leaf samples were taken from different treatments and analysed using a portable Vis-NIR instrument (800 – 1800 nm) in reflectance. Partial least square (PLS) calibrations between reference and NIR data were developed using cross validation. Preliminary NIR calibrations yielded good correlation coefficients between predicted and measured stem and leaf water potential ($r > 0.60$, Figure 9). These functions appear to hold within a variety for several weeks of measurement. It is also possible to determine clear trends in the spectra that would allow a rapid detection of the onset of water stress.

Objectively defining Pinot Gris and Pinot Grigio wine style

Pinot Gris is a white grape variety that is growing in importance in the Australian wine sector. However, because this variety has two different names (Pinot Gris and Pinot Grigio), and has traditionally been used to make wines of very different styles, there is potential for considerable confusion amongst consumers which could impact on the variety's ultimate success in the marketplace. Our work on this variety aims to gain a greater understanding of Australian styles of Pinot Gris/Grigio wines, in a global context. A combination of sensory analysis, chemical analysis and rapid spectroscopy techniques has demonstrated that objective measures of Pinot Gris/Grigio style can be achieved. This could provide wine producers with information to better communicate with consumers, and potentially label wines more consistently. Communication of the findings to the wine sector will be a focus of the coming year.

In summary, the key to the success of the Industry Applications model will be to ensure that all of these technologies are combined into packages which have relevance to the Australian grape and wine sector – what we term 'integrated solutions', and that those 'integrated solutions' are actually implemented into production practices.

Applications of mass spectrometry to ensure the quality and integrity of Australian wine

Staff

Dr Yoji Hayasaka, Gayle Baldock

The members of the Mass Spectrometry team work collaboratively across teams within the AWRI and also with external researchers. Their collaborative activities are included elsewhere within this report, however, the highlights from the year are shown here.

Investigation of taint problem using mass spectrometry

Collaborators

Winemaking and Extension team of the Industry Development and Support Group

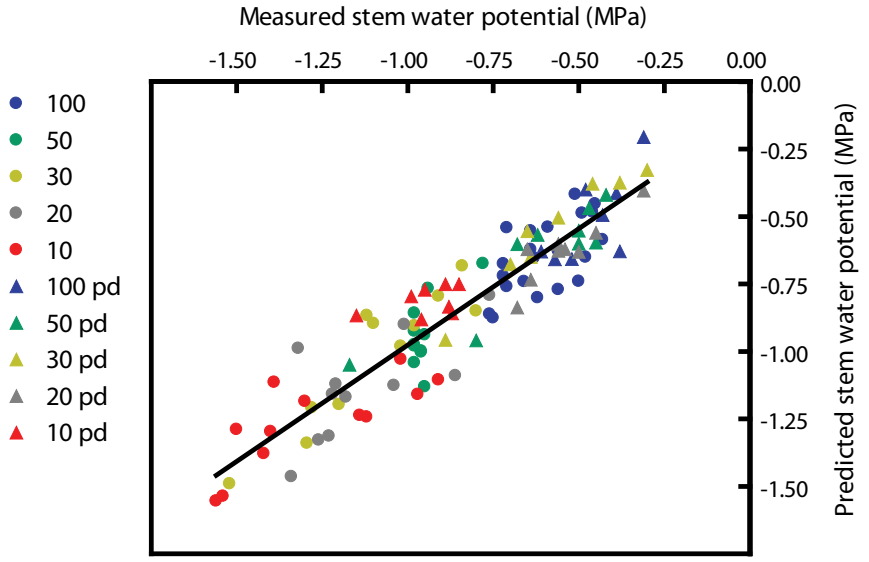

Figure 9. Predicted Stem (Petiole) Water Potential (SWP) versus measured SWP using NIR spectra for Chardonnay vines measured in January and February. Each absorption spectrum was measured on the underside of a leaf on the same shoot as an adjacent bagged leaf used to measure SWP. Irrigation treatments are indicated with different coloured symbols (pd = predawn)

Mass spectrometric techniques were applied for various separate taint investigations. These included investigations into 'burnt rubber-like' aroma in red wine, off-flavour and taint caused by containers in various wines, wine affected by filtering through suspected tainted earth, wines exposed to excessive heat in a warehouse and an unusually high production of indole in wines. See the 'Technical problem solving and consulting' report published in this Annual Report for further details.

Investigations into smoke-affected grapes and wine

Collaborators

Technical problem solving team of the Industry Development and Support

Dr Kerry Wilkinson and Kerry Dungey of the University of Adelaide

In recent years, the negative impact of bushfire on grapes and their resulting wines is an increasing concern. The wines made from grapes exposed to smoke in vineyards are often characterised by objectionable 'smoky', 'dirty' and 'burnt' aromas and tastes. This can result in significant reductions in their market values or even make the wine not fit for sale.

Guaiacol has been used as a marker to assess the smoke-affected grapes and wine. The reasons for that are (i) guaiacol is one of the most common smoke-derived compounds; (ii) has a strong smoky character; and (iii) most importantly, its concentration in juice and wine samples has been strongly correlated with the intensity of the smoke affect (2003 AWRI annual report). However, in some instances, grapes containing very low or not detectable (less than 1 µg/kg) concentrations of guaiacol have produced smoke-affected wine. This observation suggests that a better marker or way to assess the degree of smoke affect to grapes and wine is needed.

We are currently working on two approaches to develop a better way to assess the potential of grapes to produce smoke-affected wine. These are:

1 Can guaiacol precursors be used as markers to indicate a degree of smoke affect to grapes and wine?

It has been reported that guaiacol precursors are biotransformed from guaiacol and accumulated in grapes as a consequence of grapevine exposure to smoke. A portion of these precursors release free guaiacol during fermentation and possibly during maturation and even in-bottle. Therefore the precursors are good candidates for markers to assess the degree of smoke-affect.

Initially, the identification of guaiacol precursors was carried out using stable isotope technique. A brief protocol of this experiment is as follows: (i) the aqueous guaiacol solution (5 mg or 30 mg/L) containing the unlabelled (*d0*) and labelled (*d3*) guaiacol at an equal amount ratio was prepared; (ii) two pots of grapevines (Cabernet Sauvignon) were prepared: one was treated with guaiacol and another was untreated (control); (iii) the *d0*- and *d3*-guaiacol solution was poured in a plastic bag with a seal and then a leaf or a bunch of grapes was placed in the bag, allowing to directly contact with the guaiacol solution for one or two days; (iv) the leaves and grapes were collected from the treated and untreated pots of grapevines at six weeks after contact with the guaiacol solution; and (v) the leaf and grape samples were analysed by LC-MS and LC-MS/MS techniques.

Team reports

(L to R) Yoji Hayasaka, Heather Brooks, Gayle Baldock, Markus Herderich, Sarah Ballantine, Randell Taylor, Marcel Essling, Con Simos

As a result, the following observations and findings were made:

- Significant abundance of seven different glycosides (shown below) of *do*- and *d3*-guaiacol was found in the leaves and berries which had been directly contacted with the guaiacol solution.
- Only trace amounts of the unlabelled (do-) and labelled (d3-) glycosides (*do* >> *d3* in abundance) were found in the leaves and berries with no contact with the guaiacol solution, collected from the treated grapevine. This observation demonstrates that the translocation of the guaiacol glycosides is limited within the grapevine.
- Trace amounts of the unlabelled guaiacol glycosides were found in the control leaf and grape samples collected from the control grapevine. This observation demonstrates that these guaiacol glycosides are present as natural components of grape berries and leaves.
- The leaf and berry samples contained the same guaiacol glycosides but had a different relative abundant distribution of the glycosides. The monoglucoside is most abundant in the leaf accounting for nearly 70% of total glycosides but is the least abundant in the berry samples in which total diglycosides are dominant accounting for more than 75%.

The seven guaiacol precursors were also found in the leaves and grapes which had been exposed to smoke resulting from the Black Saturday Bushfire that occurred in February 2009 in Victoria. Hence, the stable isotope trace experiment simulated the fate of guaiacol taken in by leaves and berry which had been exposed to smoke.

A method for the semi-quantification of total guaiacol precursors (sum of seven precursors) in berries using HPLC-MS/MS was developed and applied for analysis of smoke-affected grapes obtained from the bushfire-affected areas in Victoria. In total, 26 samples were analysed and the lowest and highest concentrations were at 0.026 and 3.041 mg/kg, respectively. Importantly, all samples were found to contain a detectable level of guaiacol precursors, including a control sample (not smoked). On the other hand, 11 (42%) out of the same sample set (26 samples) showed no-detectable level of guaiacol and the 38% showed only very low concentrations (1 to 5 µg/kg).

In conclusion, guaiacol precursors are promising markers to assess a degree of smoke-affect to grapes and wine. The analysis of these precursors is expected to be useful as a risk management tool for making decisions regarding whether suspected smoke-affected grapes can be used for winemaking. The investigation is in progress.

2 Can smoke sampling be used to indicate smoke exposure intensity in a vineyard (airborne analysis)?

This approach aims to measure smoke intensity in a vineyard during a bushfire event and use the smoke intensity data to assess the extent to which smoke has impacted on grape quality. In addition, sampling of smoke during a particular fire event may provide an indication as to what other compounds might be present in concentrations that might cause a smoke affect in grapes and wine (other than guaiacol).

Passive (diffusion) and active (pump) smoke sampling techniques using two different adsorbents (TENAX and PDMS) were tested in tents enclosing grape vines filled with smoke from burning straw (model experiment). The trapped smoke-derived volatile compounds on the adsorbents were analysed by GC-MS combined with TDU (Thermal Desorption Unit) and CIS (Cooled Inlet System). These sampling techniques were also tested in a prescribed burn-off in the Adelaide Hills in order to better understand the applicability of these methods in a realistic smoke event.

In all experiments, all methods were able to analyse a broad range of smoke-derived components. Guaiacol and methyl guaiacol were easily measured, as were other typical 'biomass burn'-generated compounds including a variety of alkylbenzenes, naphthalenes, phenols, methooxyphenols and

syringols, etc. The active methods (pump) were found to be a better way to analyse smoke than the passive methods, which sensitively detected a greater number of compounds. The active TENAX method was able to pick up more compounds than all other methods. The passive TENAX 'badge' was the least sensitive method both in the model experiments and in the real situation.

The twister (PDMS) passive method was able to pick up a similar number of compounds as the PDMS active method with less intensity. When the twisters were left for 24 hours in the bush during and after the prescribed burn in different areas (low and high visible smoke intensity) it appeared to be capable of quantitatively measuring the range of smoke intensity. Also, results from duplicate samples in each area illustrated good repeatability of the method.

In conclusion, the twister is a practical method of sampling smoke in the vineyard. It is suited to rapid deployment in a bushfire situation and would provide suitable results for this type of effort. More work is required to further assess the potential of the twister for this application (including a better design for deployment, understanding the sorbents ability to retain compounds under laboratory and natural variations in weather such as temperature and rain etc.).

If, in the future, we could combine air sampling during a smoke exposure event with grape and wine analysis from the specific vineyard we could possibly develop an early warning risk assessment tool for grapegrowers who are concerned about their grapes being smoke affected.

Technical problem solving and consulting

Staff

Adrian Coulter, Geoff Cowey, Matt Holdstock, Gayle Baldock, Emma Kennedy, Con Simos and Dr Yoji Hayasaka

During the year, the name of the former Industry Services was changed to 'Winemaking and Extension Services' in order to better reflect the assistance provided to the Australian wine sector; such assistance includes a range of advisory, problem solving, extension and information services. In addition to its extension and information transfer activities, which are discussed elsewhere in this report, the Winemaking and Extension Services team provides a technical problem solving and analysis service to the Australian wine sector. The primary aim of the service is not only to provide diagnoses of the causes of various problems, but to offer preventative and remedial advice based on the cumulative problem solving and practical winemaking experience of the staff. As previously reported, it is clear that quality loss during wine processing and packaging represents a major cost to the Australian wine sector. Consequently, all the activities of the Winemaking and Extension team, in terms of problem solving, extension and information transfer, aim to address this issue in a targeted manner, such as previously exemplified by the closure trial and 'Brett' projects, and currently illustrated by our 'taints' workshop 'The avoidance of taints and chemical instabilities during winemaking'; our 'Packaging' workshop, which is currently being developed; and our involvement with research on smoke taint from bushfires.

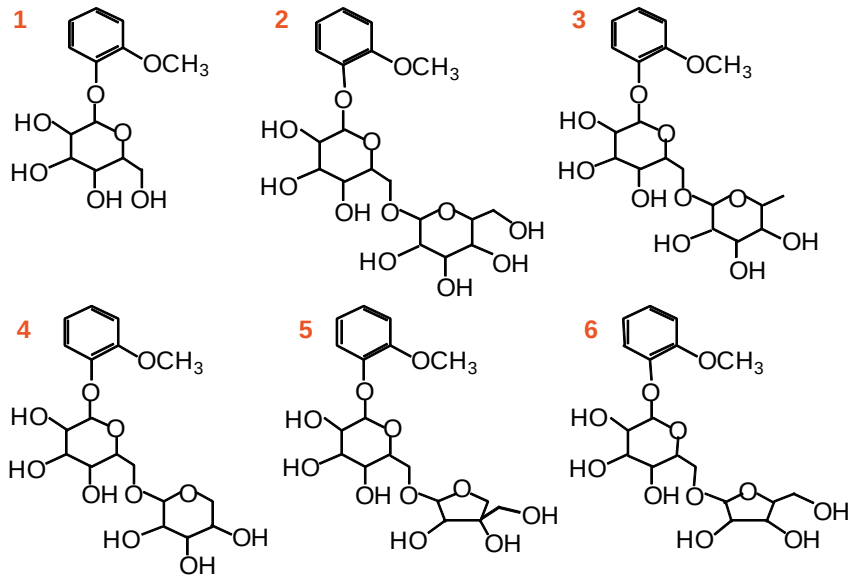

Figure 10. Proposed structures of guaiacol precursors found in the grapes and leaves directly contacted with guaiacol solution, **1:** glucoside (glu), **2:** gentibioside (glu-glu), **3:** rutinoside (glu-rhamnose), **4:** glu-xylopyranoside and – arabinopyranoside, **5:** glu-apiofuranoside, **6:** glu-arabinofuranoside

The technical problem solving service represents a significant proportion of the team's workload and is provided according to strict Terms and Conditions, and client confidentiality is an important aspect of the provision of the services. This facilitates a frank exchange of information between the AWRI and its clients, which in turn allows the maximisation of the knowledge gained from the provision of these services. If a particular problem is considered to be of interest to the wider wine sector, the results of investigative work are made available through relevant publications, and the Winemaking and Extension Services team contributes regular articles to *Technical Review*. However, under no circumstances is the name of the winery or company concerned, or any possible identifying references, ever published.

A summary of the enquiries received by Con Simos, Adrian Coulter, Geoff Cowey and Matt Holdstock for the year is shown in Table 1, with comparison figures for the previous two years.

Table 1. Enquiries received by Winemaking and Extension Services advisory staff in the period 2006/2007 to 2008/2009

	2006/2007	2007/2008	2008/2009
Wineries	1285	1244	1024
Government organisations	36	48	36
Other	369	150	160
Students	21	15	16
Total	**1711**	**1457**	**1236**

Compared with the previous year, the figures for 2008/2009 show a 15% decrease in the total number of enquiries received. The decrease has largely occurred through the 'wineries' category and may partly reflect the decline in the winegrape crush in 2009, which was about 7% lower than the previous year. Nevertheless, the number of enquiries received from wineries is significant and indicates that a large number of Australian wine producers continue to regard the AWRI as a trusted, reliable, and an important source for quality technical information and problem solving solutions.

A proportion of the investigations conducted by the team relate to disputes arising between levy-payers or between levy-payers and suppliers of either materials or contract services. Consequently, and with great sensitivity, Winemaking and Extension Services staff members often 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. Fortunately however, the majority of disputes are settled before formal court proceedings are instigated, which is of great benefit to clients.