting lower numbers of multi-residue assays performed.

The sensory assessment service had been a significant growth area in the previous year due to a large number of samples being assessed for taints, faults or general quality parameters. This year, the sample numbers declined for no apparent reason. A sensory analyst was appointed by the Institute during the year to coordinate sensory evaluation for the AS, Industry Services and the research projects. Procedures for sensory evaluation and training of panel members have been progressively improved and the reporting of data streamlined through the use of the FIZZ software.

Staff have worked closely with the Industry Services' team to analyse the 30 month and 36 month samples from the closure trial (see report on page 43). The analysis of large numbers of free and total sulfur dioxide and OD₄₂₀ were performed with staff working in shifts to complete the work within two to three days of commencement. Similar testing has also been performed for samples from the *Studies on random oxidation of bottled wines* trial under GWRDC-funded project AWR 9. Staff have commenced performing bentonite fining trials on samples submitted under the *Characterisation of unstable wine proteins involved in haze formation* trial under GWRDC-funded project AWR 8. Analytical support for other research projects is also provided from time to time on a commercial basis.

The Analytical Service continues to hold NATA accreditation based on ISO 17025. Considerable progress has been made to estimate measurement uncertainty for all approved methods as required

under this standard. Matt Holdstock and Randell Taylor have been working on the estimation of measurement uncertainty. Don Buick continued to perform assessments of other accredited laboratories in the wine industry as a volunteer assessor for NATA. Greg Ruediger attended a training course and has begun the planning and preparing the documentation necessary to achieve Good Laboratory Practice accreditation through NATA which will be required from 2003 to provide analyses and winemaking for chemical companies seeking registration of new agrochemicals from the National Registration Authority.

The Trace Analysis Laboratory (TAL) performs specialised testing including a multi-residue screen using gas chromatography/mass spectrometry to detect and quantify trace levels of 30 agrochemical residues where present. In addition, a target residue screen using high pressure liquid chromatography has been developed to include cyprodinil, fludioxinil, pyrimethanil, fenhexamid and tebufenozide.

An increasing number of samples have been analysed during the year for chloroanisoles – the principle contributor to cork taint in wine – and for 4-ethylphenol which is an indicator of *Brettanomyces/Dekkera* yeast spoilage activity. Some of the latter testing is for the Industry Services team as part of the *Brettanomyces* investigation.

During the year the TAL performed surveys of exported Australian wine for agrochemicals, histamine, ochratoxin A and for ethyl carbamate on behalf of the Australian Wine and Brandy Corporation. Some of the data from 2001 was included in posters presented at the 11th AWITC.

Several residue trials have been performed for chemical companies during the year to establish the levels of residue present or absent in grapes or wine following the application to vines of newly developed agrochemicals. These trials involve winemaking and testing of grapes and wine following development and validation of methods of analysis. Some trials included sensory evaluation of the wine made and the monitoring of ferments. Analytical methods for spinosad, quinoxifen and emamectin have been validated in the last few months as part of these trials. Spinosad and emamectin are insecticides whilst quinoxifen is a fungicide for application to grapevines.

David Boehm has validated a high performance liquid chromatography method during the year to measure histamine and tyramine, both biogenic amines found in wine. As an extension of the development work on histamine, a method utilising HPLC with pre-column derivatisation has been validated for many amino acids commonly found in grape juice and wine. The amino acids included are aspartic acid, glutamic acid, hydroxyproline, asparagine, glutamine, citrulline+serine, histidine, glycine, threonine, alanine, gamma amino butyric acid, proline, tyrosine, arginine, valine, methionine, isoleucine, leucine, phenylalanine, tryptophan, cystine, ornithine and lysine.

These amino acids are of interest for several reasons. Amino acids are the most prevalent form of nitrogen in grape juice and wine. Amino acids are, therefore, important in the fermentation

process being the principle source of nitrogen, along with ammonium ions, for yeast and their measurement can, therefore, be useful for evaluating the fermentability of a juice or must (Ribereau-Gayon, 1999). Proline and arginine have been proposed as indicators of ripeness of grapes as the level increases significantly during the period immediately prior to picking (Ribereau-Gayon, 1999). This capability will be of interest to research projects within the Institute and to winemakers.

The TAL completed the analysis of spiked samples of organic wine by GC/MS and HPLC and provided samples to CSIRO (Plant Industry) for ELISA kit analysis as part of the validation of the ELISA kits for use in detecting selected residues in wine.

Staff of the AS contributed to the 11th Australian Wine Industry Technical Conference in many ways. Three posters were presented by staff (see Appendix 1) with all three of these winning prizes recognising the quality of the posters.

The posters were *Ethyl Carbamate in wines* authored by Gayle Baldock, Yogi Hayasaka and Greg Ruediger. *Ochratoxin A in wine* authored by Greg Ruediger and David Boehm and *Development of a multi-residue analysis for grapes and wine using HPLC and small scale extraction techniques* authored by Randell Taylor and Greg Ruediger. Greg Ruediger, Matt Holdstock, John Hughes and Don Buick all presented workshops (see Appendix 1). Jeremy Hack coordinated the large poster exhibition and most other AS staff assisted with arranging the supply of audio visual facilities, glasses for tasting workshops, pouring of wine and other tasks in support of the workshops. The AS maintained an information booth throughout the conference as part of the suppliers' trade exhibition and provided information about analytical services to the large number of delegates attending the conference.

The AS sponsored a trophy for *The Best Riesling* at the Adelaide Wine Show in September 2001. AS staff contributed to the successful running of the Advanced Wine Assessment Course conducted in 2001.

Several staffing changes have occurred during the year. David Boehm and Amanda Cook were offered contract positions after working as casual employees for several years. Jeremy Hack, Radka Kalouch and Andrea Kemp all resigned as casual employees and chose to pursue alternative careers. All three have been employed by the Analytical Service for several years and have made significant contributions to its success. Jeremy had been responsible for the DNA typing service, IT support and was the unofficial AWRI photographer. Andrea has worked in the Analytical laboratory providing export certification and routine analysis. Radka has worked in the Trace Analysis Laboratory performing agrochemical analysis and has assisted Jeremy with DNA typing analysis. Athina Massis,

Anna Catalano, Danielle Leedham and Heather Brooks have been recruited as casual Laboratory Technicians and have been trained in various duties. Maria Mills transferred from Administration to provide part time assistance with the AS administration of samples.

Matt Holdstock, Analytical Service Supervisor, has continued his part-time studies towards a Graduate Diploma in Oenology. Several staff have attended specialised instrument training courses to ensure new equipment is well maintained and used to its maximum capability. The Analytical Service aims to support the continual upgrading of staff skills and considers this as being important in expanding our portfolio of services. John Hughes and Matthew Cream took leave during the 2002 vintage to gain experience in commercial wineries.



TERRY LEE

GUEST SPEAKER 11 AWITC

(USA)

Statement of Financial Performance

For the Year Ended 30 June 2002

	2002	2001
Revenue from operating activities	(\$)	(\$)
Grape and Wine Research and Development Corporation		
Project funds	3,879,947	3,569,186
Capital grants		
Building	0	0
Equipment	333,866	426,000
Other project funds	543,035	496,598
Commercial collaborations	77,548	61,773
Analytical Service	1,224,875	1,167,825
Sundry income	212,647	180,830
Expenses from operating activities		
Employee benefit expense	3,857,053	3,437,532
Consumables used	502,466	514,417
Depreciation and amortisation expense	439,572	551,179
Repairs and maintenance	68,846	99,282
Travel expenses	109,085	96,512
Borrowing cost expense	2,112	12,406
Other expenses from ordinary activities	1,010,735	959,196
Profit from operating activities	282,049	231,688
Net gain (loss) on disposal of assets		
Motor vehicles	14,503	14,935
Equipment	(5,323)	397
Profit from ordinary activities	291,229	247,020
Write back of asset revaluation reserve on reversion to measuring buildings at cost	0	(603,067)
Transfer of accumulated amortisation on buildings	0	125,536
Total changes in equity	291,229	(230,511)

Statement of Financial Position

As at 30 June 2002

	2002	2001
Current assets	(\$)	(\$)
Cash assets	1,428,325	920,798
Receivables	300,761	307,584
Commercial bills	1,192,000	670,000
Other current assets	64,729	52,326
Total current assets	2,985,815	1,950,708
Non current assets		
Leasehold buildings	1,559,247	1,596,247
Plant and equipment	1,834,661	1,918,127
Australian Wine Industry Chair of Oenology	840,000	840,000
Total non current assets	4,233,908	4,354,374
TOTAL ASSETS	7,219,723	6,305,082
Current liabilities		
Payables and other accruals	1,352,485	924,288
Project funds not expended		
GWRDC	208,055	37,150
CRCV	22,340	13,936
Interest bearing liabilities	0	120,000
Provisions	575,068	391,490
Total current liabilities	2,157,948	1,486,864
Non current liabilities		
Provisions	43,298	90,970
Total non current liabilities	43,298	90,970
TOTAL LIABILITIES	2,201,246	1,577,834
NET ASSETS	5,018,477	4,727,248
EQUITY		
Reserves	966,750	966,750
Retained profits	4,051,727	3,760,498
TOTAL EQUITY	5,018,477	4,727,248

Statement of Cash Flows

For the Year Ended 30 June 2002

	2002	2001
Cash flows from operating activities	(\$)	(\$)
Grants and other income	6,196,141	5,874,426
Interest received	57,727	68,845
Payments to suppliers and employees	(4,794,415)	(4,605,745)
Net cash provided by operating activities	1,459,453	1,337,526
Cash flows from investing activities		
Payment for commercial bills	(522,000)	(22,000)
Payments for building improvements	0	(8,858)
Payments for plant and equipment	(347,017)	(507,390)
Proceeds from sale of plant and equipment	37,091	51,500
Net cash used in investing activities	(831,926)	(486,748)
Cash flows from financing activities		
Repayment of loans	(120,000)	(50,000)
Proceeds from loans	0	0
Net cash provided by financing activities	(120,000)	(50,000)
Net increase (decrease) in cash held	507,527	800,778
Cash at 1 July	920,798	120,020
Cash at 30 June	1,428,325	920,798
Reconciliation of net cash provided by ordinary activities with profit		
Profit from ordinary activities	291,229	247,020
Non cash flows in operating profit		
Amortisation and depreciation	439,572	551,179
(Profit) loss on the sale of plant and equipment	(9,180)	(15,332)
Charges to (reduction in) provisions	135,906	55,366
Changes in assets and liabilities		
(Increase) decrease in inventories	(12,470)	(29,332)
(Increase) decrease in receivables and prepayments	(18,050)	41,456
Increase (decrease) in sundry creditors and accruals	607,506	487,169
Net cash provided by operating activities	1,459,453	1,337,526



Trudy Wallis

Hans Muhlack

Liz Morgan

Maria Mills

Sandra Lloyd-Davies

Appendix I. External seminars, talks and poster papers presented by Institute staff during 2001/02

External seminars and talks

Author	Title	Date	Organisation/location
M.J. Herderich	Red wine colour and mouthfeel: analysis of phenolic pigments and tannins	2 Jul 2001	E. & J. Gallo Winery, Modesto USA
S.J. Bell	Effects of water stress on the phenolic composition of Shiraz grapes	3 Jul 2001	University of Oulu, Sotkamo, Finland
P.A. Henschke	Wine microbiology and biotechnology projects overview	13 Jul 2001	Graduate Certificate in Viticulture Students and staff, University of Western Australia, Urrbrae SA
M. Gishen	Update on the NIR project		
P.B. Høj	Chair of Session	9 Aug 2001	Who's running this Show, ASVO Seminar, Melbourne Vic
M. Gishen	Quality and colour: the science and the usefulness of NIR spectroscopy	10 Aug 2001	SA Farmers Federation — winegrapes section AGM, Langhorne Creek SA
P.B. Høj	From photons to wine - new insights into an ancient craft	18 Aug 2001	International Photosynthesis 2001 conference, Brisbane Qld
P.W. Godden	Screening wine for random (fault) characters	23 Aug 2001	Australian Wine and Brandy Corporation Wine Inspectors' General Meeting, Adelaide SA
P.B. Høj	An overview of the 11 th AWITC	5 Oct 2001	11 th National Conference for the Australian Society of Wine Educators, Adelaide, SA
C.S. Stockley	Women and wine—dispelling the myths and legends		
J.M. Eglinton	Non-conventional wine yeasts. Flavour and aroma diversity using <i>Saccharomyces bayanus</i>	7 Oct 2001	11 AWITC Workshop W05, Adelaide SA
E.J. Waters	Impacts of grape and wine tannins and enotannins on red wine structure	7 & 11 Oct 2001	2 presentations: 11 AWITC Workshop W23/W63, Adelaide SA
A.D. Coulter	Troubleshooting wine instability problems	7 & 11 Oct 2001	2 presentations: 11 AWITC Workshop W22/W77, Adelaide SA
M. Gishen	Opportunities for NIR and chemometrics in the wine industry	7 Oct 2001	11 AWITC Workshop W19, Adelaide SA
M.J. Herderich	The 2001 large scale Shiraz trial at the Hickinbotham Roseworthy Wine Science Laboratory	7 & 11 Oct 2001	2 presentations: 11 AWITC Workshops W23/W63, Adelaide SA
M.J. Herderich	Impacts of grape and wine tannins and enotannins on red wine structure: summary and outlook	7 & 11 Oct 2001	2 presentations: 11 AWITC Workshops W23/W63, Adelaide SA
E.J. Waters, K.F. Pocock, D. Tattersall ¹ , Y. Hayasaka, I. Dupin ² , B. McKinnon ² , R. van Heeswijk ² , P.B. Høj	The unstable proteins of wine: a summary of their properties and of factors influencing the levels of these proteins in wine	8 Oct 2001	11 AWITC, Adelaide SA
P.W. Godden	An evaluation of the technical performance of various types of wine closure	8 Oct 2001	
I.L. Francis, R. Gawel ¹⁰ , P.G. Iland ² , S. Vidal, V. Cheynier ¹³ , S. Guyot ¹⁵ , M.J. Kwiatkowski, E.J. Waters	Characterising mouth-feel properties of red wines	9 Oct 2001	
M.A. de Barros Lopes	Hybrid wine yeasts with unique fermentation characteristics	9 Oct 2001	
A.P. Pollnitz	Some applications of analyses of volatile flavour compounds in wine	9 Oct 2001	
E.J. Bartowsky	Management of malolactic fermentation – wine flavour manipulation	9 Oct 2001	
M.J. Herderich	Polyphenols, pigmented polymers and red wine colour: results of the 2001 large scale winemaking trial	9 Oct 2001	
C.S. Stockley	Do wine-derived phenolic compounds have a role in improving human health	11 Oct 2001	11 AWITC Workshop W84, Adelaide SA
M.A. Sefton	Flavour compounds and advanced measurement techniques	11 Oct 2001	11 AWITC Workshop W79, Adelaide SA
M. Gishen	Introduction to NIR spectroscopy and calibration	11 Oct 2001	11 AWITC Workshop W79, Adelaide SA
C.S. Stockley	Research efforts in Australia – do wine-derived phenolic compounds have a potential beneficial role in improving human health	11 Oct 2001	11 AWITC Workshop W84, Adelaide SA
M. Gishen	Opportunities for NIRS and chemometrics in viticulture	12 Oct 2001	OIV General Assembly, Adelaide SA
P.W. Godden	An evaluation of the technical performance of various types of wine closure	13 Oct 2001	
P.A. Henschke	Fermentation with <i>Saccharomyces bayanus</i> and <i>Candida stellata</i> yeasts can increase the aroma diversity of wine	13 Oct 2001	

Author	Title	Date	Organisation/location
P.B. Høj	Section President, Oenology Sub-commission	12-17 Oct 2001	OIV General Assembly, Adelaide SA
C.S. Stockley	Section President, Wine and Health Section 4	18 Oct 2001	
P.W. Godden	<i>Dekkera/Brettanomyces</i> and the production of 4-ethylphenol during winemaking (presentation and tasting)	13 Oct 2001	The University of Adelaide Wine Club, Adelaide SA
P.B. Høj	Research at The Australian Wine Research Institute	30 Oct 2001	Yalumba Technical Seminar, Barossa Valley, SA
M.J. Herderich	Red wine colour and mouth-feel: analysis of phenolic pigments and tannins	7 Nov 2001	Seminar, University of Adelaide, Urrbrae SA
P.W. Godden	<ul style="list-style-type: none"> Update on AWRI trial on the technical performance of various types of wine closure Prevention and management of stuck fermentation 	19-21 Nov 2001	Roadshow seminar to winemakers and grapegrowers, Toowoomba Qld
A.D. Coulter	<ul style="list-style-type: none"> <i>Brettanomyces</i>, and the production of 4-ethylphenol during winemaking. The pro's and con's of the use of ascorbic acid in white winemaking 		
S-J. Bell	<ul style="list-style-type: none"> Agrochemical issues for grapegrowers winemakers Managing Botrytis in your vineyard. 		
P.B. Høj	<ul style="list-style-type: none"> Grape protein research: Possible identification of proteases which may aid protein stability in wine, and the authentication of juice (and wine?) by characterisation of the proteins present. GMO issues - what is genetic engineering? How could it be used to improve grape and wine quality? The improvement of wine yeast using molecular genetics. The link between vineyard management and wine protein stability 		
P.A. Henschke	<ul style="list-style-type: none"> Which bacterial strain (and how many) are conducting your MLF?, and the use of lysozyme to control MLF Microbial induced oxidative spoilage of bottled red wine 		
M. Gishen	<ul style="list-style-type: none"> Quality management systems - ISO 9000, HACCP - what are they, do you need them? Introduction to the AWRI Analytical Service The use of NIR to measure the composition of grapes, juice and wine 		
P.W. Godden	Results of a trial to evaluate the technical performance of various types of wine closure	30 Nov 2001	Interwinery Analysis Group Inc, Clare SA
E.J. Bartowsky	Microbiology of Wines	21 Nov 2001	Australian Science Communicators - SA Branch, The Investigator Science Centre, Adelaide SA
E.J. Bartowsky	Microbiology of spoiled wines	23 Nov 2001	IMVS, Adelaide SA
C.S. Stockley	Walking a straight line: the protective effect of wine	7 Dec 2001	Modbury Public Hospital Annual Diabetics Refresher Day, SA
P.B. Høj	Canopy management and quality wine – a perspective	15 Feb 2002	King Valley Cool Canopies Seminar, Whitfield Vic
P.B. Høj	Sustainable aquaculture	15 Mar 2002	Coordination Committee for Science and Technology, Canberra ACT
P.J. Costello, P.A. Henschke and A.J. Markides ⁴	Standardised method for testing malolactic bacteria and wine yeast compatibility	22-23 Apr 2002	Lallemand Scientific meeting in Biarritz, France
C.S. Stockley	Potential cardiovascular benefits of moderate wine consumption	4 May 2002	Your invitation to a cardiovascular journey: drinking to your health symposium of the 14 th World Cardiology Congress, Sydney, NSW
R.G. Damberg, M.B. Esler, W.U. Cynkar, A. Kambouris ⁹ , L. Janik, D. Boehm, I.L. Francis, P.B. Høj and M. Gishen	Non-linearity and matrix effects in NIRS calibrations for measurement of grape quality variables - or 'straightening the PLS banana.'	6-7 May 2002	10 th Australian Near Infrared Spectroscopy Group conference in Coffs Harbour NSW
M. Gishen, R.G. Damberg, L. Janik, W.U. Cynkar, D. Boehm, I.L. Francis, P.B. Høj and M.B. Esler	Spectroscopic analytical techniques in the wine industry (NIRS and FT-IR)	8-9 May 2002	FOSS Directions 2002 conference at Coffs Harbour NSW Beer and wine users group meeting
Y. Hayasaka	Screening for potential pigments in red wine using nano-electrospray tandem mass spectrometry	15-17 May 2002	50 th Annual Conference on Mass Spectrometry, Kyoto, Japan

Author	Title	Date	Organisation/location
S. Vidal	Mouth-feel properties of tannins and anthocyanins	16 May 2002	Graduate Diploma in Oenology students, Urrbrae SA
M. Gishen, I.L. Francis, R.G. Damberg, M.B. Esler, W.U. Cynkar, D. Boehm, A. Kambouris ⁹ and P.B. Høj	NIR spectroscopy to measure the composition of grapes, juice and wine.	21 May 2002	Visiting group of grapegrowers from SA organised by Elders at the AWRI, Urrbrae SA
S.J. Bell	Agrochemical issues for grapegrowers and winemakers.		
P.B. Høj	Research at The Australian Wine Research Institute		
P.W. Godden	Science and technology, their importance in the production of quality wines for the consumer	22 May 2002	3 rd Foro Mundial Del Vino (3rd World Wine Forum), Logroño (Rioja), Spain
P.B. Høj	Sustainable aquaculture	31 May 2002	PMSEIC Committee, Parliament House, Canberra ACT
I.L. Francis	Wine quality – a researcher's perspective	5 Jun 2002	South Australian Farmer's Federation, McLaren Vale Wine Grapes Branch 2002 Grower's Day, McLaren Vale SA
PA. Henschke	Chair of session IV: 'Curing stuck fermentations under practical conditions'	10 Jun 2002	13 th International Oenology Symposium, Montpellier, France
PA. Henschke	The influence of non- <i>Saccharomyces cerevisiae</i> yeasts on wine quality	11 Jun 2002	
M. Gishen, R.G. Damberg, L. Janik, W.U. Cynkar, D. Boehm, I.L. Francis and P.B. Høj	Near Infrared Spectroscopy - future directions	17-18 Jun 2002	CRCV 2002 Symposium, Mildura Victoria
M.A. de Barros Lopes	Metabolonomics and proteonomics		
G.M. Eley	Grape and wine – flavour		
M.J. Herderich	Grape and wine tannins		
A.J. Heinrich	What makes a wine yeast a wine yeast?		

Workshops			
S.J. Bell	<i>Research to Practice</i> TM - Grapevine Nutrition	25-26 Sept 2001	Clare Valley SA
S.J. Bell	<i>Research to Practice</i> TM - Grapevine Nutrition	3 Oct 2001	McLaren Vale SA
M.G. Holdstock, D.R. Buick	W24 – Practical laboratory applications for a UV/V is spectrophotometer	7 Oct 2001	11 AWITC, Adelaide SA
S.J. Bell, A.N. Sas ⁹	W08/W49 Future trends in agrochemical use	7 & 9 Oct 2001	
J.M. Eglinton	W05 Winemaking with non-conventional yeasts	7 Oct 2001	
E.J. Waters, M.J. Herderich	W23/W63 Impacts of grape and wine tannins and enotannins on red wine structure	7 & 11 Oct 2001	
A.D. Coulter, P.J. Valente, E.M.C. Robinson, P.W. Godden	W22/W77 Troubleshooting wine instability problems	7 & 11 Oct 2001	
E.J. Bartowsky, P.J. Costello	W26 Malo cultures – can you taste the difference? An exploration of the sensory effects of MLF	7 Oct 2001	
J.B. Hughes	W67 Riesling – the next viticultural challenge	11 Oct 2001	
M. Gishen	W42 Quality management – options for growers	8 Oct 2001	
	W53 Quality management – options for wineries	9 Oct 2001	
	W85 Quality management – introduction to HACCP	11 Oct 2001	
	W86 Quality management – HACCP plan development (Advanced)	11 Oct 2001	
M. Gishen, I.L. Francis	W79 Objective measures of grape and wine quality	10 Oct 2001	
C.S. Stockley	W84 Do wine-derived phenolic compounds have a role in improving human health	11 Oct 2001	
M. Gishen, P.W. Godden	<ul style="list-style-type: none"> Quality management systems-description and options Developing HACCP plans for food safety Analyses available to the wine industry through the AWRI Analytical Service Enhancing your quality system through analysis Efficient sampling and testing – what, why when and how? A winemaker's perspective. 	20-21 Nov 2001	Winemakers and grapegrowers, Toowoomba Qld.
A.D. Coulter	<ul style="list-style-type: none"> Isolation of hazes/deposits and use of microscope What bug is that? Microbiological techniques 		
P.W. Godden	Persistent Wine Instability Issues / Winemaking do's and don'ts – general winemaking theory and practice		
R.G. Damberg	Vintage colour measurements	30 Nov 2001	Interwinery Analysis Group annual review workshop, Clare, SA
S.J. Bell	<i>Research to Practice</i> TM Grapevine Nutrition	20 Jun 2002	Clare Valley, SA
		21 June 2002	McLaren Vale, SA

Posters presented at 11 AWITC, Adelaide SA: 7-10 October 2001

Author	Title
A.J. Heinrich, J. Bellon, B. Van Wegen, G. Currie ¹ , V. Jiranek ² , M.A. de Barros Lopes	Identifying genes that are involved in the stress response of the yeast <i>Saccharomyces cerevisiae</i> during fermentation
J. Gardner ³ , M. Wenk ² , Miguel de Barros Lopes, V. Jiranek ²	Identification of genes contributing to a 'high nitrogen efficiency' (Hne) phenotype in a modified wine yeast
J. Bellon, J.M. Eglinton, A.P. Pollnitz, M.A. de Barros Lopes	Hybrid wine yeasts with unique fermentation characteristics
E.J. Bartowsky, J. McCarthy, P.A. Henschke	Diacetyl production by five commercial MLF bacteria strains in red and white wine
N. D'Incecco ³ , E.J. Bartowsky, P.A. Henschke	Release of glycosidically bound flavour compounds by <i>Oenococcus oeni</i> during MLF
P.J. Costello, P.A. Henschke, A.J. Markides ⁴	A model system for predicting interactive effects of wine yeast and malolactic bacteria
P.J. Costello, M.J. Kwiatkowski, I.L. Francis, P.A. Henschke	Novel yeasts to produce novel wines: flavour profile of Chardonnay wines made with <i>Candida</i> yeast
K. Poole ⁵ , J.-M. Salmon ⁶ , M.A. de Barros Lopes, V. Jiranek ²	Oxygen addition leads to improved fermentation kinetics in a <i>Saccharomyces cerevisiae</i> strain capable of proline transport
K. Poole ⁵ , M.A. de Barros Lopes, V. Jiranek ²	Modification of <i>Saccharomyces cerevisiae</i> to utilise proline as a nitrogen source during oenological fermentation
K. Howell, E.J. Bartowsky, G. Fleet ⁷ , P.A. Henschke	Use of DNA microsatellites to monitor <i>Saccharomyces cerevisiae</i> proportions during mixed culture fermentation
K. Howell, E.J. Bartowsky, G. Fleet ⁷ , P.A. Henschke	Mixed inocula of <i>Saccharomyces cerevisiae</i> can alter wine aroma
J.M. Eglinton, M.A. de Barros Lopes, P. Langridge ⁸ , T. Lithgow ⁹ , P.A. Henschke	Localisation of aldehyde dehydrogenase Ald4p in mitochondria of <i>Saccharomyces cerevisiae</i>
J.M. Eglinton, P.A. Henschke	Evaluation of practical strategies for ensuring complete fermentation with a non-conventional yeast
J.M. Eglinton, A.J. Heinrich, A.P. Pollnitz, P. Langridge ⁸ , P.A. Henschke, M.A. de Barros Lopes	Deletion of ALD6 decreases acetic acid accumulation in a glycerol overproducing strain of <i>Saccharomyces cerevisiae</i>
J.M. Eglinton, I.L. Francis, P.B. Høj, P.A. Henschke	Flavour modification by using <i>Saccharomyces bayanus</i>
M.B. Esler, M. Gishen, I.L. Francis, R. Damberg, A. Kambouris ⁹ , W.U. Cynkar, D. Boehm	Effects of variety and region on near infrared reflectance spectroscopic analysis of quality parameters in red wine grapes
P. Blake ⁹ , M. Frankel ⁹ , R.G. Damberg	High throughput malic acid analysis using a microplate reader
R. Ristic ¹⁰ , I.L. Francis, R. Gawel ¹⁰ , P.G. Iland ²	Relationships between seed composition and grape and wine quality
C. Puglisi, G.M. Eley, G.K. Skouroumounis, M.A. Sefton, R. Prager ¹¹	On the formation of naturally occurring β-damascenone in grapes and wine
K. Wilkinson, M. Raunkjaer, G. Eley	Precursors to oak lactone: synthesis of gallate ester derivatives of 3-methyl-4-hydroxyoctanoic acid
W.U. Cynkar, I.L. Francis, H. McCarthy ² , E. Gomez ¹² , E.J. Bartowsky	Evaluation of a 'chemical sensor' instrument for rapid fingerprinting of wine volatiles
R.G. Damberg, A. Kambouris ⁹ , N. Schumacher ¹³ , I.L. Francis, M.B. Esler, M. Gishen	Wine quality grading by near infrared spectroscopy
Y. Hayasaka, K. Adams ¹⁴ , K.F. Pocock, G.A. Baldock, E.J. Waters, P.B. Høj	Differentiation of grape (<i>Vitis vinifera</i>) varieties by electrospray mass spectrometry of juice pathogenesis related proteins
Y. Hayasaka, S. Vidal, E.J. Waters, V. Cheynier ¹³ , M.J. Herderich	Characterisation of highly polymerized proanthocyanidins in seeds by electrospray mass spectrometry

Author	Title
A.P. Pollnitz, J. Campbell ² , M.A. Sefton	The effects of oak chip size and age on wine composition
J. Kennedy ² , Y. Hayasaka, S. Vidal	Composition of grape skin proanthocyanidins at different stages of berry development
G.A. Baldock, Y. Hayasaka, G.A. Ruediger	Ethyl carbamate in wine
R.L. Taylor, G.A. Ruediger	Development of a multi-agrochemical residue analysis for grapes and wines using HPLC and small-scale extraction techniques
G.A. Ruediger, D. Boehm	Ochratoxin A in wines
Z.K. Peng, E.J. Waters, P.W. Godden, C. Day ² , S.J. Clarke ² , M.J. Herderich	Polyphenols, pigmented polymers and red wine colour: results of the 2001 large scale winemaking trial
E.J. Bartowsky, I.L. Francis, J. Bellon, P.A. Henschke	Buttery aroma in commercial Australian wines: relationship with diacetyl concentration
G.K. Skouroumounis, M.J. Kwiatkowski, E.J. Waters	Non-destructive measurement of white wine colour
G. Eley, I.L. Francis, M.A. Sefton	Sensory properties of some grape and wine aroma compounds
Y. Hayasaka, R. Asenstorfer ²	Screening for potential wine pigments in red wine using tandem mass spectrometry
R. Asenstorfer ² , Y. Hayasaka, G.P. Jones ²	Isolation and structures of oligomeric wine pigments by bisulphite-mediated ion-exchange chromatography
D. Capone, S. Franek ² , H. McLean ¹⁴ , M.A. Sefton	Absorption of flavour by wine packaging
S. Dillon, E.J. Bartowsky, P.A. Henschke, P.B. Høj, L. Dulau ⁴	Yeast affect accumulation of phenolic compounds in Shiraz wine
K.F. Pocock, P.B. Høj, E.J. Waters	Less bentonite needed after short time high temperature heat treatment of wine either with or without proteolytic enzyme addition
S. Brown, M.A. de Barros Lopes, P.B. Høj, E.J. Waters	Investigation into the mechanism of action and biological role of <i>Saccharomyces cerevisiae</i> mannoproteins which reduce visible haziness in white wine

Posters presented at 22nd International Specialised Symposium on Yeast held in Pilanesberg, South Africa: 25-28 March 2002

J.M. Eglinton, I.L. Francis, P.B. Høj, and P.A. Henschke	Flavour modification of wine by using <i>Saccharomyces bayanus</i>
K.S. Howell, E.J. Bartowsky, G.H. Fleet ⁶ and P.A. Henschke	Use of DNA microsatellites to monitor <i>Saccharomyces cerevisiae</i> proportions during mixed culture fermentation
K.S. Howell, E.J. Bartowsky, G.H. Fleet ⁶ and P.A. Henschke	Mixed inocula of <i>Saccharomyces cerevisiae</i> can alter wine aroma and composition
C. Charoenchai ⁵ , G.H. Fleet ⁶ and P.A. Henschke	Growth and fermentation properties of some non- <i>Saccharomyces</i> wine yeasts

Poster presented at International workshop on anthocyanins, organised by The Cooperative Research Centre (CRC) for Bioproducts, Wirrina Cove, SA: 18-19 April 2002

M.J. Kwiatkowski, Z.K. Peng, E.J. Waters, P.W. Godden, C.J. Day ² , S.J. Clarke ² , and M.J. Herderich	Polyphenols, pigmented polymers and red wine colour: First results of the 2001 large scale winemaking trial.
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- School of Botany, University of Melbourne
- Department of Horticulture, Viticulture and Oenology, Adelaide University
- Department of Agricultural Biotechnology, Padova University, Italy
- Lallemand
- Institut des Produits de la Vigne, Institut National de la Recherche Agronomique, France
- Department of Food Science, University of New South Wales
- Department of Plant Science, Adelaide University
- University of Melbourne
- BRL Hardy
- Roseworthy Wine Tasting Programs Pty Ltd
- School of Chemistry Physics and Earth Sciences, Flinders University
- Department of Food Technology, University of Murcia, Spain
- Unité de Recherche des Produits de la Vigne, INRA, France
- Orlando Wyndham
- Unité de Recherche Cidricole-Biotransformation des Fruits et Légumes, INRA

Other activities

Staff	Activity	Date
P.W. Godden, E.M.C. Robinson, J.B. Hughes, M. Gishen, T.A. Wallis, A.D. Coulter, I.L. Francis	Advanced Wine Assessment Course, Adelaide SA	17-20 Jul 2001
P.W. Godden	Judge at the Australian Alternative Varieties Wine Show, Mildura Vic	19 Nov 2001
P.W. Godden	Associate Judge at the Royal Adelaide Wine Show, Adelaide, SA	25-28 Sept 2001
P.B. Høj	Associate Judge at the Sydney Wine Show, Sydney NSW	3-7 Feb 2002

Appendix 2. Teaching responsibilities of Institute staff during 2001/02

Subject	No. of lectures	Institute staff
<i>2001 – Semester 2</i>		
Adelaide University		
9685 Advances in Oenology	1	P.W. Godden
9685 Advances in Oenology/ 9086 Advances in Oenology (supplemented)	6	Dr P.A. Henschke
9685 Advances in Oenology/ 9086 Advances in Oenology (supplemented)	3	Dr E.J. Bartowsky
9685 Advances in Oenology/ 9086 Advances in Oenology (supplemented)	1	Dr P.J. Costello
3011WT Oenology	2	Dr M.A. Sefton
9685 Advances in Oenology	2	Dr I.L. Francis
4789 Sensory Studies	7	Dr I.L. Francis
1958 Wine packaging and quality management	1	M. Gishen
2580 Stabilisation and Clarification	1	P.W. Godden
2580 Stabilisation and Clarification	1	A.D. Coulter
3009WT Advanced Sensory Practice	4 hours	P.W. Godden
Viticulture B	1	S-J. Bell
The Flinders University of South Australia		
MMED 3921 Industrial and Pharmaceutical Microbiology	1	Dr P.A. Henschke
<i>2002 – Semester 1</i>		
Adelaide University		
3000WT Grape Industry Practice Policy and Communication	Approx. 56 hours	Professor P.B. Høj and C.S. Stockley
1005/3113 Winemaking	2	Dr P.A. Henschke
Final year wine science students	1	M. Gishen
Graduate Diploma of Oenology	1	M. Gishen
Graduate Diploma of Oenology	1	Dr M.J. Herderich
Graduate Diploma of Oenology	1	Dr S. Vidal
Graduate Diploma of Oenology	1	Dr M.A. de Barros Lopes
2582 Biotechnology in the Food and Wine Industries	2	Dr M.A. de Barros Lopes
2943 Advanced Sensory Practice	9	Dr I.L. Francis
2580 Stabilisation and Clarification	3	Dr E.J. Waters
LaTrobe University		
Viticulture and Oenology (Wodonga campus)	1	M. Gishen

Appendix 3. Graduate and Honours student supervision responsibilities of Institute staff for 2001/02

Student	Supervisor/s	Source of funds
<i>PhD</i>		
S. Brown	E.J. Waters, M.A. de Barros Lopes, P.B. Høj	GWRDC
M. de Sa	M.J. Herderich, I.L. Francis	CRCV
J. Gardner	V. Jiranek ⁷ , M.A. de Barros Lopes	DHVO – University of Adelaide
R. Gawel	I.L. Francis, A. Markides ⁶	
W. Greenrod	M.Fenech ¹ , M.Abbey ¹ , P. Burcham ² , C.S. Stockley	GWRDC
A. Grimaldi	E.J. Bartowsky, V. Jiranek ⁷	DHVO – University of Adelaide
A. Heinrich	M.A. de Barros Lopes, V. Jiranek ⁷	CRCV
K. Howell	P.A. Henschke, G. Fleet ³ , P.A. Henschke	University of NSW/GWRDC
A. Janusz	G.M. Eley, M.A. Sefton, M. Perkins ³	CRCV
C. McBryde	V. Jiranek ⁷ , M.A. de Barros Lopes	DHVO – University of Adelaide
R. Muhlack	C. Colby ⁴ , E.J. Waters, P.B. Høj, B. O'Neill ⁴ , A. Lim ⁵	University of Adelaide/BRL Hardy/ GWRDC
A. Oberholster	E.J. Waters, I.L. Francis, G.P. Jones, P.G. Iland ⁷	GWRDC
C. Puglisi	G.M. Eley, M.A. Sefton, R. Prager ³	CRCV
R. Ristic	P.G. Iland, I.L. Francis	GWRDC
H. Smyth	I.L. Francis, M.A. Sefton, M.J. Herderich	GWRDC
K. van Leeuwen	G.M. Eley	Flinders University
K. Wilkinson	G.M. Eley, M.A. Sefton, R. Prager ³	GWRDC
<i>Masters</i>		
D. Coates	E.J. Bartowsky	DHVO – University of Adelaide
<i>Honours</i>		
J. Cartwright	M.A. de Barros Lopes, A.J. Yap ⁷	DHVO – University of Adelaide
M. Fetke	P.A. Henschke, E.J. Bartowsky, P.J. Costello	DHVO – University of Adelaide

Industry placement student

C. Hillier	M.A. de Barros Lopes (21 January to 1 March 2002)	Flinders University
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Theses completed

Honours/PhD			
Student	Hon/PhD	Title of thesis	Supervisors
A. Matthews	Hons	Investigation into the wine flavour profiles produced during malolactic fermentation	E.J. Bartowsky, I.L. Francis
M. Mercurio	Hons	The use of multi-layer countercurrent chromatography in the isolation of glycosidically bound aroma compounds from grape leaves.	G.K. Skouroumounis, G.M. Eley
M. Smith	Hons	Formation of TDN: the effect of glycosylation in the hydrolysis of 3,6-dihydroxymegastigm-4-en-9-one	G.M. Eley
A. Walkenhorst	Hons	Rapid methods for the quantitation of red wine phenolic parameters	I.L. Francis, R.G. Dambergs
N. D'Incecco	PhD	Cellular localisation of beta-glucosidase enzyme in oenological microorganisms	P. Spettoli ⁸ , E.J. Bartowsky, P.A. Henschke

¹ CSIRO Health Sciences and Nutrition² Department of Clinical and Experimental Pharmacology, The University of Adelaide³ Flinders University⁴ Department of Chemical Engineering, The University of Adelaide⁵ BRL Hardy⁶ Lallemand Australia⁷ Department of Horticulture, Viticulture and Oenology, The University of Adelaide⁸ University of Padova, Italy⁹ University of New South Wales

Appendix 4. Institute staff publications 2001/02

666	Godden, P.; Francis, L.; Field, J.; Gishen, M.; Coulter, A.; Valente, P.; Høj, P.; Robinson, E. Wine bottle closures: physical characteristics and effect on composition and sensory properties of a Semillon wine. 1. Performance up to 20 months post-bottling. <i>Aust. J. Grape Wine Res.</i> 7: 64–105; 2001.
667	Smith, B.P.; Alcaraz Zini, C.; Pawliszyn, J.; Tyler, M.J.; Hayasaka, Y.; Williams, B.; Bastos Caramao, E. Solid-phase microextraction as a tool for studying volatile compounds in frog skin. <i>Chem. Ecol.</i> 17: 215–225; 2000.
668	van Heeswijck, R.; Stines, A.P.; Grubb, J.; Skrumsager Møller, I.; Høj, P.B. Molecular biology and biochemistry of proline accumulation in developing grape berries. Roubelakis-Angelakis, K.A., ed. <i>Molecular biology and biotechnology of the grapevine</i> . Dordrecht, The Netherlands: Kluwer Academic Publishers; 2001: 87–108.
669	Tattersall, D.B.; Pocock, K.F.; Hayasaka, Y.; Adams, K.; van Heeswijck, R.; Waters, E.J.; Høj, P.B. Pathogenesis related proteins — their accumulation in grapes during berry growth and their involvement in white wine heat instability. Current knowledge and future perspectives in relation to winemaking practices. Roubelakis-Angelakis, K.A., ed. <i>Molecular biology & biotechnology of the grapevine</i> . Dordrecht, The Netherlands: Kluwer Academic Publishers; 2001: 183–201.
670	Stockley, C.S. The effectiveness of strategies such as health warning labels to reduce alcohol-related harms — an Australian perspective. <i>Int. J. Drug Policy</i> 12: 153–166; 2001.
671	Godden, P.; Francis, L.; Field, J.; Gishen, M.; Coulter, A.; Valente, P.; Høj, P.; Robinson, E. Results of an AWRI trial investigating the technical performance of various types of wine closure: part 2 — wine composition up to 20 months post-bottling. <i>Aust. Grapegrower Winemaker</i> (452): 89–98; 2001.
672	Gawel, R.; Iland, P.G.; Leske, P.A.; Dunn, C.G. Compositional and sensory differences in Syrah wines following juice run-off prior to fermentation. <i>J. Wine Res.</i> 12: 5–18; 2001.
673	Puglisi, C.J.; Eisey, G.M.; Prager, R.H.; Skouroumounis, G.K.; Sefton, M.A. Identification of a precursor to naturally occurring β-damascenone. <i>Tetrahedron Lett.</i> 42: 6937–6939; 2001.
674	Tattersall, D.B.; Bak, S.; Jones, P.R.; Olsen, C.E.; Nielsen, J.K.; Hansen, M.L.; Høj, P.B.; Møller, B.L. Resistance to an herbivore through engineered cyanogenic glucoside synthesis. <i>Science</i> 293: 1826–1828; 2001.
675	Godden, P.; Francis, L.; Field, J.; Gishen, M.; Coulter, A.; Valente, P.; Høj, P.; Robinson, E. Results of an AWRI trial investigating the technical performance of various types of wine closure: part 1 — physical measurements up to 20 months post-bottling. <i>Aust. Grapegrower Winemaker</i> (451) : 67–70, 72–73, 75–77; 2001.
676	Godden, P.; Francis, L.; Field, J.; Gishen, M.; Coulter, A.; Valente, P.; Høj, P.; Robinson, E. Results of an AWRI trial investigating the technical performance of various types of wine closure: part 3 — wine sensory properties up to 20 months post-bottling. <i>Aust. Grapegrower Winemaker</i> (453) : 103–110; 2001.
677	Hayasaka, Y.; Asenstorfer, R.E. Screening for potential pigments derived from anthocyanins in red wine using nano-electrospray tandem mass spectrometry. <i>J. Agric. Food Chem.</i> 50: 756–761; 2002.
678	Vidal, S.; Cartalade, D.; Souquet, J.-M.; Fulcrand, H.; Cheynier, V. Changes in proanthocyanidin chain length in wine-like model solutions. <i>J. Agric. Food Chem.</i> 50: 2261–2266; 2002.
679	Henschke, P.A.; Eglinton, J.M.; Costello, P.J.; Francis, I.L.; Gockowiak, H.; Soden, A. Fermentation with <i>Saccharomyces bayanus</i> and <i>Candida stellata</i> yeasts can increase wine aroma diversity. 26 th World Congress and 81 st General Assembly of the Office International de la Vigne et du Vin: meeting the consumer challenge: 11–17 October 2001; Volume II. Oenology. Adelaide, SA: OIV; 2001.: 84–94.
680	Costello, P.J.; Lee, T.H.; Henschke, P.A. Ability of lactic acid bacteria to produce N-heterocycles causing mousy off-flavour in wine. <i>Aust. J. Grape Wine Res.</i> 7 : 160–167; 2001.
681	Raunkjaer, M.; Sejer Pedersen, D.; Eisey, G.M.; Sefton, M.A.; Skouroumounis, G.K. Precursors to oak lactone: synthesis of gallate ester derivatives of 3-methyl-4-hydroxyoctanoic acid. <i>Tetrahedron Lett.</i> 42 : 8717–8719; 2001.
682	Kennedy, J.A.; Hayasaka, Y.; Vidal, S.; Waters, E.J.; Jones, G.P. Composition of grape skin proanthocyanidins at different stages of berry development. <i>J. Agric. Food Chem.</i> 49 : 5348–5355; 2001.
683	Asenstorfer, R.E.; Hayasaka, Y.; Jones, G.P. Isolation and structures of oligomeric wine pigments by bisulfite-mediated ion-exchange chromatography. <i>J. Agric. Food Chem.</i> 49: 5957–5963; 2001.
684	Eglinton, J.M.; Heinrich, A.J.; Pollnitz, A.P.; Langridge, P.; Henschke, P.A.; de Barros Lopes, M. Decreasing acetic acid accumulation by a glycerol overproducing strain of <i>Saccharomyces cerevisiae</i> by deleting the ALD6 aldehyde dehydrogenase gene. <i>Yeast</i> 19:295-301; 2002.
685	de Barros Lopes, M.; Bellon, J.R.; Shirley, N.J.; Ganter, P.F. Evidence for multiple interspecific hybridization in <i>Saccharomyces sensu stricto</i> species. <i>FEMS Yeast Res.</i> 1: 323–331; 2002.
686	Peng, Z.; Iland, P.G.; Oberholster, A.; Sefton, M.A.; Waters, E.J. Analysis of pigmented polymers in red wine by reverse phase HPLC. <i>Aust. J. Grape Wine Res.</i> 8: 70–75; 2002.

687	Damberg, R.G.; Kambouris, A.; Francis, I.L.; Gishen, M. Rapid analysis of methanol in grape derived distillation products using near infrared transmission spectroscopy. <i>J. Agric. Food Chem.</i> 50: 3079-3084; 2002.
688	Francis, I.L.; Gawel, R.; Iland, P.G.; Vidal, S.; Cheynier, V.; Guyot, S.; Kwiatowski, M.J.; Waters, E.J. Characterising mouth-feel properties of red wines. <i>Aust. NZ Wine Ind. J.</i> 17(3): 18, 21-22, 24-25; 2002.
689	Bartowsky, E.; Costello, P.; Henschke, P. Management of malolactic fermentation-wine flavour manipulation. <i>Aust. NZ Grapegrower Winemaker</i> (461a): 7-8, 10-12; 2002.
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Appendix 5. Institute Committees

Staff member	Management Advisory	Research Steering	Industry Services Steering	Communication Steering	Analytical Service Steering	Information Technology	Biosafety	Occupational Health and Safety	Staff Code Negotiation
Peter Høj	C	C	X	X	X	X			X
Eveline Bartowsky						X		X	X
Sally-Jean Bell	X	X	X	X	X				
Rae Blair	X	X		C					
Shauna Brown								X	
Don Buick	X		X		X				
Adrian Coulter	X	X	X		X				
Hans Muhlack	X								X
Catherine Daniel				X					
Miguel de Barros Lopes	X	X					X		
Rachel Edwards						X			
Jeff Eglinton				X		C	C		
Leigh Francis	X	X	X						
Mark Gishen	X	X	X		C				
Peter Godden	X	X	C	X	X				
Jeremy Hack						X			
Yoji Hayasaka	X	X	X						
Paul Henschke	X	X			X			C	
Markus Herderich	X	X							
John Hughes								X	
Ingrid Oats									X
Ken Pocock							X	X	
Alan Pollnitz						X			
Mark Sefton	X	X			X				
Creina Stockley		X		X					
Elizabeth Waters	X	X					X		

C = denotes holder of Chair



The staff of The Australian Wine Research Institute

Front row (L to R)

Creina Stockley
Amanda Cook
Kate Howell
Claire Balmer
Maria Mills
Miguel de Barros Lopes
Carolyn Puglisi
Mark Gishen
Yoji Hayasaka
Jenny Bellon
Anthina Massis

Second row

Sandra Lloyd-Davies
Les Janik
Jelena Jovanovic
Kerry Wilkinson
Heather Donnell
Merran Smith
Gayle Baldock
George Skouroumounis
Kevin Pardon
Danielle Leedham
Hans Muhlack
Greg Ruediger
Simon Dillon

Third row

Matthew Cream
Jeff Eglinton
Kate Lattey
Rachel Edwards
Melissa Franics
Emma Darling
Rae Blair
Heather Smyth
Maria de Sa
Weis Cynkar
Bob Dambergs
Dimitra Capone
David Boehm
Daniel Cozzolino

Fourth row

Matthew Holdstock
Ingrid Oats
Randell Taylor
Adrian Coulter
Elizabeth Waters
Shauna Brown
Ella Robinson
Peter Hoj
Paul Henschke
Holger Gockowiak,
Mariola Kwiatkowski
John Hughes
Peter Costello

Back row

Anthony Heinrich
Katryna van Leeuwen
Leigh Francis
Sally Bell
Tracey Siebert
Alan Pollnitz
Markus Herderich
Mark Sefton
Ken Pocock
Peter Godden
Don Buick
Gordon Elsey

Absent

Eveline Bar towsky
Anna Catalano
Catherine Daniel
Agnieszka Janusz
Jane McCarthy
Mango Parker
Stephane Vidal
Trudy Weber