DEPARTMENT OF
PRIMARY INDUSTRIES

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Understanding the sensitivity to timing and management options to mitigate the negative impacts of bush fire smoke on grape and wine quality – Scoping study.

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CMI Number: 101284

Project Leader: Dr Mark Krstic

Victoria
The Place To Be

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Executive Summary

Smoke from bushfires and prescribed burns of forests have over the years negatively impacted on the grape and wine quality in various regions in Australia and overseas. Large bushfires in early 2003 in north east Victoria caused considerable downgrading of fruit quality and initiated a program of R&D to investigate the issue and attempt to alleviate the effects of smoke taint. Bushfires have again impacted on north east Victoria, in 2006-07 growers were unable to salvage much of the crop leading to an estimated loss of value of wine of approximately $75-90m.

In response to industry concerns, the Department of Primary Industry was able to obtain funding from the Victorian Government for further R&D into the issue during and just after the bushfire period in 2006-07. In addition to the R&D program in north east Victoria, DPI organised and conducted a series of industry meetings utilising the resources of an R&D group from Western Australia who had also been dealing with smoke taint issues in that state. Around 130 people attended the four grower meetings held throughout Victoria, at Knoxfield, Milawa, Oxley and Nagambie, where they were presented with the most up to date information on smoke taint. The concentration of indicators of smoke taint in the grape (guaiacol and 4-methylguaiacol) was greater in the King Valley compared with the Alpine Valleys region and other regions in the state (e.g. Yarra Valley, Strathbogie Ranges, west Gippsland and Mornington Peninsula). Localities in the King Valley that were closer to the fires, and in locations where smoke naturally accumulated in higher concentrations, had higher levels of smoke taint compound in the fruit and wine.

Results from grape sampling suggested that uptake of smoke taint compounds by grapes was greatest during the immediate post-veraison period (as was suggested from WA R&D activities from smoke applied during the veraison to harvest period only) yet other sites did not show such a pattern. An early white grape, Chardonnay, and a later ripening red grape, Shiraz, showed similar patterns of uptake, but the rate of uptake of smoke taint compounds differed markedly between localities. The spatial patterns of variation in smoke taint compounds in grapes on individual vineyard blocks showed that those zones closest to the fires and the prevailing wind direction of the smoke had the highest values and there was potential for selective harvesting to separate relatively high and low smoke affected parcels of fruit. The analysis of vineyard block variation has enabled optimum sample sizes to be calculated to get accurate estimates of mean smoke taint indicator compound values across a block.

Substantial variations in the uptake of smoke taint compounds into the grapes were observed between varieties with Sangiovese > Cabernet Sauvignon > Chardonnay > Shiraz > Merlot. The conversion of grapes into wine demonstrated variations in extraction rate of smoke taint compounds between varieties. Both wine and juice aroma and taste assessments correlated well with the measurements of principle indicators of smoke taint, guaiacol and 4-methylguaiacol, in both grape juice and wine. A commercial winery provided data on various options for minimising and/or mitigating smoke taint character in wines. Chilled grapes, early press fractions, hand

harvested grapes, and charcoal fining of wine all contribute to minimising the development of smoke taint in the wine. Suggestions for further R&D to address the smoke taint issue were identified.

The aim is to continue on with this research and industry liaison effort throughout 2007/08, to continue to assist the Victorian and Australian Wine Industry in providing more insights into the management and mitigation of bushfire smoke impacts. This will involve close planning and collaboration between key researchers and industry personnel across Australia.

Contents

1. Project Background

After the Alpine bushfires in January and February 2003, the Australian Wine Research Institute (AWRI) in Adelaide, South Australia received many enquires about issues relating to the presence of 'smoke' taint in grape and wine. At this time, the industry services team at AWRI considered this problem to be the single largest problem that the team had dealt with in terms of value and number of growers and wineries affected.

The problem was initially raised by technical staff of several wine companies in Victoria and South Australia who thought that there was a problem with 'smoke' taint in fruit that they had either processed or had contracted to purchase. Initial sensory trials by AWRI concluded that there was a 'smoky' taint to wines and juices submitted to the AWRI. The AWRI's sensory panel described the characters as smoky, burnt, ash, ashtray, salami, and smoked salmon.

Further to this, the AWRI established that guaiacol and 4-methylguaiacol were the most important compounds contributing to this sensory taint. These compounds occur naturally in wines that have been matured in toasted oak products. The concentration of these compounds was strongly correlated with the presence of the 'smoky' taint. While these compounds are most likely to contribute to the 'smoky' taint, it is likely that other compounds may also be involved.

The research by AWRI showed conclusively that tainting of grapes and wine by bushfire smoke can and does occur. Tainting can have a severe economic impact. In 2003, many white wines and juices, particularly sparkling base wines, were deemed unfit for purpose and severely downgraded in terms of value. Loss of value included super premium sparkling downgraded to bulk wine, or worse to distillery grade.

The potential economic impact of prescribed burning is determined by the proportion of grapes and/or wine that is affected by smoke and ash. This is presumably determined by the factors such as proximity of grapes to prescribed burns, direction of wind at time of burning, etc. At present there are no published guidelines for growers or winemakers so they can reduce risk of smoke taint (e.g. not using grapes within 1 km of controlled burns) or to dilute smoke taint to undetectable levels (e.g. using a maximum of 10% tainted grapes with untainted grapes). No data is available to estimate lost value due to downgrading of wines (i.e. proportion of grapes affected by region in 2003).

The research was conducted between December 2006 – June 2007, during a period when bushfires in north eastern Victoria, particularly around the King Valley, were severe, and there was a need to understand more about the impacts of bushfire smoke on grape and wine quality.

2. Objectives

1. To coordinate a series of smoke taint seminars in key smoke affected wine growing regions within Victoria in collaboration with key researchers from Western Australia (Associate Professor Mark Gibberd and Dr Kerry Wilkinson, Curtin University of Technology, Margaret River, WA; Ms Kristen Kennison and Mr Glynn Ward, Department of Agriculture and Food, Western Australia) and South Australia (Mr Con Simos, Australian Wine Research Institute).
2. To coordinate a technical workshop with key smoke taint researchers and industry personnel in the King Valley.
3. To carefully review previous research and development around smoke damage on grapes and wine. In particular, the research conducted during the 2003 Alpine Valley fires in Victoria.
4. To determine the primary pathway of smoke entry into premium wine grape varieties.
5. To understand the pattern of guaiacol and 4-methylguaiacol (smoke taint indicator compounds) accumulation in grapes leading up to commercial harvest.
6. To understand the varietal sensitivity to accumulation of guaiacol and 4-methylguaiacol indicator compounds in the grapes, and their extraction and persistence in small scale wines made without oak treatments.
7. To understand the spatial variability of guaiacol and 4-methylguaiacol indicator compounds within individual vineyards and the implications this has in regards to vineyard sampling.
8. To understand the relationship between guaiacol and 4-methylguaiacol indicator compounds measured in both grapes and wine and their associated extraction efficiency.
9. To understand the relationship between levels of guaiacol and 4-methylguaiacol indicator compounds and detection of smoke taint aroma in small scale wines made without oak treatment. This includes identifying potential aroma thresholds.
10. To understand varietal sensitivity to the incidence of smoke taint aroma in small scale wines made without oak treatment.
11. To examine a number of key winemaking treatments that may be used to alleviate and/or mitigate the effects of smoke taint on wines (carbon fining, reverse osmosis, pressing intensity, hand versus machine harvesting).
12. To benchmark and cross-validate two main laboratories in South Australia (AWRI) and Victoria (commercial laboratory) with respect to their measurement accuracy of guaiacol and 4-methylguaiacol indicator compounds.
13. To develop emergency response capability within DPI to better inform the regional grape and wine industry and the Department of Sustainability and Environment (DSE) about the likely impacts of smoke taint from bushfires (wild or prescribed burning).

14. To identify opportunities for future research and development in relation to smoke damage of grapes and wine.

3. Previous work on smoke taint

3.1 Brief history of smoke taint in wine

Wines with smoky attributes have been described over many years. They have been detected in a wide range of regions in Australia (Hunter Valley, south east Western Australia, north east Victoria, southern Victoria) and in other countries (Okanagan Valley Canada, California USA and South Africa). For example, in August 2003 wildfires that encroached on Kelowna in the Okanagan Valley in Canada, affected 15 wineries and vineyards in the area. Impacted vineyards produced smoke affected wines which were observed to have a wet-ashtray taste.

In Australia, the bushfires of January-March 2003 in north east Victoria demonstrated the potential impact of broad scale damage. Many wineries were unsure on how to deal with smoke tainted fruit and much experimentation occurred during that vintage. The Australian Wine Research Institute (AWRI) provided an analytical service and some guidance on treatments to ameliorate the smoke taint. Private consultant work, e.g. Ms Jill Kuchel, and the involvement of other agencies also contributed to a greater understanding of this issue. A summary of the outcomes of that work is presented below in section 3.3.

In Western Australia, repeated issues with smoke taint in wines led to the formation of a working group comprising scientists from Curtin University and the Department of Agriculture and Food, forest managers from the Department of Environment and Conservation, and the WA Wine Industry Association. An active research program and close liaison between agencies has produced a focused effort on addressing the issue.

3.2 Economic impact

The impact of smoke taint from bushfires, and to a lesser extent controlled burns, can be substantial. Bushfires in the Pemberton region of Western Australia were estimated to cost the wine region in excess many millions in lost wine revenue from unmarketable product during 2003 and 2004 (Kennison 2005).

The cost to the Alpine Valley wine industry from the 2003 fires was estimated at $4m. The value of grapes with smoke taint in the 2006-07 fires in north east Victoria was estimated at $15-20m by the taskforce established in that area. The value of the loss of wine not produced in north east Victoria was much higher at around $75-90m.

Climate change is predicted to increase the frequency and intensity of bushfires in Australia. Forest Fire Danger Indices are expected to rise by between 15 and 70 percent by 2050 (www.csiro.au/science/ps17j.html). This scenario will reduce the window available for prescribed burns of forest to be undertaken and the risk of bushfires will be increased. Without changes to current forest fire management procedures it is likely the economic impact on the wine industry will be greater in the future.

3.3 Summary of 2003 and 2004 work

The AWRI was involved in investigating the issue of smoke taint in grapes and wine resulting from bushfires in southern NSW and north east Victoria in January and February 2003. Due to an overwhelming number of approaches by vineyards and wineries, the AWRI supported additional targeted trial work in north east Victoria. A summary of the AWRI report is available at www.awri.com.au/infoservice/current/Smoke_taint.asp

Jill Kuchel was engaged to conduct the trials and supply samples to the AWRI for testing. Given the timing of the smoke damage, which was just before harvest, most work focused on attempting to reduce the taint on grapes going into the winery and on processing methods to reduce smoke taint transfer into the wine. The trial work was supported by companies such as Brown Brothers and Gapstead Wines, the Alpine Valleys Vignerons and the Department of Primary Industries in Victoria.

The main conclusions of the initial work were:

- Juices and wines submitted to the AWRI sensory panel exhibited aroma characters such as smoky, burnt, ash, ashtray, salami, and smoked salmon. On the palate there was an excessively drying character and a retro-nasal ash character.
- Guaiacol and 4-methylguaiacol were considered the most important compounds contributing to the sensory taint, but not the only compounds responsible for the taint.
- Samples of wine from bushfire affected areas showed in excess of 70 µg/L of guaiacol. Sixty percent of the AWRI guaiacol analyses database were below 20µg/L. Samples from regions where there were no bushfires had no detectable guaiacol or 4-methylguaiacol.
- The concentrations of guaiacol and 4-methylguaiacol in juice and wine were strongly correlated with the sensory panel rating of the intensity of the taint.
- Sensory thresholds for guaiacol determined at the time were 6 µg/L in white juice and between 15 and 25 µg/L in red wine.
- Various vineyard washing treatments (cold water, cold water plus wetter, warm water, cold water plus 5% ethanol and milk) did not reduce guaiacol concentration in free run juice or macerated grapes.
- Maceration time increased the concentration of guaiacol in the juice and maceration with leaves increased guaiacol concentration further again.
- Mechanically harvested fruit was observed to have greater smoke taint than hand harvested fruit, and free-run juice from whole bunch pressing had less smoke taint than pressing fractions.
- Particulate matter (ash) washed off bunches contained little or no guaiacol.

- The most likely way to minimise the taint was to leaf pluck the vines, apply a high volume, high pressure wash in the vineyard, followed by hand picking and whole bunch pressing.
- Of a number of fining agents tested only activated carbon was found to remove guaiacol by 5% at a rate of 300 mg/L.
- Peeling grapes demonstrated that guaiacol and 4-methylguaiacol were associated with the skin but not the pulp of the grapes. Washing berries in aqueous ethanol, silicon oxide and hexane resulted in little removal of guaiacol from the berries. It was presumed that ethanol and hexane removed the wax from the berries but this was not confirmed – other solvents known to remove the wax were not tested.
- During fermentation, the concentration of guaiacol in the must increased over 3-4 days and then remained relatively stable. Free run, light and heavy pressings of fermented must all contained the same concentration of guaiacol and 4-methylguaiacol. A small amount of guaiacol and 4-methylguaiacol remained in the marc after pressing.
- Around 25-33% of total guaiacol in the grapes was extracted when grapes were crushed and macerated for 24 hours. Approximately 75% of total guaiacol was extracted from grape skins when homogenised in a 10% ethanol solution.
- Examination of a limited number of samples from reverse osmosis showed encouraging results by a diminution of measured guaiacol and less sensory assessed smoke taint. Wineries were urged to proceed with caution until more commercial applications had been conducted.
- Samples of grapes from a vineyard in December 2004 that had experienced 6-8 hours of ‘high intensity’ smoke about 3-4 weeks before veraison contained high concentrations of guaiacol and 4-methylguaiacol. The concentrations only declined marginally in the 3 months leading to harvest and the wine was rated as badly tainted.

3.4 Components of smoke

Smoke comprises visible airborne by-products of combustion and is composed of water vapour, particulates (tar, ash, carbon, and unburnt fuel fragments) and gases (CO_2, CO, N_2O, S_2O, NH_3, CH_4, NOx, ozone and other non-methane hydrocarbons). Smoke represents around 1.5-2% of the quantity of fuel burnt (Ref: Firenote, April 2006, Bushfire CRC).

Lignin appears to be the main source of the phenols of interest in smoke taint (i.e. guaiacol, 4-methylguaiacol, eugenol, etc.). Lignin comprises about 25% of the wood and itself is composed of long chains of phenol monomers. Pyrolysis, during the combustion of wood, breaks up the long chains into their smaller phenol fragments, and different burn temperatures produce a different range of products (Wittkowski *et al.* 1992).

Smoke generated from woods of different plant species have varying concentrations of the components of smoke. Thus, different forest types are likely to produce different chemicals in the smoke and hence different transfer compounds to the grapes. It has not been determined whether this makes any perceptible difference to the sensory characteristics of wines.

Measurement

The Western Australia working group have measured smoke intensity with a particulate matter device which captures particle sizes down to 10 μm (PM_{10}). These devices are expensive and commonly used as a dust tracking device. They are less useful for measuring smoke density because the particle size of many smoke related compounds is less than 10 μm. At a meeting in April 2007 with wine industry members at Knoxfield, Victoria it was suggested that other devices could be used and reference was made to an ionising type device (similar to that in smoke detectors) which could be developed relatively cheaply.

Using a PM_{10} device, a reading of 50 $\mu g/m^3$ is regarded as hazardous and readings of the smoke used in experiments by the WA group were around 200 $\mu g/m^3$.

Some weather stations record visibility over distance and smoke haze and these can be useful if smoke is the only contributing factor.

3.5 Use of guaiacol and 4-methylguaiacol as indicators of smoke taint

Guaiacol and 4-methylguaiacol have been identified as being associated with smoky characteristics in wine. They have more commonly been measured in relation to toasted oak wine barrels, which release these and other compounds into the wine. Guaiacol and 4-methylguaiacol are formed in oak barrels during the toasting process and these compounds are absorbed into wine during barrel maturation. A combination of their high solubility and low aroma threshold (see p12) contribute to them being most obvious in grapes and wine. The two compounds are now regarded as indicators of smoke character in grapes and wine, but a range of other contributing compounds have also been identified.

Guaiacol (2-methoxyphenol) has aromas described as smoky, phenolic, aromatic, burnt, and burnt bacon (cited in Pollnitz *et al*. 2004). The compound 4-methylguaiacol has smoky, leather, and spicy aromas (cited in Pollnitz *et al*. 2004 and others). The relationship between guaiacol and 4-methylguaiacol may be of interest since individually they have different aroma and taste profiles and this may influence overall perceived aroma or taste. All the Victorian derived measurements for 2006-07 in the report below only included guaiacol and 4-methylguaiacol. Wine samples from a range of grape varieties and localities have been provided to the University of NSW (Professor Mark Adams – Bushfire CRC) to measure other smoke related components that may be in the wine. Results are not available at this stage and will be reported next year.

3.6 Extraction methods

Whole grapes have been commonly used to measure the two main smoke taint compounds, guaiacol and 4-methylguaiacol. Whole berries (skin, pulp and seeds) were ground up and measurement presented on a unit weight basis. Where grapes were sent from Phylloxera Infested Zones the grapes were frozen for quarantine purposes and this procedure could alter the extraction of compounds from the berries compared to non-frozen samples, as has been found when measuring organic acids and potassium.

Free-run juice may include a small contribution of smoke taint compounds from the skin, as the free-run juice produces relatively low concentrations compared to whole berries. The concentrations in free-run juice are potentially influenced by the degree of maceration of the skins, pressing fraction and the period of time skins spend in contact with the juice. In 2003, the concentration of smoke taint compounds in juice from macerated grapes was shown to increase in a linear fashion up to about four days on contact with skins.

The concentration of smoke taint compounds observed in wine will be influenced by many factors such as processing temperature, press fraction, period on skins, and fining agents used. These aspects need to be considered when comparing wine results.

Solubility

No previous research has investigated the solubility of smoke taint compounds in juice or wine. In related work, the solubility of smoke related compounds was greater in a model wine solution than in water (Barrera-Garcia *et al.* 2006). Barrera-Garcia *et al.* (2003) attribute this to the effect of the alcohol increasing the solubility. This difference may have some impact when comparing measurements between grapes and juice (water solution) and wine (alcohol and water solution) given that the high solubility of many aroma compounds in alcohol reduces their volatility.

Of the compounds of interest in smoke affected grapes, guaiacol has the highest solubility (17.9 g/L in model wine at 25ºC), then, in decreasing order, 4-methylguaiacol (10.4 g/L), 4-ethylphenol (5.9 g/L), 4-ethylguaiacol (4.2 g/L) and eugenol (2.1 g/L) (Barrera-Garcia *et al.* 2006). Thus, the measurement of guaiacol and 4-methylguaiacol captures the two compounds that are most likely to be absorbed into grapes and wine.

Measurement

The extraction method used by the main provider of analytical services, the Australian Wine Research Institute (AWRI), only collects free guaiacol and 4-methylguaiacol. There may well be further amounts of the compounds of interest bound to glycosides that may be released as free conjugates over time or under particular conditions.

Pollnitz *et al.* (2004) examined the extraction and measurement of guaiacol, 4-methylguaiacol and other volatile oak compounds and concluded that artefacts in measurement may occur resulting in the observation of up to 10 times the level of guaiacol. These artefacts were particularly obvious with diethyl ether extraction and gas chromatography injector block temperatures above 225˚C. Data from previously published determinations for guaiacol should be examined carefully with respect to sample preparation and analysis.

Testing of the same 16 wine samples was conducted at two laboratories, the AWRI and a commercial independent laboratory in Victoria. In the range between 10 and 550 µg/L, the results showed a close relationship between the data from both laboratories (r^2 = 0.997) but it was not a 1:1 relationship. The ratio of the commercial laboratory readings to those of the AWRI was 1.47:1. With 4-methylguaiacol, the relationship was also very close (r^2 = 0.995) but again the ratio was 1.4:1 with the Victorian independent laboratory showing relatively higher readings. Similar techniques were used, i.e. liquid-liquid extraction with pentane and pentane:diethyl ether (2:1), and measurement by gas chromatography. Both techniques take into account potential issues with inaccurate guaiacol extraction and measurement identified by Pollnitz *et al*. (2004).

Further investigation is required to determine the reasons for the different results observed between the two laboratories and to validate whether the relationship holds with samples of less than 10 µg/L. Grape samples should also be compared between the laboratories particularly at the lower end of the concentration spectrum, i.e. less than 10 µg/kg guaiacol, since this is the critical range in which grape samples may be rejected by wineries.

Detection limits

Most of the analyses reported in this report were conducted by the AWRI. The level of detection in wine was 1.0 µg/L with an uncertainty of ±1.0 µg/L or ±10% (whichever was greater). The commercial laboratory in Victoria also had a minimum detection limit of 1.0 µg/L with an uncertainty of ±1.0 µg/L or ±10% (whichever was greater).

3.7 Impact of smoke taint

Sensory

In the literature, various descriptors have been applied to smoke tainted grapes and wine. These include smoky, burnt, earthy, burnt toast, smoked salmon, tobacco, beetroot, drying, ash, cigar box, truffle, charcoal, charred, cold ash, fungal, tar, ashtray, bacon, roast meat, leather, salami, coffee, chocolate and gamey. In general, they are regarded as negative characteristics but some characteristics come through in low concentrations from toasted barrels and are regarded as providing some complexity to the finished wine.

Grapes

The smoke taint characters are less easily detected through sensory analysis of grapes. The high concentration of sugars may mask many of the compounds and the concentrations in the berry are generally lower than in the resultant wine. This indicates that taint compounds may be present in conjugated forms within the grape.

Wine

The sensory components that have been ascribed to smoke tainted wines (see above) are primarily aroma and retronasal sensed characters. The taste characters are described as bitter, astringent and drying.

Thresholds

Guaiacol

The threshold concentrations of compounds at which the aroma or taste can be perceived is of great interest. If a compound has a low aroma/taste threshold then it only requires a small amount of that compound to be present for it to be detected organolepticly.

The aroma and taste threshold for guaiacol has previously been evaluated in wines. Simpson *et al.* (1986) determined the aroma threshold in a neutral dry white wine to be 20 µg/L. Other estimates are 21 µg/L in water (Wasserman 1966) and 30 µg/L in water:alcohol (Riboulet 1982 – cited in Simpson *et al.* 1986). Boidron *et al.* (1988) obtained aroma thresholds of 5.5 µg/L in water, 20 µg/L in a model wine, 95 µg/L in a Ugni blanc wine and 75 µg/L in a Merlot red wine. These odour thresholds are higher than that determined by the AWRI in white wine but some are similar to that determined in red wine (6 µg/L in white juice and between 15 and 25 µg/L in red wine).

Wine matured in oak typically contains between 10 and 100 µg/L of guaiacol. However, guaiacol above 80 µg/L in wine has been found to have a negative effect on wine aroma (cited in Pollnitz *et al.* 2004).

Guth (1997) used a water+alcohol (90+10 %v/v) mix to assess the threshold of guaiacol where samples were taken into the mouth and odour assessed retronasally. The threshold was determined as 10 µg/L.

The taste threshold for guaiacol in water has been determined as 13 µg/L (Wasserman 1966). However, 90% of the panel reported a loss of taste when their noses were pinched closed and the author concluded that there was a significant retronasal component of 'taste'.

Guaiacol is a recognised taint of apple and other juices, being produced by bacterial contamination (e.g. *Alicyclobacillus acidoterrestris*). Sensory studies have determined the aroma and taste thresholds of guaiacol in water and apple juice (Table 1). Pre-trained panels can detect a lower threshold than un-trained panels and juice results appear much lower than for those observed in wine previously.

The detection threshold for guaiacol varies markedly between panellists, up to 80-fold with aroma and 470-fold with taste. A similar wide range of detection thresholds for smoke taint aroma of up to 245-fold were demonstrated in the WA project wine tastings (Kennison pers. comm. 2007). Such broad ranges of detection thresholds need to be considered when tasting panels are developed.

Table 1. Thresholds for guaiacol determined in apple juice, and water.

Medium	Best Estimate Threshold (µg/L)	Range (µg/L)	Reference
Aroma – water	0.48[1]	0.06 to 4.71	Eisele and Semon (2005)
Aroma – apple juice	0.91[1]	0.17 to 4.71	Eisele and Semon (2005)
Aroma – apple juice	2.23	1.84 to 2.92	Orr *et al.* (2000)[2]
Aroma – juice	2	-	Pettipher *et al.* (1997)[2]
Aroma – apple juice	0.57[1]	CV[3] 15.8%	Siegmund and Pollinger-Zierler (2006)
Taste – water	0.17[1]	0.01 to 4.71	Eisele and Semon (2005)
Taste – apple juice	0.24[1]	0.01 to 4.71	Eisele and Semon (2005)

[1] Panel pre-trained to recognise guaiacol
[2] Cited in Eisele and Semon (2005)
[3] Coefficient of Variation

4-methylguaiacol

The aroma profile of 4-methylguaiacol has been described as burning wood, smoky, leather, spicy and ash. Boidron *et al.* (1988) reported a threshold of 10 µg/L in water, 30 µg/L in a model wine and 65 µg/L in both Ugni blanc and Merlot wines. In wines matured in oak, the concentration of 4-methylguaiacol ranged from 1 to 20 µg/L (Pollnitz *et al.* 2004).

Wasserman (1966) also determined aroma and taste thresholds of 4-methylguaiacol in water being 90 µg/L and 65 µg/L respectively. Wittkowski *et al.* (1992) showed that the perceived aroma of 4-methylguaiacol in water changed with concentration, exhibiting musty aromas at 10 µg/L and smoky and caramel like aromas at 100 µg/L.

Other compounds

Other components from smoke affected grapes that may be transferred into wine have also been studied. These include:

- 4-ethylguaiacol - elicits odours described as smoky, clove, spice and bacon. The aroma threshold of this compound has been determined as 25 µg/L in water, 47 µg/L in a model wine, 70 µg/L in a white wine and 150 µg/L in red wine (Boidron *et al.* 1988). A threshold of 33 µg/L in a model wine is reported in Francis and Newton (2005).

- 4-ethylphenol – shows medicinal, phenolic, horse stable, horse urine, ink, band-aid and pungent descriptors, and a threshold of 130 µg/L in water, 440 µg/L in a model wine, 1,100 µg/L in white wine and 1,200 µg/L in red wine (Boidron *et al.* 1988).

- 2,6-dimethoxyphenol - in water it has an aroma threshold of 1,850 µg/L and a taste threshold of 1,650 µg/L (Wasserman 1966).

- Eugenol - has an aroma likeness with cloves. Thresholds of 7 µg/L in water, 15 µg/L in a model wine, 100 µg/L in white wine and 500 µg/L in red wine have been determined (Boidron *et al.* 1988). Thresholds of 5 and 6 µg/L in a model wine were reported in Francis and Newton (2005).

- Furfural - has been described as an almond aroma. Thresholds of 8,000 µg/L in water, 15,000 µg/L in a model wine , 65,000 µg/L in white wine and 20,000 µg/L in red wine have been determined (Boidron *et al.* 1988).

The compounds 4-ethylguaiacol and 4-ethylphenol are commonly associated with the ‘band-aid’, ‘medicinal’, ‘wet dog’ type aromas produced by *Brettanomyces/Dekkera* spp. yeasts.

In summary:

- Trained panels may recognise guaiacol at lower thresholds than un-trained panels.
- The taste thresholds for guaiacol, 4-methylguaiacol and 2,6-dimethoxyphenol are lower than the aroma thresholds.
- The taste threshold may have a significant retronasal component.
- The guaiacol threshold in water is lower than the threshold in apple juice or wine.
- There is a large range in detection thresholds between sensory panellists.
- Perceived aromas of single compounds may change depending on the concentration.
- The recognition threshold (correctly identifying the compound) is greater than the detection threshold (e.g. 2µg/L compared to 0.57 µg/L for guaiacol – Siegmund and Pollinger-Zierler 2006)
- Thresholds have been determined in different ways so it is difficult to compare one with another.

Aroma and taste thresholds described here are for individual compounds and there is good evidence that when these compounds are present in mixtures with other compounds, they can be detected when present below these thresholds. Compounds above threshold concentrations can mask the detection of other compounds (Francis and Newton 2005).

<u>Anecdotal reports from winemakers</u>

In a dry white wine, a level of 2-3 µg/L of guaiacol seems to reflect the threshold for detection of smoke taint. At 6 µg/L guaiacol, almost anyone could smell and taste the ‘smoke’ related effect. The conclusion they drew was that guaiacol was generally a good indicator of smoke taint.

4. Summary of regional seminars in Victoria and the 2006-07 fire situation

A series of regional seminars were conducted in Victoria in April 2007. Presentations were made by a group of researchers from Western Australia who are working in a cooperative project with several agencies, and an update on the Victorian work was also delivered. The Knoxfield seminar attracted 50 attendees, Oxley 34 and Nagambie 19. In addition to the regional seminars a technical group meeting was held at Milawa. This was attended by 26 industry and agency personnel. A summary of the presentations and the feedback from industry is presented below.

4.1 Ongoing work being conducted in WA

The research group from Western Australia who attended the meetings in Victoria included Assoc. Prof. Mark Gibberd and Dr Kerry Wilkinson from Curtin University of Technology, and Ms Kristen Kennison and Mr Glynn Ward from the Department of Agriculture and Food.

Smoke taint in grapes and wine, as a consequence of grapevine exposure to smoke compounds, has resulted in financial losses and decline in product quality on numerous occasions for several wine producers throughout Western Australia (WA), Australia and the world. Wine grape regions within WA, such as Manjimup and Pemberton, have increased susceptibility to smoke taint damage due to their proximity to forested areas. Indiscriminate bushfire events have created smoke taint damage to grapevines on several occasions and have affected wine grapes grown in the Perth Hills, Swan Districts, Blackwood Valley, Great Southern, Margaret River, Manjimup and Pemberton regions. Incidence of bushfire generated smoke taint is rising as vineyards are increasingly planted in bushfire susceptible regions.

The Department of Agriculture and Food, WA (DAFWA) in conjunction with Curtin University of Technology, Margaret River, initiated research to investigate the issue of smoke taint in grapes in late 2005. Research was supported through funding by the Grape and Wine Research and Development Corporation (GWRDC). The key objectives of the smoke taint research program in WA were:

- Identify and isolate the specific volatile organic compounds in smoke that produce sensory smoke taint characteristics in grapes and wine by use of quantitative chemical analysis.
- Identify possible key periods during grapevine growth and development when smoke may be of negative effect producing ‘smoky’ aromas in wine.
- Identify grapevine mechanisms of smoke compound assimilation, translocation and storage within organelles with an emphasis on grape berries.
- Determine the effect of smoke applied at various phenological stages of grapevine growth and development.
- Determine the effect of variation in smoke concentration and exposure durations on grapevines.
- Evaluate seasonal and yearly effects of smoke exposure on grapevine growth, function and development.
- Investigate possible field based amelioration and protective treatments.

Several experiments have been established to investigate these objectives.

Smoke effects on grape bunches and organoleptic perceptions in wine

Research was initiated to demonstrate the effect smoke exposure of grapes had on the chemical composition and sensory characteristics of wine in early 2006. Post harvest wine grape bunches were utilized in this experiment and exposed to smoke in purpose built smoking facilities at Kings Park and Botanic Gardens, Perth (WA). Two wine treatments were made from smoked and un-smoked grapes (1) free-run juice treatment (2) free-run juice on skins treatment. Quantitative chemical analysis of wines for guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, eugenol, furfural, 5-methylfurfural and vanillin has been conducted by the Australian Wine Research Institute (AWRI). Sensory wine analysis of difference tests and best estimate thresholds were also performed. Wines vinified from smoked grapes exhibited ‘smoky’, ‘dirt’, ‘earthy’, ‘burnt’ and ‘smoked meat’ aroma characters.

Effect of smoke exposure and duration on field-grown grapevines and subsequent wine quality

Experiments performed in early 2006 focused on the field application of smoke to grapevines from the period of veraison to harvest. Smoke was applied in both single and successive field applications to grapevines to measure the chemical composition and sensory characteristics of resultant wines. Prior to this experiment, smoke had not been applied in a field situation to grapevines to measure the effects on chemical compounds in grapes and wine. Furthermore, the effect of timing and duration of smoke exposure to grapevines had not been reported with concentration of associated smoke compounds in wines unknown.

Field based treatments applied smoke to Merlot vines for (1) successive smoke applications applied to the same vines two times per week from veraison to harvest and (2) single smoke application applied to previously un-smoked vines two times per week from veraison to harvest. In each treatment, smoke was applied at veraison then at 3, 7, 10, 15, 18, 21 and 24 days post veraison. Quantitative chemical analysis of wines for guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, furfural, eugenol, vanillin and 5-methylfurfural was conducted by the AWRI. Sensory analysis of wine with a trained sensory panel is currently in progress.

Smoke application to grapevines at various phenological stages of growth and development

Field based applications of smoke were applied to separate Merlot grapevine plots at key stages of grapevine growth and development including leaves 10cm, flowering, berries pea sized, beginning of bunch closure, beginning of veraison, berries full colour and at harvest. Smoke was applied to grapevines for 30 min per day on two successive days. Sensory and quantitative chemical analysis of field experiment wines will be conducted in 2007.

Effect of smoke timing and duration on grape berries

A rapid model for the application of smoke to grape berries has been developed for future experimentation. One such experiment initiated in

2006-07 growing season investigates the influence of smoke on waxed versus un-waxed grape berries. Smoke was applied to both waxed and un-waxed berries at two times prior to veraison and two times post veraison. Smoke treatments were applied to berries for durations for 5, 10, 20, 40 and 80 minutes. Quantitative chemical analysis of samples from this trial is currently being performed by AWRI.

Further research

Further investigation into the issue of smoke derived taint in grapes and wine currently seeks to determine:

1. Effect of smoke application apparatus on grapevine leaf physiology;
2. Investigate possible vineyard based and winemaking amelioration treatments and protective techniques that can be employed when smoke is imminent;
3. Grapevine mechanisms of smoke assimilation and translocation within and among organelles; and
4. Analysis of various fuels from native forests and their impact on grapes and wine.

Smoke Taint Working Group

The working group is an initiative of the Wine Industry Associations of WA (WIAWA) to improve the liaison between the wine industry and the forest managers (Department of Environment and Conservation, DEC) to reduce the risk of losses from smoke taint in grapes and wine and foster R&D. Their objective is to better integrate DEC's prescribed burn program with viticulture. The group is currently developing a vineyard registry and completing a "vineyard mapping project"; developing risk based approaches to minimise clashes between prescribed burning and grape development and harvest, and supporting ongoing research into the effect of smoke on grapevines and wine.

4.2 Summary of work in Victoria

Mr Stephen Lowe, Project Officer for King Valley Vignerons, provided a summary of the bushfires in the north east of Victoria. The Great Divide Fires burnt from 1st December 2006 to 7th February 2007 (69 days – longest period for a fire in Australia) and burnt about 1.05 million ha (Figure 1). Whilst only 5ha of vineyard was burnt the impact of the fires was far greater by reducing cellar door visitations, cutting deliveries due to road closures, smoke contamination of grapes leading to rejection and downgrading, and many wineries electing not to make 2007 vintage wines.

Visibility due to smoke was reduced to hazardous levels on 17 mornings during the two months of December and January and Stephen Lowe presented a series of slides demonstrating the visual impact of the smoke. Stephen Lowe assisted many wineries with sampling to determine the impact of smoke compounds in grapes and was involved in DPI's research program (the results are described in later sections of this report).

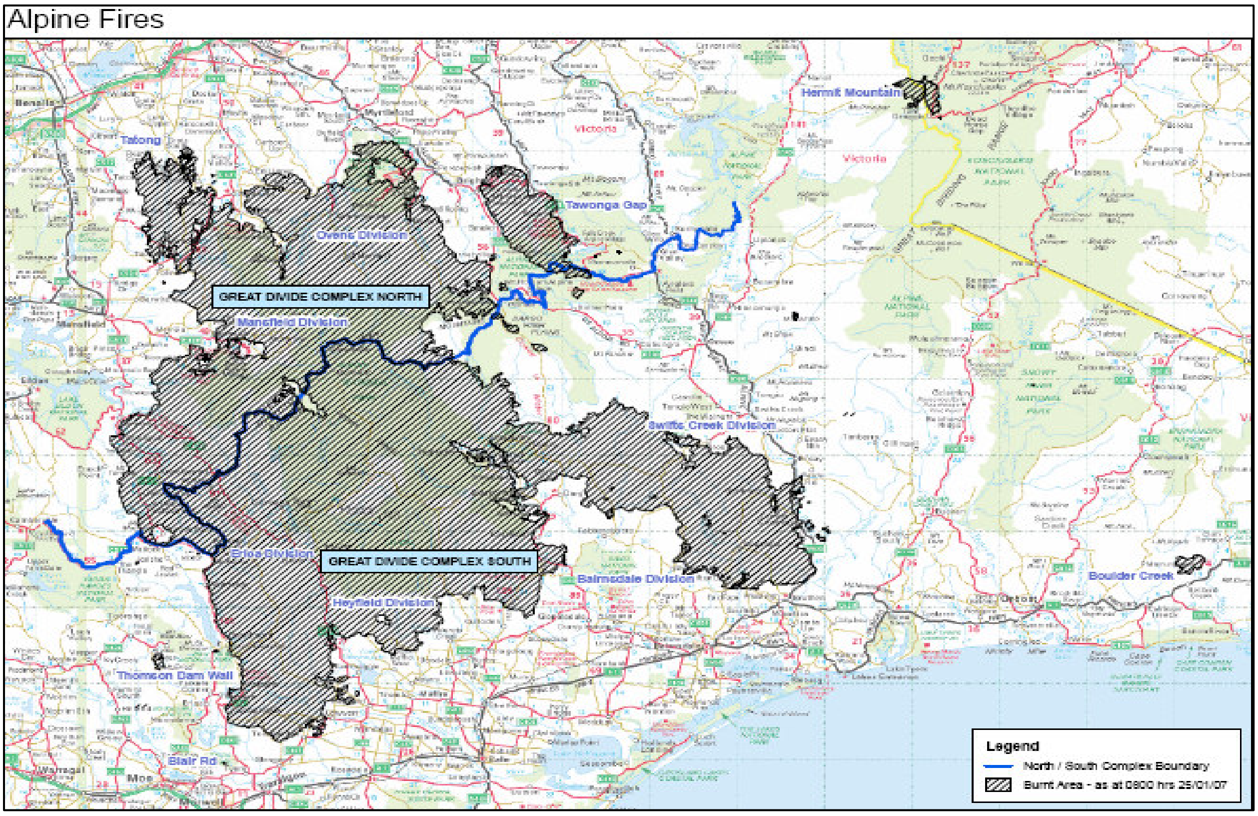


Figure 1. Map of the area burnt by the 2006-07 Great Divide Fire.

Dr Mark Krstic described the project work he had been supervising. The funding initially came from DPI and extra resources were secured from Regional Development Victoria and the Department of Sustainability and Environment. Aspects covered in the Victorian work included:

- Translocation of guaiacol from leaves and shoots to grapes.
- Changes in absorption of key smoke taint indicators during berry growth.
- Spatial variability within vineyards.
- Variation in smoke taint uptake between grape varieties.
- Transfer of smoke characteristics from grapes to wine.
- Progress towards identifying the back palate ash/drying sensation in smoke tainted wines.
- Considering and evaluating some potential management options in the vineyard and winery.

Progress with these studies are described later in this report.

Technical Group meeting – Milawa.

Mr John G. Brown presented an introduction to the smoke taint issue by describing the local impact and steps taken to seek assistance. He estimated around 15,000t was involved with an estimated loss of value of $15-20m for Brown Brothers Wines alone. As the potential impact of the bushfires in early

December 2006 became evident, the local regional associations instigated the formation of a task force.

Concerns about loss of markets for grapes and the image of the region were at the forefront of concerns. A consultant was engaged to prepare a communication plan which included such things as key messages for wineries to use when asked for comment about the issue. For example, the use of the term ‘smoke affected’ rather than ‘smoke taint’, and making it an industry issue rather than a north east Victoria issue. This approach was successful in providing consistent messages to the media and produced a measured response from the media.

The task force, through John Brown, also established links with State and Federal parliamentarians to provide them with information on the situation and to push the case for assistance. Regional Development Victoria and DPI committed funding across various projects in the region. The task force also instigated meetings between growers and banking/financial groups and a session with Beyond Blue which was well attended.

The Western Australia working group presented their information (as described above) to the meeting. Some additional comments were made with respect to smoke taint being an issue with other crops such as hops, and maybe olives and other processed foods. Also the issue of carbon credits was raised and how to deal with the trend towards considering carbon credits in food manufacture. With respect to forest burning, a low heat reduction burn is likely to produce far less carbon emissions than a wildfire.

Stephen Lowe and Dr Mark Krstic presented information from the Victorian based research (described later). Wendy Cameron from Brown Brothers Wines, summarised their experiences with processing smoke tainted grapes. They compared hand picking and machine harvesting, pressing fractions, processing grapes at different temperatures and fining agents. The results are detailed later in this report.

David Wollan described his experiences with reverse osmosis of juice and wine using the Memstar technology. Reverse osmosis of juice does not work satisfactorily. With wine about 7% of the guaiacol is removed with each pass through the machine, and 2,000 L of wine takes about one hour to reduce the guaiacol concentration by 50%. Further details are provided later in this report.

<u>Anecdotal comments from industry meetings:</u>

A number of comments from winemakers and growers were collated from the meetings. They included:

- In a sample of Pinot noir grapes there was no taint evident in grapes. After a 3 day soak on skins the smoke aroma was high, then it disappeared during the initial stages of fermentation. Taint was not detected during pressing but evident in wine two weeks later.
- In 2003, the Okanagan Valley, Canada, had smoke affected wines. A wine with 100 µg/L guaiacol tasted less smoky than Australian wines. Perhaps due to the different forest fuels and the higher moisture (lower

combustion temperatures) in Canada compared to Australia, releasing different smoke taint compounds.

- Some vineyards with spot fires in them had very little guaiacol and 4-methylguaiacol detected in the grapes. However during fermentation a meaty, smoky character came through.
- Juice from red grapes had greater smoke character than juice from white grapes.
- Concern that as fruit characters in the wine diminish over time in storage that the smoke characters will become more evident in the wine.
- Reports of up to 10 μg/kg in grapes but no smoke taint observed in the wines at an early stage.
- Reports of large differences in guaiacol readings between blocks on the one vineyard.
- $CuSO_4$ fining has taken out the smoke aroma but not the taste.
- Milk, particularly full cream milk, was suggested as taking out some of the smoke taint.

4.3 The fire situation in north east Victoria 2006-07

Two periods of intense smoke and low visibility were created by the bushfires, from the 3rd to 20th December and 7th to 15th January (Figure 3). The first period coincided with the post-flowering period for the vines in the valleys and late flowering-early set for the vines on elevated sites. The second period of smoke coincided with late veraison for early varieties (Chardonnay, Pinot gris) but prior to veraison for late varieties (Cabernet Sauvignon).

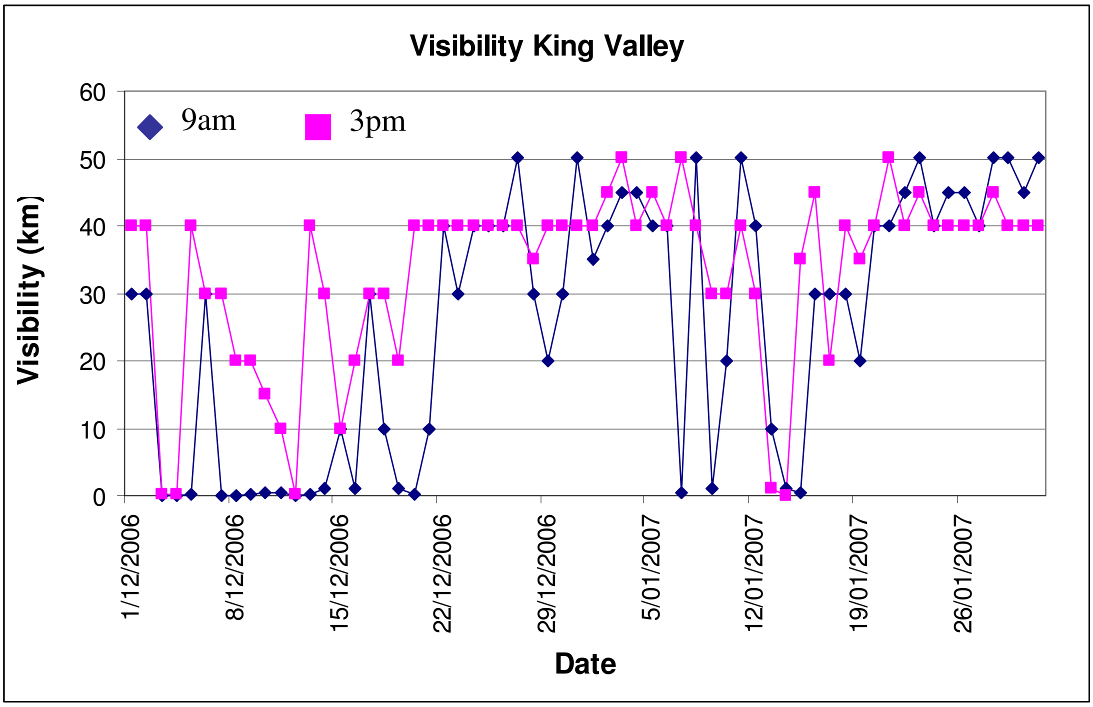


Figure 2. Visibility in the King Valley during the 2006-07 bushfires assessed from the meteorological station at Edi Upper.

Generally the morning visibility was less than the afternoon visibility due to smoke settling at night time and the wind movement picking up through the day to disperse the smoke. Visibility below 10 km is regarded as unhealthy for all people (21 mornings over the two months) and at less than 1 km the smoke is hazardous (17 mornings over the two months). Figures 4 and 5 illustrate the smoke generated from the fires.

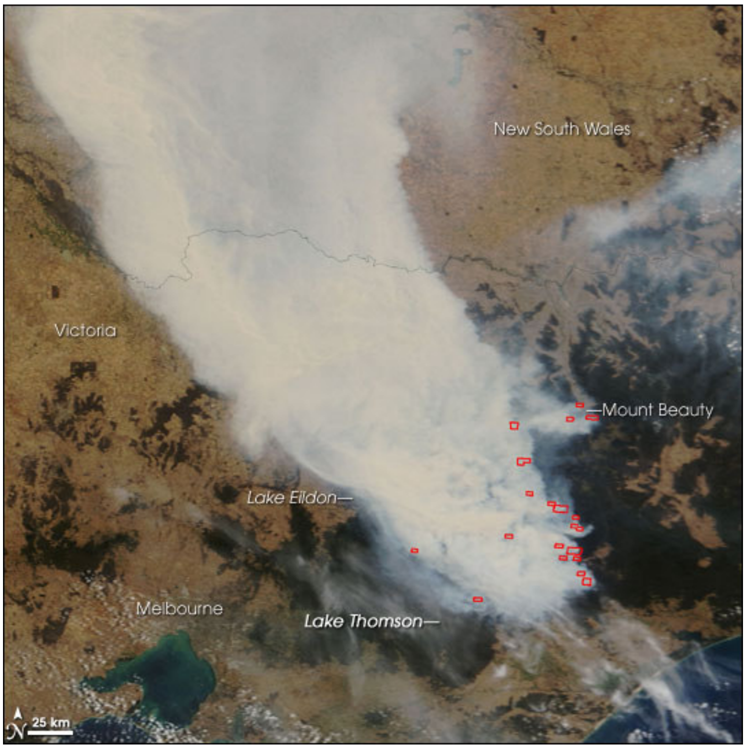


Figure 3. Satellite image of smoke from Great Divide fire 2006-07. (http://earthobservatory.nasa.gov/NaturalHazards/natural_hazards_v2.php3?img_id=14024)

Figure 4. Smoke plume from the Tatong fire – 13 January 2007 (Source: Department of Sustainability and Environment)

5. Entry of smoke into the vine and accumulation

5.1 Entry into grapes

The deliberate smoking of grapes with a smoker at Kings Park, Perth (WA), using straw and the subsequent crushing and making of wine has demonstrated that guaiacol, 4-methylguaiacol and other smoke related compounds can be transferred to the fruit and subsequently to wine (Kennison pers. comm. 2007). The descriptors used for these wines were smoky, earthy, dirt, burnt and smoked meat, which are typical of bushfire affected wines.

Whilst the Kings Park work demonstrated that smoke taint compounds can be transferred to bunches of grapes and end up in wine, the process used did not determine where the compounds were located in (or on) the bunches. They may have been on or in the stems, on the surface of the berries, or in the berries either in the cuticle, skin, pulp or seeds.

Work with AWRI in 2003 showed that washing grapes with a range of aqueous solutions did not remove smoke taint, the washings contained little or no guaiacol and washed grapes (measured in macerated juice or wine) contained just as much guaiacol as un-washed grapes. Also a range of solvents did not extract much guaiacol from the surface of the berry. It was assumed some of those solvents removed the waxy cuticle but it was never confirmed by microscopy or analysis of residues from the evaporated solvents. Further work is required to clearly elucidate the location of smoke taint compounds in grape berries.

5.2 Entry through leaves and translocation

<u>Entry through the leaf</u>

External feeding of smoke taint compounds to leaves alone and an analysis of the grapes appears not to have been done to assess if smoke taint compounds can be absorbed by leaves and translocated to the fruit. An understanding of this potential mechanism could help with treatments to ameliorate the impact of smoke taint.

Possible modes of entry of smoke related compounds into a leaf include diffusion through the upper cuticle and palisade cells, diffusion through the lower cuticle and hypodermis into spongy mesophyll cells or air spaces, or diffusion into the air spaces through the stomata. Wound sites may also be potential entry pathways for smoke related compounds.

<u>Translocation from leaf and shoot to fruit</u>

A short-term experiment was conducted in 2007 to assess whether guaiacol could be transported within the vine and into the grapes through the vascular pathway via the leaves and/or shoots. Field grown Pinot Noir vines were used in this experiment. The trial took place in the Coal Valley wine region, Tasmania, as this region had experienced little impact by smoke from bushfires. Two treatments were applied (1% and 2% aqueous guaiacol; Sigma-

Aldrich, Australia) and replicated six times on single vine plots. The solutions were stored at 4ºC in bottles wrapped in aluminium foil to ensure that guaiacol standard did not degrade as it is believed to be light sensitive.

Each vine was fed the guaiacol through three leaf flaps and three decapitated shoots. The leaves and shoots were cut under water to ensure no embolisms (air blockages) or plugging of phloem sieve plates would occur. A flap of leaf was cut using a scalpel in a section parallel to the main vein. The leaf flaps were 5mm wide and 60mm long and inserted into a 10ml centrifuge tube also covered in foil. A 50ml falcon tube was used to insert the cut end of a shoot into the guaiacol solution. The leaves on the shoots where guaiacol was applied were removed. The vials were hung in the vine or on trellis wires and held by twist-ties.

Uptake of guaiacol was rapid, and within 16 hours (the following morning), the vials of the shoot applied samples were half empty, compared to the leaf treatments where uptake was only about 1 mL. The vials were topped up every three days over a two week period to ensure the leaf flaps and shoots remained in contact with the guaiacol. A limitation to the study was the uncertain length of time the leaf flaps were in contact with the solution, as many were out of the tubes by the time the grapes were harvested two weeks later. All fruit from the treated shoots and some additional fruit on the same vine were collected and pooled per vine. Untreated grapes were collected from nearby vines in a different vine row.

The harvested grapes were washed in water, frozen at -18ºC for 24 hours and transported under permit to the AWRI for analysis of guaiacol and 4-methylguaiacol. No 4-methylguaiacol was detected in the grapes. Substantial uptake of guaiacol was detected in the grapes of the 1% and 2% treatments (Figure 5), far higher than seen with samples from smoke affected regions. Whilst bunches were not covered to exclude the possibility of guaiacol vapour reaching the berries, it is highly unlikely the concentrations seen in the berries could have come from guaiacol vapour alone.

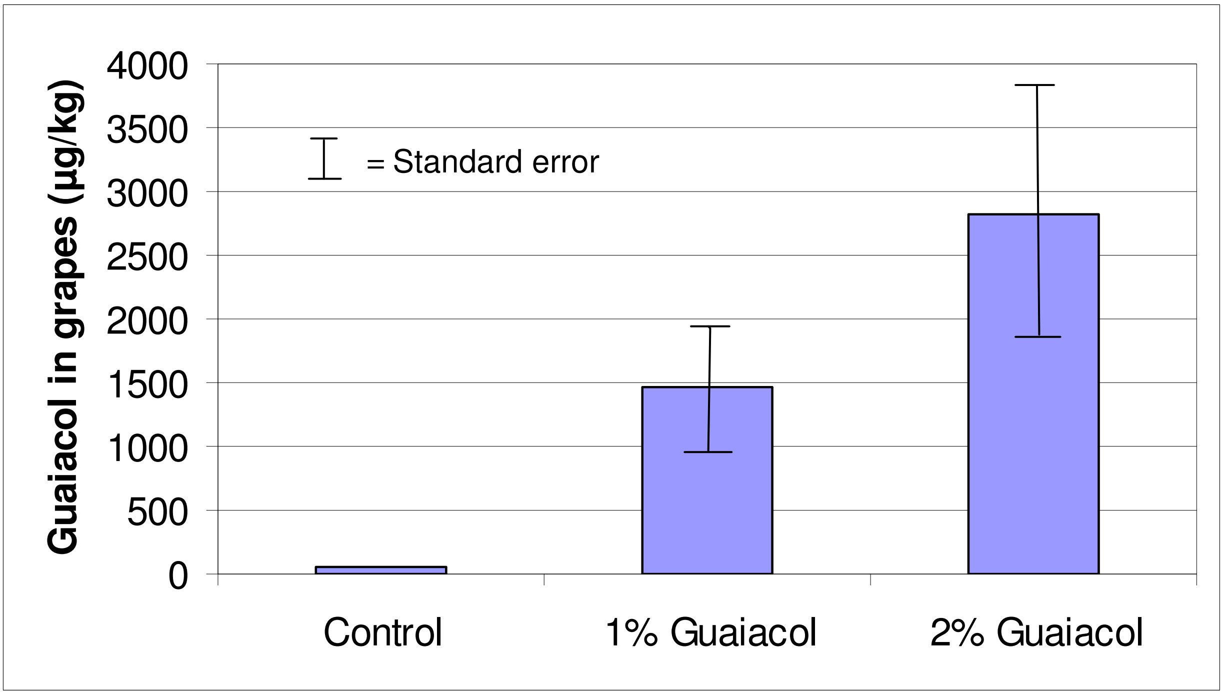


Figure 5. Guaiacol content in grapes after feeding shoots and leaves with 1% and 2 % guaiacol, compared with adjacent un-fed vines.

The untreated vines showed an unexpected level of guaiacol content (49 µg/kg) equivalent to levels reported in grape samples from smoke affected vineyards in north east Victoria. However no 4-methylguaiacol was detected. Thus, it is unlikely that smoke contributed to the results. Data from north east Victoria would have predicted a concentration of around 15 µg/kg of 4-methylguaiacol if smoke had been responsible for that level of guaiacol concentration in the untreated vines. It was noted that the guaiacol was quite volatile and a smoky aroma pervaded the area where the trial was conducted. There were some bushfire events in Tasmania and local domestic wood burning fires cannot be excluded from contributing to the result in the control treatment.

There remain some unanswered questions from this research regarding the mechanism of guaiacol uptake into the grapes. The results presented here do not distinguish between leaf fed or shoot fed guaiacol treatments because the grapes from the separately fed leaves and shoots were pooled. This preliminary study has demonstrated that guaiacol can be absorbed into cut shoots and/or cut leaf flaps and translocated to bunches. There remains a need to distinguish between guaiacol being absorbed from the exterior of the leaf through the lamina and directly into the cut ends of the lamina or conducting tissue in the veins.

5.3 Entry through the root system

Given that guaiacol is water soluble, smoke related compounds that are washed onto the soil by rainfall or irrigation could potentially be absorbed by the roots and translocated to the berries. This mechanism for guaiacol uptake has yet to be tested. However, entry via this pathway would be inhibited by the casparian strip in the roots.

5.4 Timing of accumulation into grapes

WA work

Progressive smoking and sampling of Merlot vines from veraison has demonstrated a period just after veraison as the most sensitive to the accumulation of smoke related compounds into wine. The highest concentration of guaiacol observed in the wine occurred in vines that were smoked seven days after veraison (an apparent period of high sensitivity to smoke absorption). Thereafter the guaiacol concentration in the resultant wine dropped away to less than half the maximum value when vines were smoked up to 24 days after veraison.

The compounds 4-methylguaiacol and 4-ethylphenol also peaked in the seven days post veraison smoking treatment but concentrations were generally 15-20% of the observed guaiacol concentration. Furfural concentration in wine was relatively consistent irrespective of time of smoking of the grapes and 4-ethylguaiacol was present in very low concentrations in the wine made from smoke treatments. It should be noted that Merlot has a comparatively lower uptake of smoke taint compared to other varieties (see section 7.2) and this trial work should be repeated on other varieties.

DPI (Victoria) work

Grapes were sampled from vineyards in north east Victoria (King and Alpine Valleys) at particular stages to determine changes in guaiacol and 4-methylguaiacol concentration from just after fruit set to harvest. Both Chardonnay and Shiraz grapes were sampled. 30 bunch samples were collected from the same block at three different times in the season. Grapes were frozen prior to extraction and measurement of guaiacol and 4-methylguaiacol conducted by the AWRI. The main smoke periods were from 3rd to 20th December 2006 and 7th to 15th January 2007 (Figure 2).

In the region with high smoke intensity from fires (King Valley), samples were collected on 20 December 2006 just at the end of the first period of smoke. Berries were green and hard (peppercorn to pea size – E-L stages 29-31). The second sampling was conducted on 20 January 2007 just after the end of the second period of smoke. Berries were beginning to soften and change colour (veraison – E-L stages 34-35). The third sampling was conducted on 17 February 2007 when the grapes were at intermediate sugar values before ripeness (E-L 36-37) and after the fires had been put out. Only the guaiacol concentration is reported here, as the 4-methylguaiacol concentration in grapes tended to follow similar patterns.

Most of the increase, averaged across eight sites for each variety, occurred in the period after veraison with both Shiraz and Chardonnay (Figure 6). The variability of the data is high because values at some sites went up substantially, yet at other sites values increased by much lower amounts (see section 7.1). Given that the major smoke periods were prior to the January sample period it is interesting to see the largest increase after the second sample date. The second sample date coincided with veraison and the WA research indicated that the period just after veraison was when the grapes were most susceptible to uptake of smoke taint compounds.

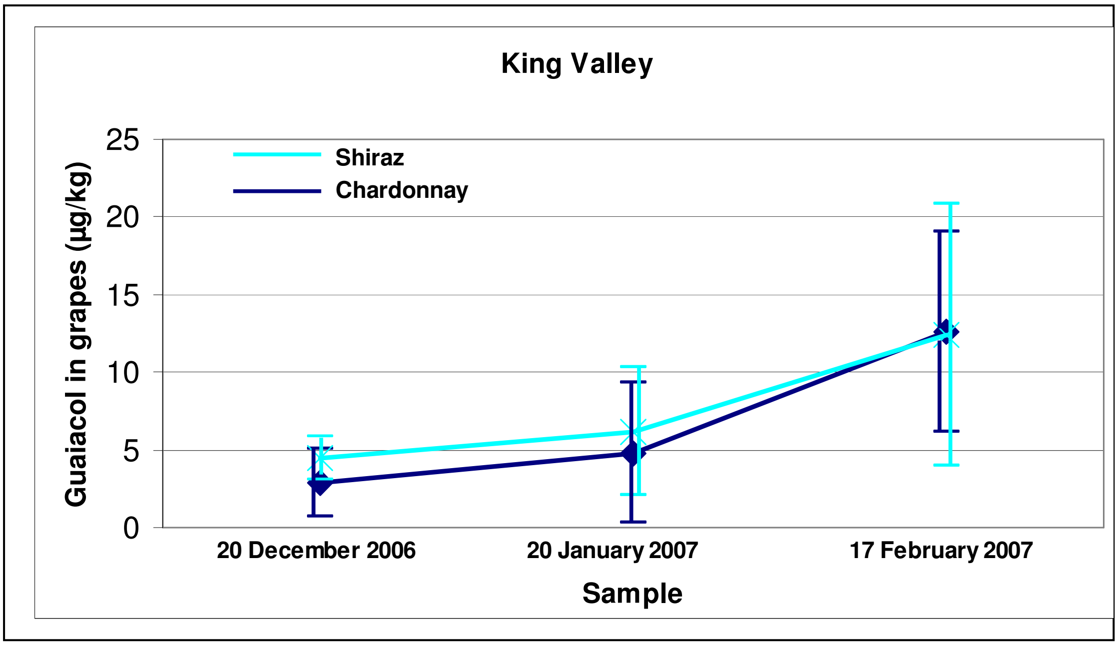


Figure 6. Mean guaiacol concentrations in grapes sampled from Shiraz and Chardonnay vines in the King Valley at three sample dates (± standard deviation).

A second group of vineyards in the Alpine Valleys, further away from the fire location, were sampled twice for guaiacol and 4-methylguaiacol in the grapes. The first sample date was 26 January 2007 which coincided with veraison (E-L stage 34-35). The second sample date was 23 February 2007 when the grapes were at intermediate sugar levels pre-harvest (E-L 36-37). Again only the guaiacol results were reported here as the 4-methylguaiacol results followed similar patterns.

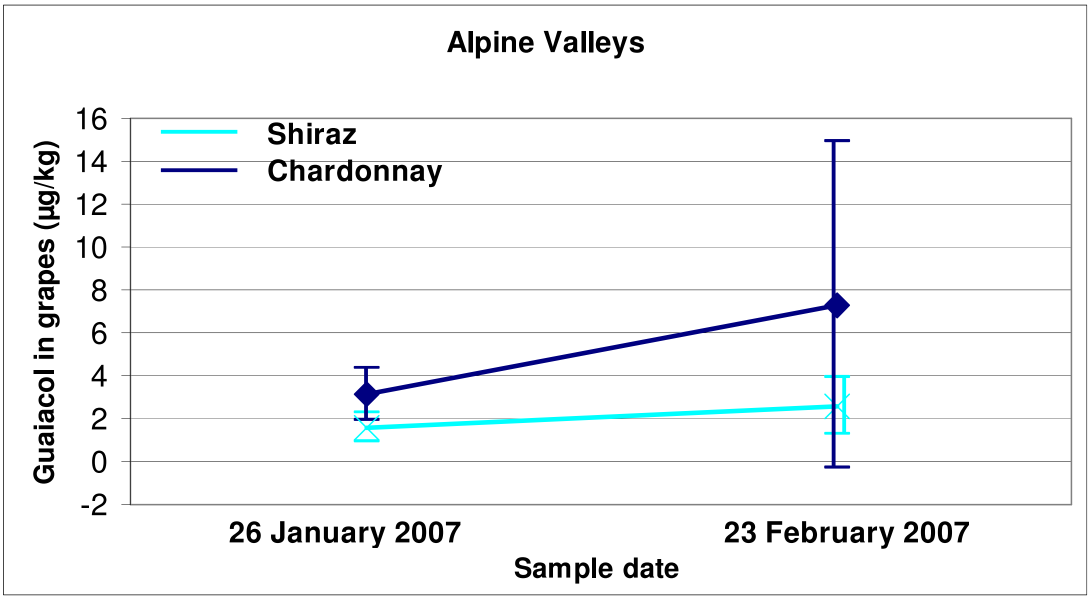


Figure 7. Mean guaiacol concentrations in grapes sampled from Shiraz and Chardonnay vines in the Alpine Valleys at two sample dates (± standard deviation).

The average grape guaiacol values were lower in the Alpine Valleys than the area closer to the fires. The Shiraz grapes showed only a slight increase between veraison and pre-harvest, whilst the average guaiacol concentration in Chardonnay grapes showed an increase of around 130% (Figure 7). The increase for Chardonnay was largely influenced by one site where the guaiacol concentration rose from 5 µg/kg to 22 µg/kg, well above the rise observed in the other sites. Removing the high value from the calculations showed the Chardonnay rose by a similar amount as the Shiraz.

The sampling of three vineyards to determine vine-to-vine variability (see section 6.2) did not necessarily show the same response described above. A Chardonnay block which went through veraison on 31 December 2006 (after the first period of smoke but just prior to the second period of smoke) and was sampled on 19 February 2007 (after the second period of smoke) had relatively low levels of guaiacol in the grapes (mean 7 µg/kg). A block of Pinot Gris which went through veraison on 20 January (at the end of the second period of smoke) and was sampled on 14 February had relatively high concentrations of guaiacol (mean 61 µg/kg). A block of Cabernet Sauvignon which went through veraison on 7 February (well after the second smoke period) and was sampled on 2 March also had a quite high concentration of guaiacol in the grapes (mean 45 µg/kg).

Thus, the Chardonnay block where post-veraison coincided with a smoke period had relatively low guaiacol and a Cabernet Sauvignon block where veraison occurred well after the smoke period had relatively high guaiacol concentrations. The results for these three sites are contrary to the results across a broader range of sites and also the Merlot results from WA. This perhaps indicated that there are varietal (genetic) or other differences influencing the phonological stage when uptake of smoke taint compounds is greatest.

Anecdotal reports

In the Yarra Valley, one producer with an earlier ripening first crop of grapes had moderate concentrations of guaiacol and 4-methylguaiacol, but in vines that had been frosted and had produced later ripening second crop, these compounds were not detected. Similar views were expressed by others in the region, apart from a second crop from an early ripening variety (Pinot Noir) which was detected with a smoke taint.

5.5 Rate of absorption into/adsorption onto grapes

No research was found assessing the absorption of smoke related compounds into grapes or wine. Limited research has been conducted on the sorption of phenolic compounds from a model wine into pieces of oak wood (Barrera-Garcia *et al*. 2006). In that work the authors suggested a two stage sorption process of i) a fast initial sorption onto active sites of the wood surface within the first 26 hours; and ii) a slower diffusion process between the second and eighth day. The sorption equilibrium across the phenolic compounds studied was around 6-7 days.

In addition, the authors described a two step sorption process into the wood dependant on the concentration of the phenolic compound in the model wine (Barrera-Garcia *et al*. 2006). These authors suggested up to a particular concentration (10 mg/kg) in the model wine, the aroma compounds were sorbed linearly without competition via weak interactions with the wood surface. Above the 10 mg/kg concentration, a polymerisation process was likely. Whilst these results were specific to wood, similar experiments should be conducted to assess sorption rates into or onto grapes.

6. Spatial variability and impacts on sampling

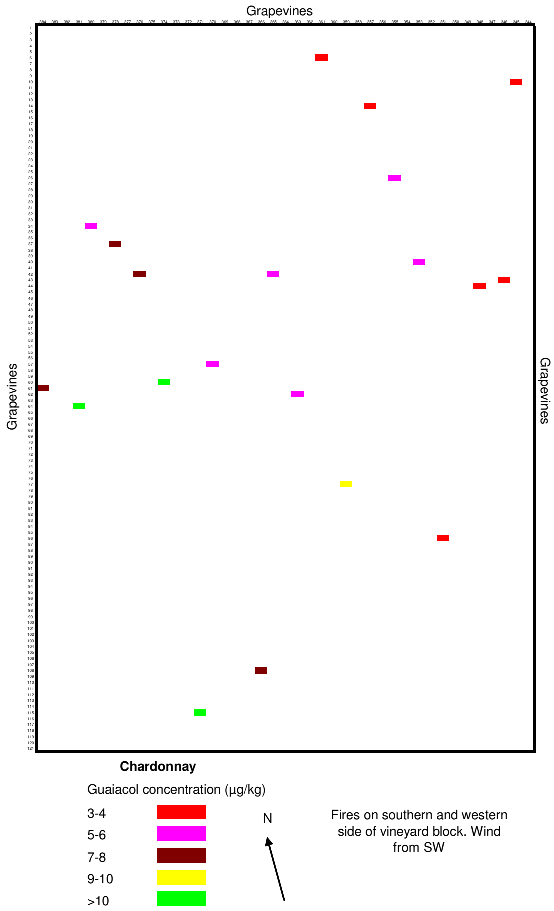
6.1 Spatial variability within vineyard blocks

The data collected to determine variability within vineyard blocks (see section 6.2) was plotted over a vineyard plan to determine whether there were any spatial patterns relating to the guaiacol readings. Figure 8 (a, b & c) show concentration ranges across the vineyard blocks (plans not to scale).

In Figure 8a, the average guaiacol reading across the whole Chardonnay block was 7 µg/kg and the grapes were rejected for a vintage wine in 2007. This was based on a cut-off value for guaiacol of 6 µg/kg used in the region for rejecting smoke affected grapes. Spatial analysis of sample results reveal that the average guaiacol reading was largely influenced by three high readings nearer the source of the smoke on the western/south western corner. If the section of the block with the highest readings was excluded from harvesting then the remaining section of the block would have had an average guaiacol concentration of 5.3 µg/kg and potentially be considered for a vintage wine.

In Figures 8 b and c the mean guaiacol readings are well above limits set locally and differential harvesting could not be considered.

Figure 8 (a). Spatial distribution of guaiacol concentration in grapes in a Chardonnay vineyard block. Fires were on the southern and western boundaries of the block. Wind was from south-west.



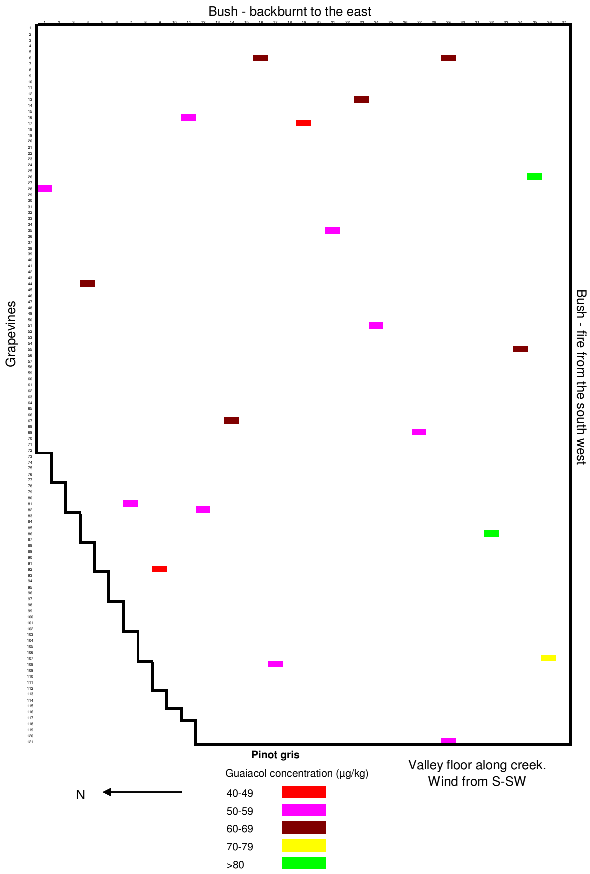


Figure 8 (b). Spatial distribution of guaiacol concentration in grapes in a Pinot Gris vineyard block. Fires were on the southern and eastern side of the block. The western edge is in a valley floor along a creek. Wind from south to south-west.

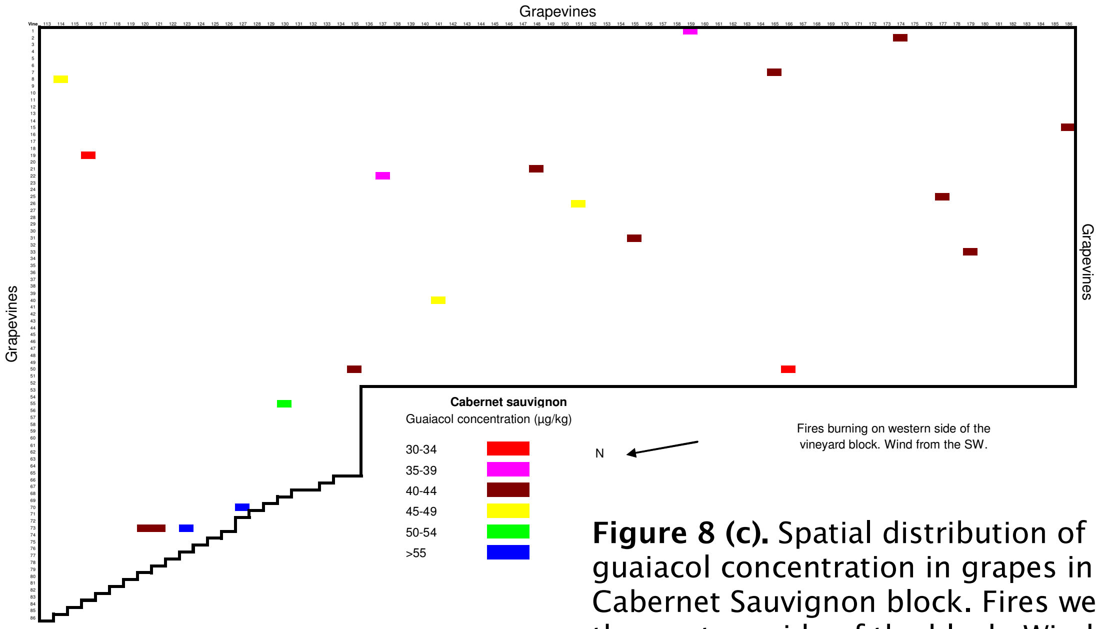


Figure 8 (c). Spatial distribution of guaiacol concentration in grapes in a Cabernet Sauvignon block. Fires were on the western side of the block. Wind from the south-west.

In general, vines closest to the fire source and smoke from the prevailing winds had the highest concentrations of guaiacol. Similar trends were observed with 4-methylguaiacol (data not presented). The spatial variation suggests differential harvesting may be of value in separating grapes with higher guaiacol (and presumably other smoke taint related compounds) from grapes with less 'smoke taint' where the average is near the level of rejection.

6.2 Sampling variability

In order to collect samples for routine testing, an estimate of the variability across a block of vines is useful for determining the optimum sample number per vineyard block. Three sites were sampled to estimate the variability. Twenty vines were sampled at random and sufficient bunches collected for around 200 g of grapes for each sample. These were frozen at -18ºC and transferred under permit to the AWRI for guaiacol and 4-methylguaiacol analysis. Results for guaiacol show differences between sites with the Chardonnay site having much lower guaiacol values than the other two sites (Table 2). The variability (expressed as % coefficient of variation) differed across the three sites and was greatest in the Chardonnay site and least in the Pinot Gris site. Hence in lightly smoke affected sites, sampling may need to be more comprehensive to cover potentially higher variability.

Table 2. Guaiacol analysis in grapes from twenty samples at three sites showing highest and lowest readings, the mean and variability (highest, lowest values).

	Guaiacol in grapes (µg/kg)		
Sample number	**Site 1 Pinot Gris**	**Site 2 Chardonnay**	**Site 3 Cabernet Sauvignon**
1	55.8	3	48
2	63.1	3	31
3	56.8	4	40
4	48.2	3	44
5	52.9	5	71
6	55.1	5	76
7	62.7	4	53
8	61.3	9	43
9	58.1	4	36
10	48.4	6	47
11	55.3	5	43
12	60.5	7	45
13	58.2	5	41
14	56.6	18	39
15	66.2	13	40
16	55.1	8	31
17	81.6	8	44
18	66.8	5	41
19	86.5	17	41
20	74.4	7	41
Mean	61.2	7	45
Standard Deviation	9.9	4	11
% Coefficient of Variation	16.3	62.5	24.8

The coefficient of variation data can be used to calculate optimum sample size. For Site 1, five samples would have been sufficient to determine the mean guaiacol value at a level of confidence of 95% and a tolerance of doubt of 15%. At a doubt of 10% the number of samples required is 12. Similar calculations for Sites 2 and 3 generate sample sizes of 74 and 12 for 15% doubt and 168 and 27 for 10% doubt. Usually 15% doubt is used so sufficient samples were collected at Sites 1 and 3 to get a good estimate of the mean guaiacol value, but at Site 2, more samples needed to be collected to give an accurate estimate of the mean value.

With Site 2 (Chardonnay), fires burnt up close to the vineyard and a few vines with relatively high guaiacol readings were detected. If the three high values for guaiacol are removed from the assessment of variability, as may be the case where remotely generated smoke covers a vineyard, then the coefficient of variation is reduced to 35%. At this level of variability, 24 samples would be needed to determine an accurate mean guaiacol value across the block.

Table 3. 4-methylguaiacol analysis in grapes from twenty samples at three sites showing highest and lowest readings, the mean and variability (■ highest, ■ lowest).

	4-methylguaiacol in grapes (µg/kg)		
Sample number	**Site 1 Pinot Gris**	**Site 2 Chardonnay**	**Site 3 Cabernet Sauvignon**
1	11.7	1	10
2	14.6	1	10
3	13	1	12
4	11.7	1	13
5	12.3	2	12
6	12.7	1	13
7	13.9	1	14
8	13.1	3	13
9	12.6	1	13
10	11.3	2	13
11	12	2	14
12	13.1	3	14
13	12.9	2	15
14	14.8	7	16
15	14.8	6	17
16	12.7	3	16
17	17.6	2	16
18	15	2	16
19	17.9	6	21
20	17	3	23
Mean	13.7	3	15
Standard Deviation	1.9	2	3
% Coefficient of Variation	14.2	73	20

The coefficient of variation for 4-methylguaiacol was slightly lower than guaiacol on two sites but higher on the site where the values were very low and close to the limits of detection (Table 3). Thus the number of samples calculated for estimating the mean guaiacol concentration in the grapes (at a level of confidence of 95% and a tolerance of doubt of 15%) for Sites 1 and 3 would have been sufficient to get an 'accurate' estimate of mean 4-methylguaiacol concentration. At Site 2, to get an equivalent estimate of the mean 4-methylguaiacol, 103 samples would need to be collected.

In summary, more research is required to determine the optimal sampling regime for grapes in smoke affected regions, based on a better understanding of the inherent variability. This is particularly important in vineyards where low and near winery rejection levels (tentatively set at somewhere between 2-6 µg/kg at present depending on variety and wine style) of guaiacol and 4-methylguaiacol are expected. However, in the interim, in vineyard blocks where the degree of smoke taint is suspected as being low and close to any pre-determined winery rejection value for guaiacol, more intensive sampling is suggested, i.e. a minimum of 50 sample sites at random across the whole vineyard is suggested.

7. Regional and varietal observations

7.1 Regional variability

Sampling of sites over consecutive months gave an insight into variations between sites over time for two grape varieties. With Shiraz and Chardonnay in the King Valley the concentration of guaiacol appeared to decrease between some sample times and increase between others (Figures 9 and 10 respectively). Sites 4, 6 and 8 showed distinct increases between the second and third sample times with both Shiraz and Chardonnay. Sites 1 and 2 showed the least increase (even a slight decrease) for both Shiraz and Chardonnay. Sites 3 and 7 showed distinct increases in guaiacol concentration in the grapes between the second and third sampling for Chardonnay but not for Shiraz.

These results are related to proximity to the fires (intensity of smoke) to a large extent. Sites 1 and 2 are out on the broad plain of the King Valley to the north of the fires. The remaining 6 sites were in the narrower valleys of the King Valley wine region. Sites 4, 6 and 8 for Shiraz and Sites 3,4,6,7 and 8 for the Chardonnay had fires burn in close proximity to the vineyard boundaries. Sites 3, 5 and 7 for the Shiraz and Site 5 for Chardonnay were elevated sites away from the valley floor with no fires burning close by.

The variability between locations in the King Valley was high and the primary influence was proximity to bushfires and the generated smoke. Some limited data from the Yarra Valley region across all varieties tested, where no bushfires burned close to vineyards and prescribed burning contributed some smoke, showed the variability between localities within the region was low.

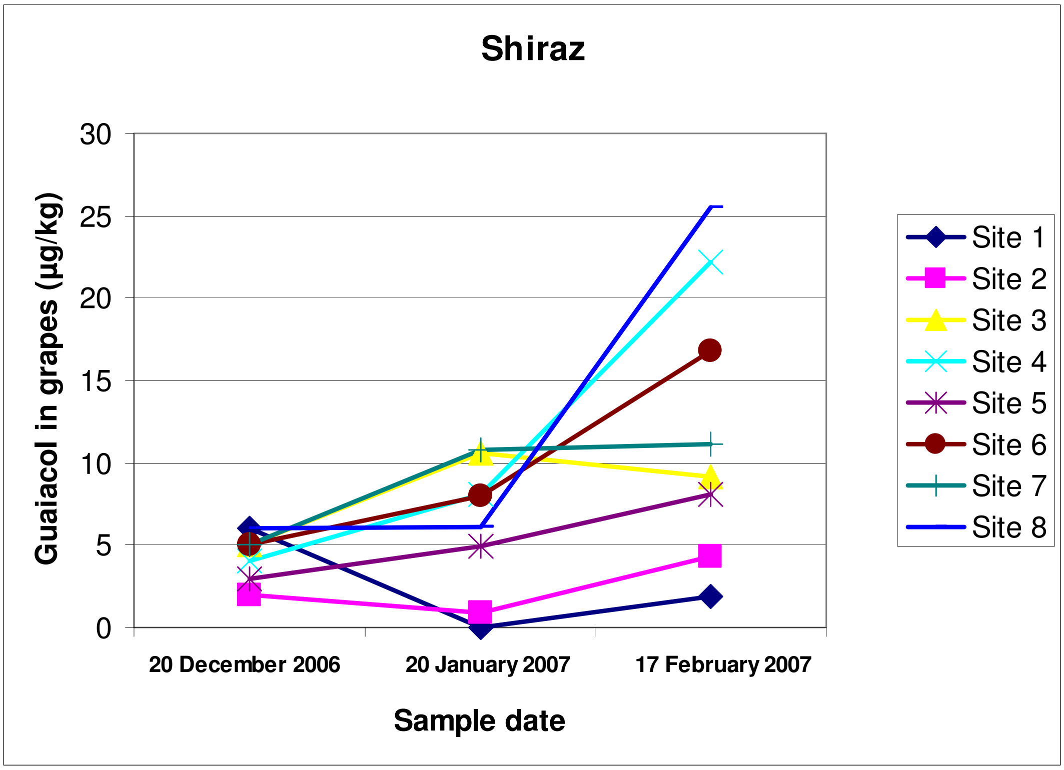


Figure 9. Guaiacol concentration in grapes at three sample times for Shiraz over eight sites in the King Valley.

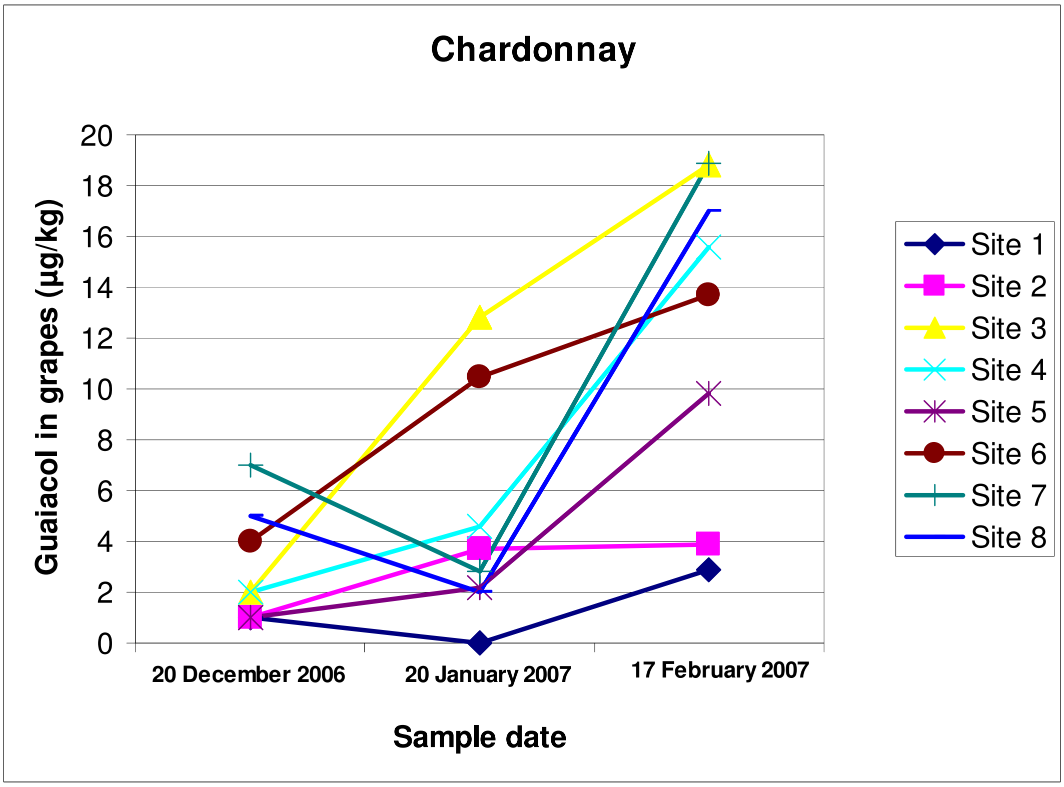


Figure 10. Guaiacol concentration in grapes at three sample times for Chardonnay over eight sites in the King Valley.

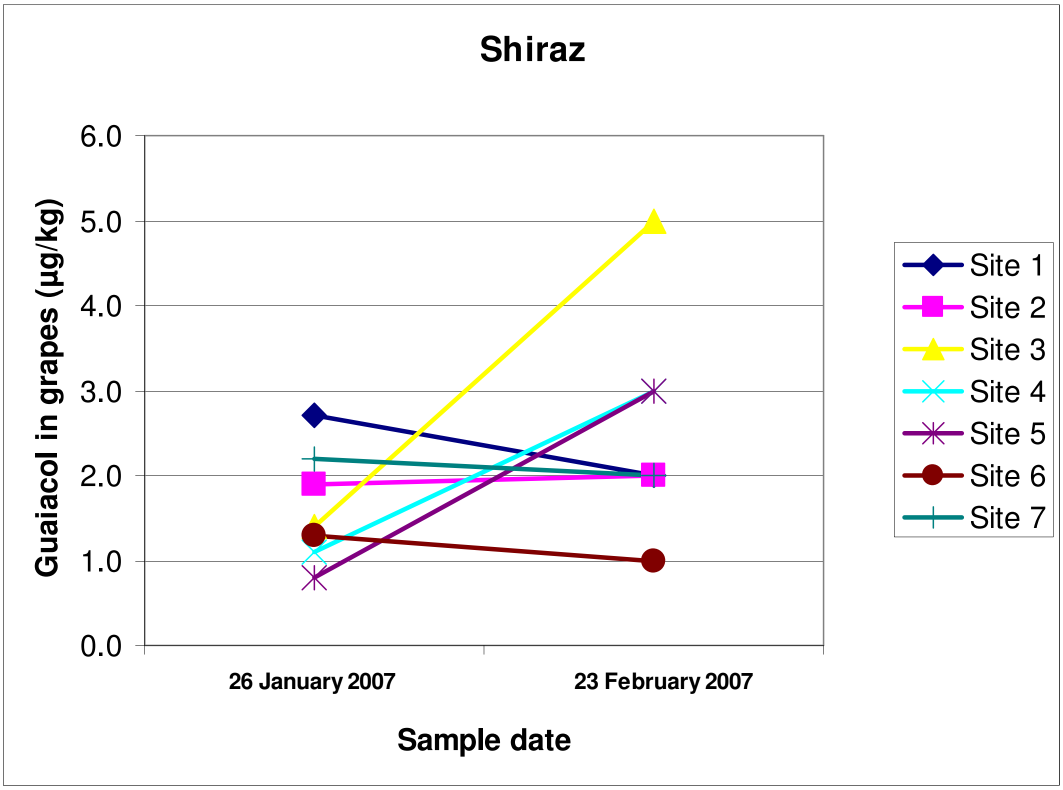


Figure 11. Guaiacol concentration in grapes at two sample times for Shiraz over seven sites in the Alpine Valleys.

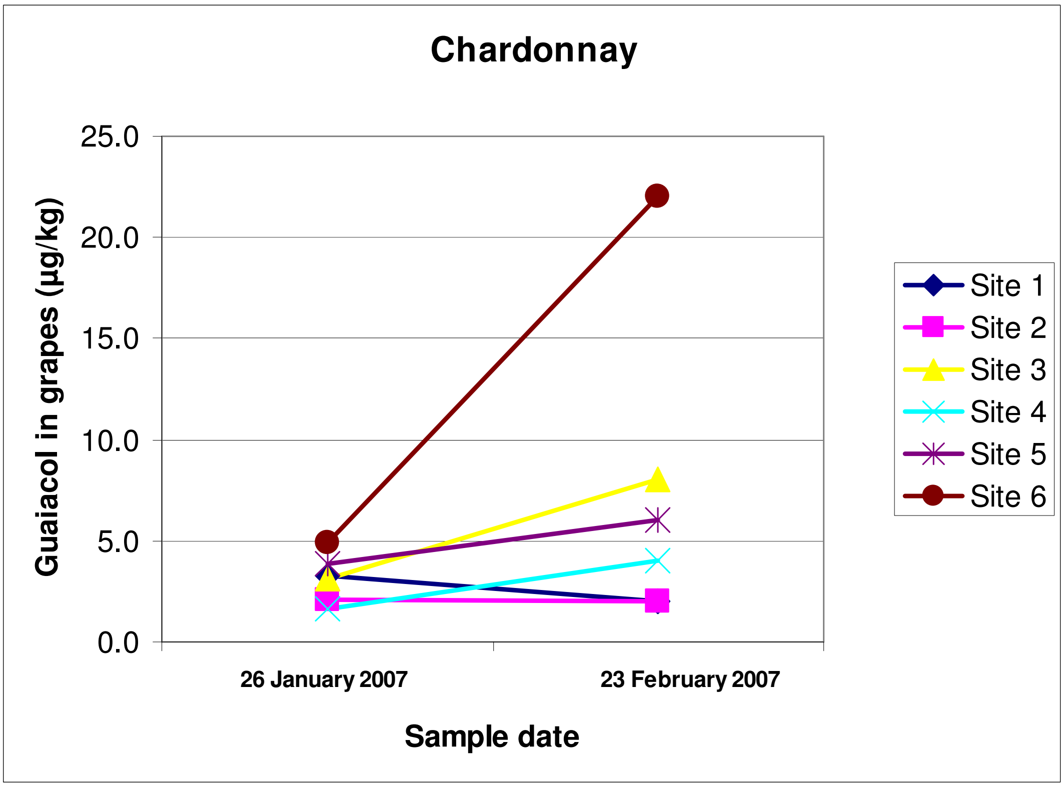


Figure 12. Guaiacol concentration in grapes at two sample times for Chardonnay over six sites in the Alpine Valleys.

In the adjacent Alpine Valleys, guaiacol concentration in the grapes was lower than in the King Valley (Figures 11 and 12 respectively). Sites 3, 4 and 5 in the Shiraz and Site 3 and 5 in the Chardonnay were closer to the fires than the other sites. Site 6 in the Chardonnay had fires along the vineyard boundary.

Limited data from a Victorian laboratory suggested far greater differences between regions than within regions. In general, across samples of all varieties, grape guaiacol concentration results were in order: north east Victoria >> east Gippsland > Strathbogie Ranges > Yarra Valley > Central Victorian High Country > west Gippsland = Mornington Peninsula. The results do not necessarily suggest all grapes in these regions were affected by smoke as samples may well have been submitted from vineyard owners who had particular concerns for diverse reasons.

Potential influence of different forest types

There is potential for different regions or localities to have different forest types and hence a different array of smoke taint compounds (see section 3.5). Only guaiacol and 4-methylguaiacol were measured in grape samples from the 2006-07 fires in Victoria, but if different forest types were involved at different locations, then a difference in the ratio of guaiacol to 4-methylguaiacol may have been evident. In the limited data available there were no differences in the ratio across a broad range of sites over a distance of around 50km.

However, in Merlot wine, the ratios of guaiacol to 4-methylguaiacol arising from the bushfires in Victoria from two sets of data were 2.3±1.5 (±standard deviation; n=10) and 2.5±0.1 (n=3), whereas where straw has been used as a fuel in Western Australia in controlled studies on Merlot the ratio was estimated to be around 5.6±1 (n=8) (Kennison pers. comm. 2007). This may be due to fuel type or differences in burn temperatures (pyrolysis) creating different smoke taint products. Thus, different sources of smoke or burn temperatures may influence the relative concentrations of smoke related compounds in grapes and wine, and requires further investigation under controlled conditions.

7.2 Varietal differences

Anecdotal information gathered at the industry meetings suggested that there was variation in smoke taint between grape varieties. Varieties classed as having high susceptibility by industry were Pinot Noir, Sangiovese and Cabernet Sauvignon, and lower susceptibility was found with Merlot and Shiraz.

To assess this issue, samples of grapes were collected for wine making across five varieties and seven sites, in all 16 samples. Guaiacol and 4-methylguaiacol were measured in the grapes and in the resultant wine. There were clear varietal differences for guaiacol concentration (and 4-methylguaiacol – data not presented) in the grapes and wine (Figure 13). Sangiovese had the highest concentration of guaiacol in the grapes and wine and Merlot the lowest in the grapes and wine (of the varieties fermented on skins). Whilst the mean data did not have representative grapes from exactly the same sites, all samples were exposed to smoke over extended periods. There was one site where each variety was sampled from and the results there showed the same trends as the mean results (in the grape samples from one location, Merlot < Shiraz < Chardonnay = Cabernet Sauvignon << Sangiovese).

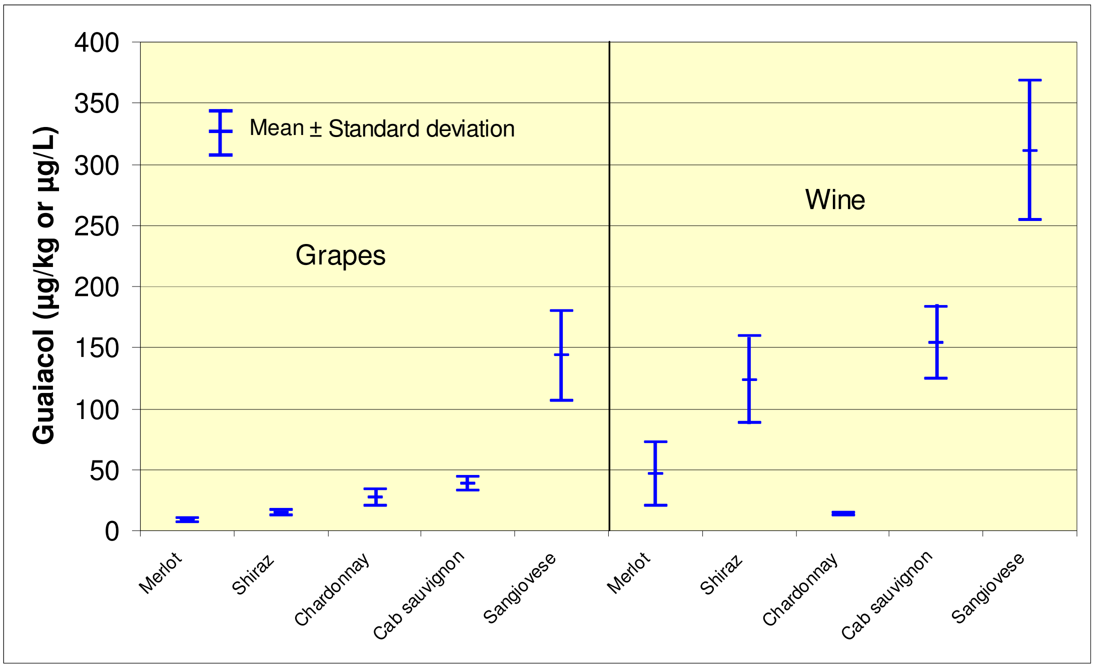


Figure 13. Guaiacol in grapes and wine for five grape varieties in King valley.

Data provided by the AWRI from approximately 450 grape samples also enables some comparisons over a broader range of varieties. Whilst the samples were not collected in a systematic manner to reduce biased sampling and the variability is high due to samples coming from a wide number of regions with varying degrees of smoke, results for the five varieties in Figure 13 show very similar trends in the AWRI data. This data could be used as a guide until more reliable data becomes available from consistent smoking of varieties under controlled conditions.

Table 4. Summary of varietal means for grape samples submitted to AWRI in 2007.

Variety	Mean	Standard deviation	Number of samples
Shiraz	6.1	6.4	83
Merlot	6.6	4.5	33
Riesling	8.4	5.7	14
Chardonnay	9.0	8.8	104
Sauvignon Blanc	11.5	9.7	37
Pinot Noir	13.7	12.6	62
Pinot Gris	14.8	15.2	43
Pinot Meunier	24.0	10.9	11
Cabernet Sauvignon	27.5	20.9	52
Sangiovese	65.5	50.4	13

Varietal differences in guaiacol concentration in wines have been noted before in a database of Spanish wines (Ferreira *et al.* 2000). Tempranillo had the highest concentration of guaiacol in the wine (5.9 µg/L) with lesser concentrations observed in Cabernet Sauvignon (3.54 µg/L) and Merlot and Grenache (2.95 µg/L).

The relationship between guaiacol and 4-methylguaiacol

The relationship between guaiacol and 4-methylguaiacol may be of interest since individually they have different aroma and taste profiles and this may influence overall perceived aroma or taste. Using larger data sets of many grape samples analysed through the AWRI, the ratio of guaiacol to 4-methylguaiacol appeared to be variety dependant. One group of varieties had an average ratio of less than 4 (range 2.5 to 3.7) and included Cabernet Sauvignon, Chardonnay, Merlot and Sauvignon Blanc. The other group of varieties had average ratios close to five and above (range 4.8 to 6.6), and included Shiraz, Sangiovese, Riesling, Pinot Noir, Pinot Meunier and Pinot Gris.

In addition, the analysis of 16 wines made from smoke affected grapes from north east Victoria also showed varietal differences, with Shiraz having the highest ratio of guaiacol:4-methylguaiacol of between 5.6 and 6.3, Sangiovese was intermediate (range 3.8 to 4.6) and Cabernet Sauvignon, Chardonnay and Merlot had the lowest ratios (2.4 to 2.8). These groupings need to be re-tested under controlled conditions as the variability of the data (covering different regions, stages of berry development, etc.) was high.

It is of interest that all the Pinot varieties fall into the higher ratio grouping. There may be something different about the berries or the vines in this group where guaiacol is absorbed in a greater proportion than 4-methylguaiacol or conversely, 4-methylguaiacol is not as well absorbed as guaiacol. Alternatively, the differences may be due to variation in the extent to which these compounds are bound to glycosides in the grapes (the analytical technique measures only ‘free’ compounds).

Within each variety the relationship between guaiacol and 4-methylguaiacol in the grapes across a wide range of concentrations was quite strong with most r^2 values of around 0.90 (range 0.84 to 0.95). Some r^2 values were lower where the number of data points was low (<15 values), e.g. Riesling and Pinot Meunier.

The strong relationship between guaiacol and 4-methylguaiacol also held up in juice samples (across all varieties) with r^2 values of 0.95 and 0.96 for juice from red and white grapes respectively. However, in wine post-fermentation the relationship was not as strong with r^2 values of 0.68 and 0.54 for red and white wine respectively (across all varieties).

Wine tasting scores

A preliminary assessment of wines made from smoke affected grapes was conducted by a panel of seven winemakers from north east Victoria who had experience with smoke affected grapes. Wines were only presented once to the panel and they were asked to rate both aroma and taste on a scale of 0 (no perceptible smoke taint) to 7 (high degree of smoke taint). The data was analysed using a Generalised Linear Model with binomial errors and logit transformation of the data.

The mean wine aroma and taste scores show significant differences between the wines (Table 5). Wines with any common letter were not significantly different (p=0.05) using a test of significance for pair-wise differences. The different numbers beside the variety identification represent different localities where the grapes were sourced.

Table 5. Mean smoke taint aroma and taste scores for wines from a range of varieties and localities in north east Victoria.

Wine smoke aroma			Wine smoke taste		
Wine	**Score**	**Difference**	**Wine**	**Score**	**Difference**
Sangiovese 175	6.3	a	Sangiovese 174	6.0	a
Sangiovese 174	6.0	ab	Sangiovese 175	6.0	ab
Cab. Sauv. 179	5.4	abc	Sangiovese 173	5.3	abc
Cab. Sauv. 177	5.4	abcd	Cab. Sauv. 179	5.1	abcd
Sangiovese 173	5.0	bcde	Cab. Sauv. 177	5.0	abcde
Cab. Sauv. 178	4.9	bcdef	Shiraz 172	4.9	abcdef
Shiraz 171	4.3	cdefg	Shiraz 170	4.6	abcdefg
Shiraz 170	4.2	cdefgh	Shiraz 171	4.3	cdefgh
Shiraz 172	4.0	cdefghi	Cab. Sauv. 178	4.3	cdefghi
Merlot 162	3.7	efghij	Merlot 162	4.1	cdefghij
Merlot 163	3.6	efghijk	Merlot 163	3.6	defghijk
Shiraz 169	3.3	ghijkl	Shiraz 169	3.6	defghijkl
Shiraz 168	2.0	klm	Shiraz 168	2.3	klm
Chardonnay 156	1.4	m	Chardonnay 156	2.0	klm
Merlot 161	1.3	m	Chardonnay 155	1.9	m
Chardonnay 155	0.9	m	Merlot 161	1.6	m

Further analysis of the data was conducted to investigate the impact of locality and variety. With locality, Moyhu had significantly lower aroma and taste scores than the other localities (Table 6). Moyhu is located out of the narrower part of the King Valley on a broader plain and would not be expected to have the smoke intensity compared with localities up in the narrower sections of the King Valley. All other localities had similar average aroma and taste scores apart from Cheshunt having a lower aroma score than Edi Upper.

Table 6. Mean smoke taint aroma and taste scores for wines from a range of localities in north east Victoria.

Wine smoke aroma			Wine smoke taste		
Wine	**Score**	**Difference**	**Wine**	**Score**	**Difference**
Edi Upper	5.4	a	Edi Upper	5.0	a
Cheshunt South	4.6	ab	King Valley	4.3	a
King Valley	4.0	ab	Cheshunt South	4.3	a
Whitfield	3.9	ab	Whitfield	4.3	a
Myrrhee	3.5	ab	Myrrhee	3.8	a
Cheshunt	3.4	b	Cheshunt	3.6	a

Moyhu	1.3	c	Moyhu	1.6	b

Significant differences in smoke aroma and taste scores in wines between varieties were demonstrated (Table 7). With aroma, Sangiovese and Cabernet Sauvignon had the highest scores, Shiraz and Merlot intermediate and Chardonnay the lowest. The same trend was observed with palate score but the differences were slightly different with a significant difference between Sangiovese and Cabernet Sauvignon.

Table 7. Mean smoke taint aroma and taste scores for wines from a range of varieties in north east Victoria.

Wine smoke aroma			**Wine smoke taste**		
Wine	**Score**	**Difference**	**Wine**	**Score**	**Difference**
Sangiovese	5.8	a	Sangiovese	5.8	a
Cab. Sauvignon	5.2	a	Cab. Sauvignon	4.8	b
Shiraz	3.6	b	Shiraz	3.9	bc
Merlot	2.9	b	Merlot	3.1	c
Chardonnay	1.1	c	Chardonnay	1.9	d

8. Understanding translation from grapes to wine

8.1 From grapes to wine

The work in WA has demonstrated the transfer of compounds from smoke into wine under controlled conditions. Work in Victoria in 2007 studied the transfer of smoke taint compounds from grapes, grown under field conditions and exposed to bushfire smoke, to wine. Small batches of grapes (approx. 25 kg) were harvested from a range of sites in north east Victoria, frozen at -18ºC and transported under permit to the Provisor wine making facility at CSIRO Merbein.

Grapes were thawed and crushed prior to pressing (white wine) or fermentation on skins. Acids were adjusted using tartaric acid. Red grapes were fermented to around 13ºBrix before pressing. Wines were racked several times and cold stabilised prior to filtering and bottling.

Guaiacol and 4-methylguaiacol were measured in the grapes prior to harvest and in the resultant wine approximately two weeks after bottling. In the red grape varieties there was a close relationship between grapes and wine for both guaiacol and 4-methylguaiacol (Figure 14 a & b). The white wine results are excluded because they were not fermented on skins and hence a different extraction rate would have resulted. The varieties are clustered and different extraction rates between varieties has produced a curve rather than a linear relationship. Within the wines from the variety Shiraz, there was a close linear relationship between grape and wine guaiacol (Figure 15). Other varieties were not tested for a linear relationship because of the limited number of wines available.

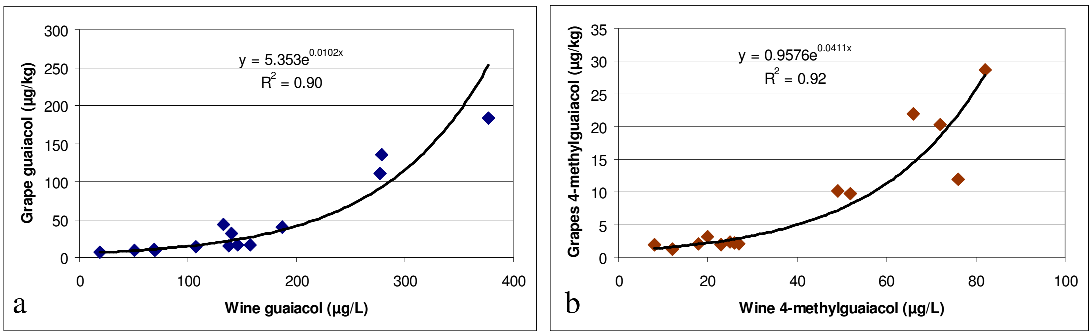


Figure 14. (a) Guaiacol and **(b)** 4-methylguaiacol in grapes prior to fermentation compared with the resultant concentration in wine for four red varieties from various locations in north east Victoria.

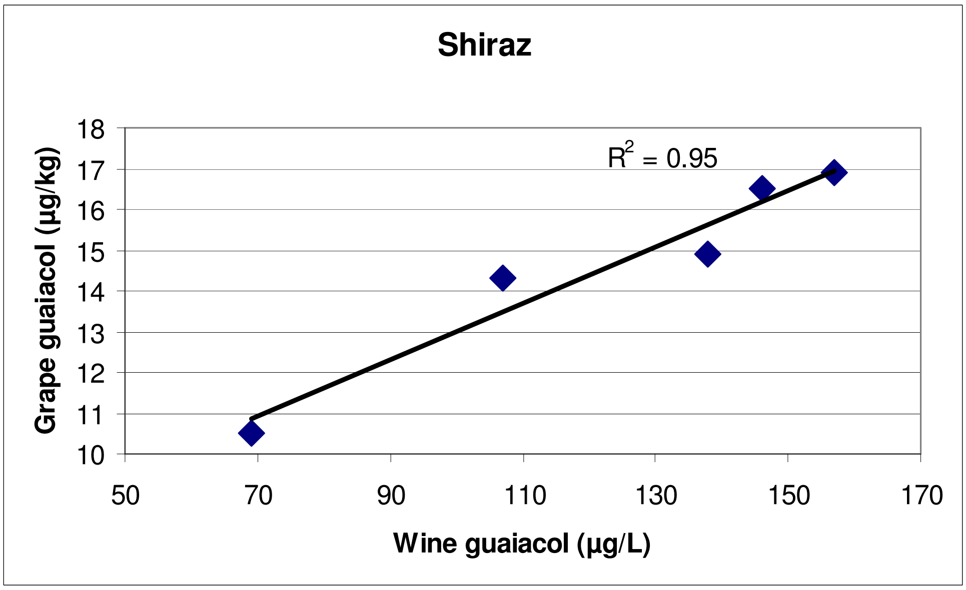


Figure 15. Guaiacol in grapes prior to fermentation compared with the resultant wine for Shiraz from various locations in north east Victoria.

The total amount of free guaiacol and 4-methylguaiacol changes from grapes to wine and varied between varieties. Using an extraction rate of 700L of wine per tonne of grapes and the concentrations from grape and wine samples, and assuming the smoke compounds were homogenously mixed in the fermenting must prior to pressing, total amounts of free guaiacol and 4-methylguaiacol can be calculated.

Shiraz showed the greatest increase in total free guaiacol with a 5.8-fold increase, Sangiovese showed the least increase of 1.5-fold, with Merlot and Cabernet Sauvignon intermediate (3.6 and 2.8-fold increase respectively). The increase in total free 4-methylguaiacol was generally 30-50% higher and followed the same varietal trends as guaiacol. It is highly unlikely these compounds were manufactured during the winemaking process but it is likely they were released from glycosidically bound precursors. Acid hydrolysis (to a very small extent) and enzymic hydrolysis (predominantly) of guaiacol has been demonstrated in grape juice (not exposed to smoke) (Sefton 1998). With Chardonnay there was a decrease of 60-65% in total free guaiacol and 4-methylguaiacol from grapes to wine reflecting the removal of skins prior to fermentation.

8.2 Extraction ratio

The ratio of the concentration of guaiacol and 4-methylguaiacol in the wine compared to that observed in grapes could be deemed an 'extraction ratio'. All wines were pressed at a predetermined concentration of soluble solids and thus time on skins varied. With Chardonnay, which was crushed and pressed and not fermented on skins, about half of the concentration of guaiacol and 4-methylguaiacol in the grapes was observed in the wine (Table 8). Of the red grape varieties, Sangiovese had the lowest extraction ratio (2.2 and 3.1 for guaiacol and 4-methylguaiacol respectively) and Shiraz the highest (8.3 and 10.6). Cabernet Sauvignon and Merlot were intermediate. The high variability of the Merlot results (high standard deviation) was due to a high extraction ratio in one wine sample which had a fermentation period twice as long as the other samples (but same period on skins) due to a higher initial sugar concentration.

Table 8. Extraction ratio (wine/grapes) of free guaiacol and 4-methylguaiacol from grapes to wine for five grape varieties grown across several sites in north east Victoria.

Variety	Guaiacol extraction ratio		4-methylguaiacol extraction ratio	
	Mean	Standard deviation	Mean	Standard deviation
Chardonnay	0.5	0.1	0.6	0.1
Sangiovese	2.2	0.2	3.1	0.3
Cabernet Sauvignon	4.1	0.8	5.5	0.8
Merlot	5.2	2.3	8.0	4.9
Shiraz	8.3	1.2	10.6	1.4

These results need to be tempered by the fact that the grapes were frozen (for quarantine purposes) and thawed prior to crushing and fermentation. This process may have released more compounds from ruptured cells than would normally occur during standard fermentation processes with unfrozen grapes. By comparison results from three ferments of Shiraz at one winery showed an average extraction ratio of 5 for guaiacol. We are confident the varietal trends will remain the same but absolute values may change with wine made from 'fresh' grapes.

9. Influence of wine making procedures

9.1 Influence of fruit handling temperature

To test the influence of fruit handling temperature, a batch of hand harvested Sauvignon Blanc grapes was split into two parts, with one half chilled in a cool room to 10ºC and the other half kept at ambient temperature (25ºC). The grapes were pressed and guaiacol and 4-methylguaiacol measured in three press fractions (Figure 16).

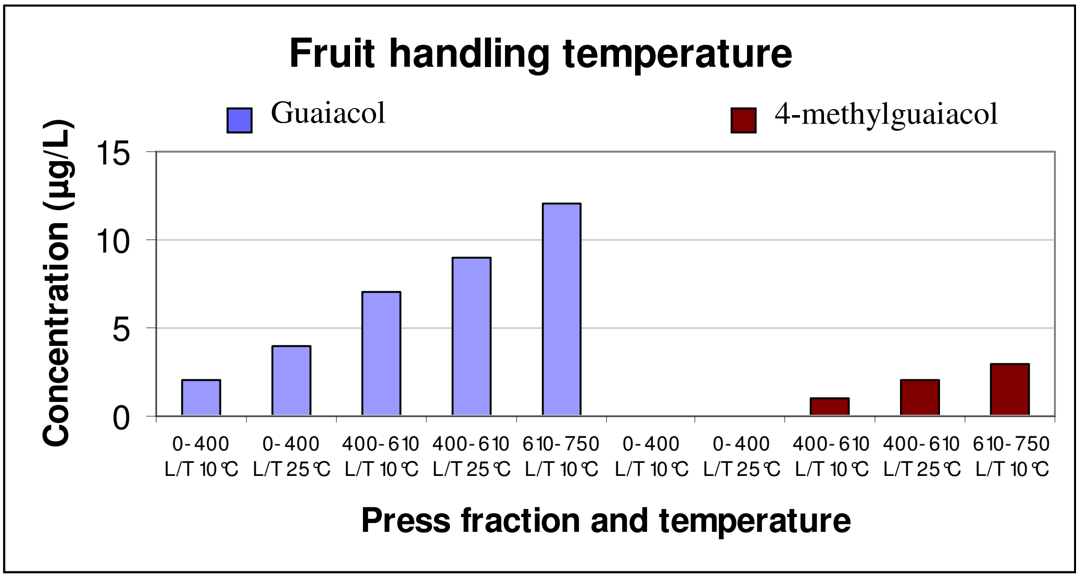


Figure 16. Influence of grape temperature on phenol concentration during pressing.

Cooler fruit temperatures during pressing resulted in less guaiacol and 4-methylguaiacol in the juice in both early and later juice fractions. In the 0-400 L/T fraction guaiacol concentration was reduced by 50% by chilling grapes to 10ºC, and in the 400-610 L/T fraction it was reduced by 22%. No 4-methylguaiacol was detected in the 0-400 L/T fraction at both temperatures and in the 400-610 L/T fraction the concentration was reduced by 50% by chilling the grapes to 10ºC.

9.2 Influence of pressing fraction

The influence of pressing fraction on guaiacol and 4-methylguaiacol concentrations in the juice was compared with the initial grape concentration and concentration in the lees at a commercial winery. Pinot Gris grapes were hand harvested and whole bunch pressed in a Willmes airbag press at ambient temperature, with juice fractions being collected in separate tanks. The juice was cold settled overnight and the samples were obtained from the clear, racked juice, along with the volume of each fraction. All the lees from the rackings were combined in one tank. Guaiacol and 4-methylguaiacol were measured and the smoke taint was rated for each press fraction.

The concentrations of guaiacol and 4-methylguaiacol in the grapes prior to harvest were 45 and 9 µg/kg respectively. The progressive guaiacol and 4-methylguaiacol concentrations are presented in Figure 17. The smoke compound concentrations remained relatively steady up to about 500 L/T, after which the concentration of guaiacol rose above 20 µg/L and up to 84 µg/L in the last press fraction. Based on the original concentration in the grapes and the concentrations and volumes of the juice collected, around 39% of the total guaiacol in the grapes came through in the press fractions and lees, and around 48% of the 4-methylguaiacol came through. These proportions are similar to that estimated for Chardonnay during small scale winemaking (see section 8.1).

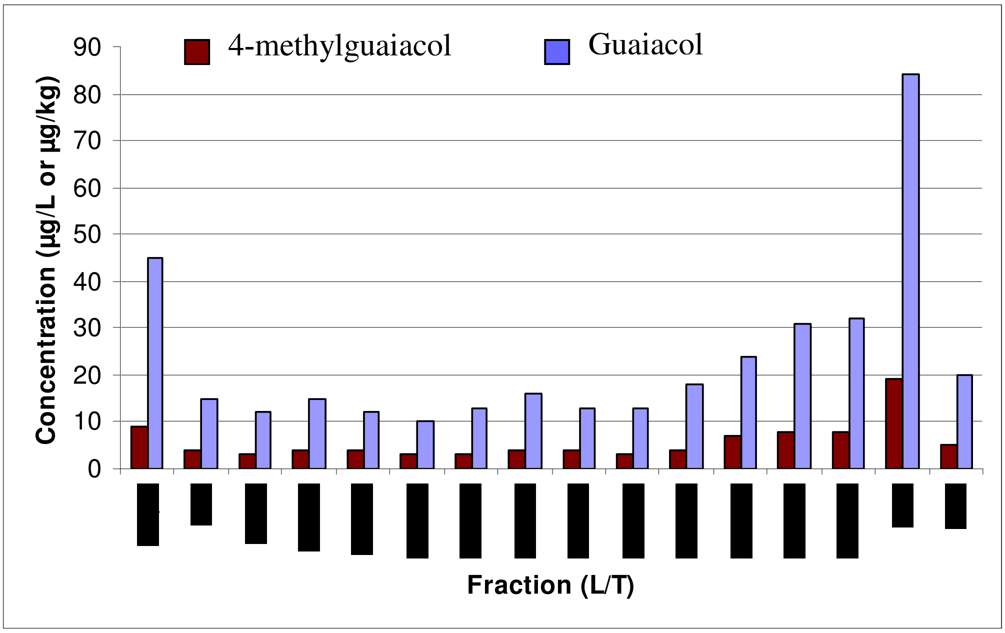


Figure 17. Influence of pressing fraction on the concentration of two smoke related compounds from hand harvested whole bunch pressed grapes.

The trends demonstrated with the Pinot Gris fruit were repeated with other varieties. In all cases, the concentration of guaiacol and 4-methylguaiacol in the pressed juice from hand harvested grapes increased with later press fractions compared with earlier press fractions (Table 9). 'Total guaiacol' increased by 3.25 to 7-fold between early and late fractions.

Table 9. Concentrations of two smoke related compounds in grapes and pressed juice fractions for a range of varieties (units are µg/kg in grapes and µg/L in wine).

Variety	Press fraction (L/T)	Guaiacol (µg/L or kg)	4-methylguaiacol (µg/L or kg)	'Total guaiacol' (µg/L or kg)
Chardonnay	Grapes	4	2	6
	0-415	3	0	3
	415+	8	3	11
Chardonnay	0-400	2	0	2
	400-500	4	2	6
	500+	10	4	14
Chardonnay	Grapes	31	13	44
	0-400	2	0	2
	400-500	4	1	5
	500+	8	3	11
Pinot Noir	Grapes	20	6	26
	0-400	11	2	13
	400-500	18	3	21
	500+	40	7	47
Pinot Meunier	Grapes	36	7	43
	0-500	6	2	8
	500+	22	4	26

9.3 Influence of hand and mechanical harvesting on pressing fractions

A block of Pinot Gris grapes was trialled for comparing hand and mechanical harvesting. A Willmes airbag press was used to provide the juice fractions. Each fraction was cold settled overnight and the samples were obtained from the clear, racked juice, along with the volume of each fraction. All the lees from the rackings were combined in one tank. Guaiacol and 4-methylguaiacol were measured but for the presentation of the results the two were added. The smoke taint was rated for each press fraction.

The initial concentration of guaiacol+4-methylguaiacol ('total guaiacol') in the grapes was 54 µg/kg (Figure 18). There is a steady rise in the concentration of 'total guaiacol' in the machine harvested grapes with each progressive press fraction whereas with hand harvested grapes this only happens above around 500 L/T extraction. The concentration of the 'total guaiacol' was much greater in the machine harvested grapes in all fractions except the 650-700 L/T fraction.

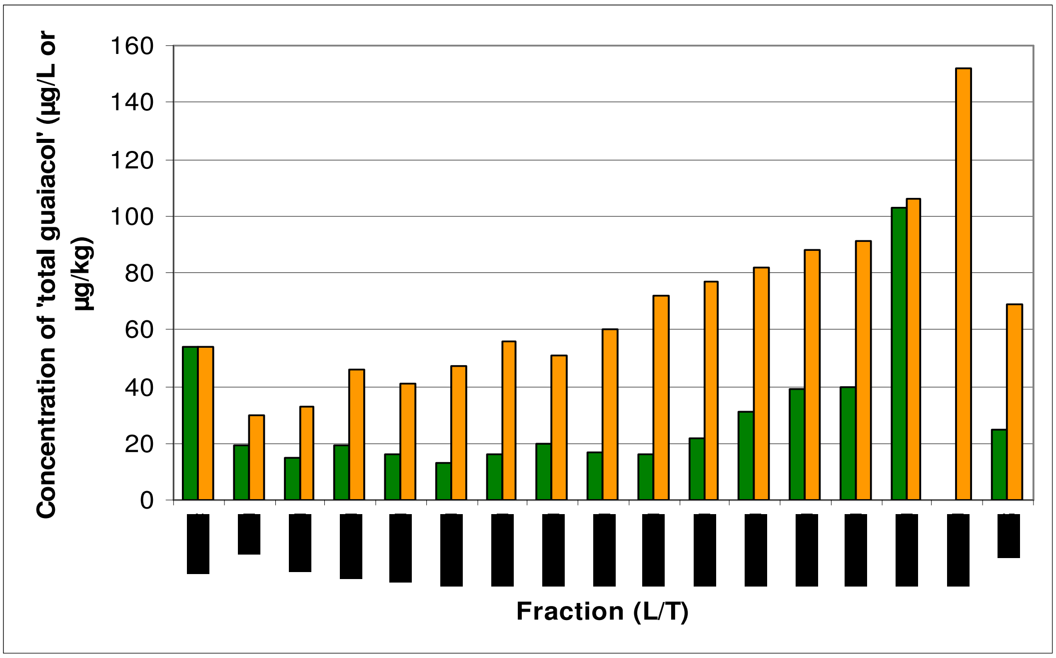


Figure 18. Smoke compound concentration (guaiacol + 4-methylguaiacol) in press fractions from hand (■) and mechanically (■) harvested grapes.

The concentration of 'total guaiacol' in the machine harvested grapes ranged up to 4.5 times that of the hand harvested grapes in the press fractions. Based on the original concentration in the grapes and the concentrations and volumes of juice collected, around 40% of the 'total guaiacol' in the grapes came through in the press fractions and lees with hand harvesting and around 98% of the 'total guaiacol' in the grapes came through in the press fractions and lees in the machine harvested sample. The total press volumes from the hand harvested whole bunch pressed and machine harvested pressed grapes were 712 and 748 L/Tonne respectively.

Thus machine harvesting of grapes, which produces a high degree of fruit maceration and skin contact with the juice, leads to much greater concentrations of guaiacol and 4-methylguaiacol in the resultant juice prior to fermentation than hand harvested grapes. Two bins of mechanically harvested fruit that had sat for different lengths of time prior to crushing were compared and the concentration of 'total guaiacol' was around 30% higher in the bin that had the longer period before crushing. Whilst only a single comparison, this observation was consistent with earlier work demonstrating progressively greater extraction of guaiacol and 4-methylguaiacol in macerated grapes over time.

9.4 Changes during fermentation

Anecdotally, several winemakers claimed there was an increase in perceived smoke taint during fermentation. Only limited data was obtained on this subject, but measurements of guaiacol and 4-methylguaiacol were observed to increase during fermentation (Table 10). Given that the analytical technique only measured the 'free' compounds, it could be that the increases observed during fermentation may be due to release of 'bound' compounds. Production of other by yeasts or bacteria may also have occurred.

Table 10. Changes in two smoke related compounds during fermentation of Chardonnay juice.

Fraction	Compound	Juice	Post-fermentation
0-415 L/T pressings	Guaiacol (μg/L)	3	7
	4-methylguaiacol (μg/L)	0	3
0-400 L/T pressings	Guaiacol (μg/L)	2	8
	4-methylguaiacol (μg/L)	0	3

Skin contact

No specific experiments were conducted on this aspect. However information from a batch of 14 red wines made from smoke affected grapes from north east Victoria suggested no relationship between period on skins and the ratio of the concentration of guaiacol in the wine to the concentration of guaiacol in the grapes (extraction ratio). All wines were pressed at the same soluble solids concentration in the ferment, i.e. 13ºBrix. The absolute concentration of guaiacol in the wine was not a good indicator of the influence of skin contact period on smoke taint compounds because the initial concentration of guaiacol in the grapes varied between ferments.

For example, three Merlot and three Cabernet Sauvignon ferments each had 6 days fermentation on skins yet the extraction ratio of guaiacol ranged from 2.8 to 7.3 in Merlot and 3.1 to 4.7 In Cabernet Sauvignon. Total fermentation period also did not relate to extraction ratio or final guaiacol concentration in the wine. Further controlled experiments are required to examine this issue. It is likely that short periods on skins (at stages >13ºBrix) will influence the extraction of smoke taint compounds, as was determined during the 2003 fires.

Length of fermentation period

A suggestion has been made that when smoke tainted grapes are made into wine the fermentation period is shortened. Within a batch of 14 red wines with a range of concentration of smoke taint compounds there was no relationship between fermentation period and guaiacol or 4-methylguaiacol concentration in the grapes or wine. The only relationship observed was that riper grapes (>25ºBrix) had longer fermentation periods (17-19 days) than less ripe (<25ºBrix) grapes (8-12 days).

9.5 Fining effects

Fining agents have been used in the past with little success, with the exception of activated charcoal. A limited re-test of some fining agents was conducted at a commercial winery on Pinot Gris wine immediately post-fermentation. The two agents were used, Hydroclair® (a gelatine based product) and PVPP (polyvinylpolypyrrolidone), which appeared to have no effect on reducing the guaiacol and 4-methylguaiacol concentrations in the wine (Table 11). The juice values for the smoke compounds were estimated from tank volumes and concentration of press fractions combined into larger volumes for fermentation. Since different press fractions were used the tests are not directly comparable but the trends are similar and the results contrast that from activated charcoal (see below).

Table 11. Effect of two fining agents on two smoke related compounds in wine.

Treatment and press fraction	Guaiacol (μg/L)		4-methylguaiacol (μg/L)	
	Estimated in juice	Wine after fining	Estimated in juice	Wine after fining
Control (100-200 L/T)	13	17	2	3
Hydroclair® (600 mg/L) (200-300 L/T)	12	17	3	3
PVPP (600 mg/L) (300-400 L/T)	14	20	4	4

A number of activated charcoal treatments were tested on wines of several varieties (Table 12). Small amounts of activated charcoal up to 0.3 g/L did not reduce the concentrations of guaiacol or 4-methylguaiacol. One treatment of 0.5 g/L of activated charcoal reduced the concentrations of guaiacol by 50% and 4-methylguaiacol to below the analytical detectable concentration.

Further tests of activated charcoal were conducted on Pinot Gris wine (Table 13). There are limitations to the interpretation of results because different press fractions were used with different rates of charcoal and the pre-fining values for guaiacol and 4-methylguaiacol refer to pre-fermentation juice and not pre-fined wines. Rates of 1.0 to 1.6 g/L of activated charcoal were effective in reducing the concentration of guaiacol (by 70 to 80%) and completely removing the 4-methylguaiacol in all hand harvested press fractions and most mechanically harvested press fractions.

Table 12. Effect of activated charcoal added post fermentation on the concentration of two smoke related compounds.

Variety and press fraction	Treatment	Guaiacol (µg/L)		4-methylguaiacol (µg/L)	
		Juice	Wine after fining	Juice	Wine after fining
Pinot Noir (0-400 L/T)	0.1 g/L charcoal	11	13	2	3
Meunier (0-500 L/T)	0.14 g/L charcoal	6	8	2	2
Chardonnay (0-400 L/T)	0.3 g/L charcoal	2	2	0	0
Chardonnay (400-500 L/T)	0.5 g/L charcoal	4	2	1	0

Table 13. Effect of activated charcoal added post fermentation on the concentration of two smoke related compounds in Pinot Gris.

Harvest method	Treatment and press fraction	Guaiacol (µg/L)		4-methylguaiacol (µg/L)	
		Estimated in juice	Wine after fining	Estimated in juice	Wine after fining
Hand	Control (100-200 L/T)	13	17	2	3
	Charcoal (1 g/L) (0-100 L/T)	13	4	3	0
	Charcoal (1 g/L) (400-500 L/T)	15	3	3	0
	Charcoal (1.6 g/L) (500+ L/T)	47	10	11	0
Mechanical	Control (0-150 L/T)	29	42	7	9
	Charcoal (2 g/L) (150-400 L/T)	41	6	10	0
	Charcoal (3 g/L) (400-600 L/T)	64	2	16	0
	Charcoal (1.1 g/L) (600-650 L/T)	97	35	22	4

The concentration of smoke related compounds were 2 to 5 times higher in the press fractions of mechanically harvested grapes compared to hand harvested juice fractions. Higher rates of charcoal were deemed necessary by the winemaker to counteract these higher concentrations. Rates of 2.0 to 3.0 g/L of activated charcoal substantially reduced the guaiacol concentration (by 85 and 97 % respectively) and completely removed the 4-methylguaiacol. The lower rate of charcoal of 1.1 g/L in the fraction with very high concentrations of guaiacol and 4-methylguaiacol reduced guaiacol by 64% but did not completely remove the 4-methylguaiacol.

9.6 Reverse Osmosis

Utilising the characteristics of variable permeability of membranes based on molecule size and high pressure gradients has produced the process of reverse osmosis to selectively (to some extent) remove compounds from wine. Some of the removed compounds are 'scrubbed' from the filtrate and remaining wine returned to the system. Guaiacol and some other smoke related compounds are relatively small molecules so they can be removed, but other larger compounds that are not removed may actually dominate and become more evident in the aroma.

There is potential for using this technique where the smoke taint in wine is low and removal of smoke taint compounds will bring them below the threshold level. In highly tainted wines it would take many passes through the system to reduce levels to the threshold concentrations of smoke taint compounds. Also, there is a concern that some fruit characters may also be removed by reverse osmosis and the smoke characters may come to the fore.

What would help with this process would be:

- Rapid measures of smoke related compounds (e.g. UV 275nm phenolic measures) as they go through the reverse osmosis process to assist in determining when appropriate reductions have been achieved.
- Better ways of selectively 'scrubbing' smoke related compounds.
- Improved understanding of the full range of smoke taint compounds to assist in the design of specific membranes to selectively filter smoke taint compounds.

9.7 Other aspects

Mitigation with lees

When neutral wine lees were added to wine that had been stored in oak barrels, some of the volatile phenols derived from the oak wood were absorbed by the lees (Moreno Azpilicueta 2007). Of the compounds of interest for smoke affected wine, 4-methylguaiacol, was absorbed by the lees but guaiacol was not. At 50 g/L of lees in the wine (around 4-5 times the European Union limit for lees addition) the concentration of 4-methylguaiacol diminished by around 22%. This method of mitigating smoke taint in wines seems to have limited commercial application, although the work should be repeated with smoke tainted wines rather than the barrel aged wines used in the previous work.

Oak barrels

Smoke related compounds can be extracted from toasted oak barrels used for wine storage and maturation. In a model wine of water, alcohol, tartaric acid and SO_2, Spillman *et al.* (1998) recorded maximum concentrations between 6 and 33 µg/L for guaiacol, and between 1 and 16 µg/L for 4-methylguaiacol. In used oak barrels the concentration of guaiacol ranged from 6 to 8.8 µg/L and 4-methylguaiacol from 0.02 to 0.06 µg/L when sampled between 8 and 18 months (Garde *et al.* 2002). Guaiacol was not detected in non or lightly heated oak-wood (Sefton *et al.* 1990).

Cork

Taints arising from corks used in wine bottling have included guaiacol. Simpson *et al.* (1986) identified high concentrations of guaiacol in tainted wine with concentrations ranging from 70 to 2,630 µg/L of wine. Non-smoke affected wines had guaiacol concentrations of 3 to 6 µg/L. Four strains of bacteria isolated from corks have been identified as being capable of producing guaiacol from vanillic acid (Alvarez-Rodriguez *et al*. 2003). At least two very high readings above 2,000 µg/L in the Simpson *et al*. (1986) work were considered not likely to have arisen from bacterial sources and they surmised that direct chemical contamination may have occurred from an external source of guaiacol during shipping or storage. Thus, corks are a potential source of contamination in wine samples being assessed for guaiacol and possibly other smoke related compounds and any trial wines or samples for measurement should not be bottled with cork.

10. Influence of smoke taint on sensory perception in juice and wine

10.1 Smoke taint rating in juice

A rating system was employed by one winery to quickly judge the potential smoke taint in pressing fractions rather than rely on guaiacol and 4-methylguaiacol measurements which take some time to conduct (currently 1-3 weeks). The rating ranged from 0 for no perceived smoke character to 10 for a really obvious combination of taste and smell. A group of winemakers and cellar staff made the assessments.

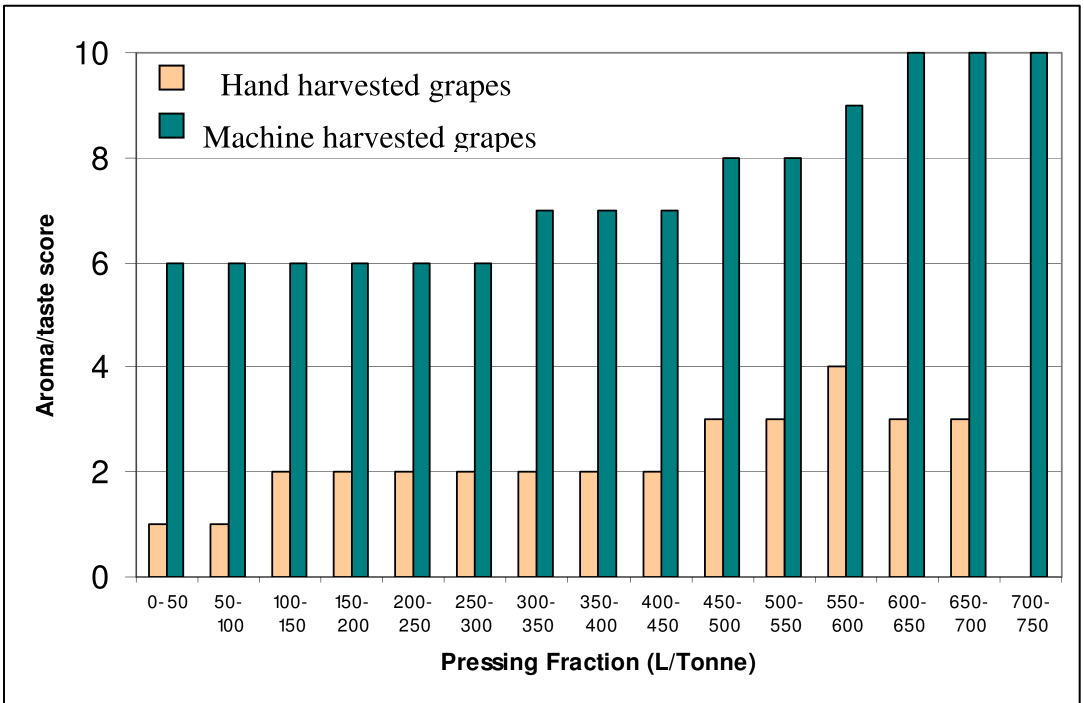


Figure 19. Aroma/taste scores for juice fractions from hand and machine harvested grapes of Pinot gris.

Smoke taint compounds in the grapes prior to harvest, measured as guaiacol and 4-methylguaiacol, were 45 and 9 µg/kg respectively. During the sequential pressing fractions the perceived smoky aroma and taste increased in both hand and machine harvested grapes (Figure 19). All fractions were assessed as having at least some smoke aroma and taste (no juice was collected in the 700-750 L/T fraction for hand harvested grapes). The mechanically harvested grapes were perceived to have much higher levels of smoke characteristics than the hand harvested grapes. This assessment reflects the measurements of guaiacol and 4-methylguaiacol in those fractions.

The combined smoke aroma/taste scores for the press fractions were compared to the guaiacol and 4-methylguaiacol measurements obtained for the same juices. A very good correlation was found with an r^2 of 0.90 and 0.89 for guaiacol and 4-methylguaiacol respectively (Figure 20 a & b). Adding the guaiacol and 4-methylguaicol ('total guaiacol') results together also gave a high r^2 of 0.89.

There were no scores of zero given for smoke aroma/taste to compare with measured values so no absolute 'threshold' could be determined. However, as an approximation, extrapolation of the graph to 0 score produces an 'apparent threshold' for smoke taint in Pinot Gris juice corresponding to around 9.4 µg/L of guaiacol and 2.6 µg/L of 4-methylguaiacol. This was not the same as a true threshold for guaiacol (and 4-methylguaiacol), only that we were using these compounds as an indicator of overall smoke taint. In reality the 'apparent threshold' would be somewhere between a score of 0 (no smoke taint detected) and 1 (low level of taint), which is therefore between 9.4 and 11.8 µg/kg. Thus, 9.4 µg/kg is the estimated minimum and the actual threshold is likely to be slightly above this.

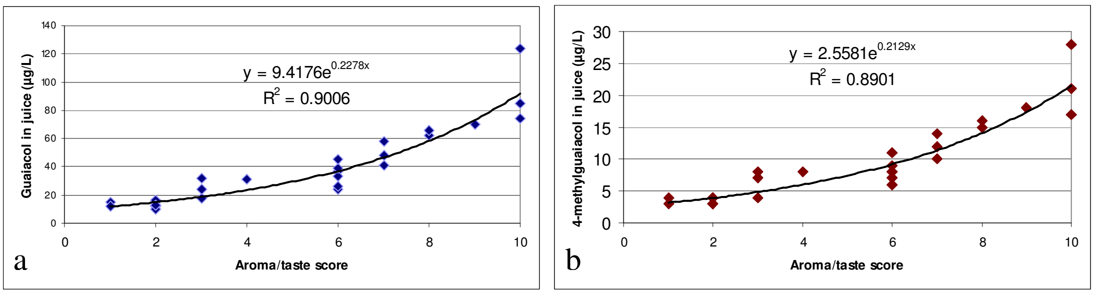


Figure 20. Correlation between aroma/taste scores for Pinot gris juice and **a)** guaiacol, and **b)** 4-methylguaiacol concentrations.

10.2 Smoke taint rating in wine

A batch of 16 wines were made in 2007 from north east Victoria covering five varieties from seven sites. These wines were submitted to seven winemakers from north east Victoria for a preliminary assessment of the wines. An analysis of guaiacol and 4-methylguaiacol was conducted by the AWRI. The wine scores were based on a range from 0 (no smoke taint) to 7 (strong smoke taint) among the wine samples. The winemaker's comments about the degree of smoke taint were mixed, with one taster from the Alpine Valley rating the taint in 2007 as less than that produced in the 2003 fires and most other tasters (from the King Valley) rating the smoke taint in 2007 as greater than 2003. The fires in 2003 had relatively more impact on the Alpine Valleys than the King Valley.

Mean wine aroma and taste scores were compared with the guaiacol and 4-methylguaiacol concentrations in the wines. Mean aroma score showed a very good correlation with both guaiacol (r^2 = 0.87) and 4-methylguaiacol (r^2 = 0.94) (Figure 21 a & b). The aroma score was more closely related to 4-methylguaiacol than guaiacol. Extrapolating the mean aroma score to 0 gives an 'apparent threshold' of smoke taint at 10.3 µg/L of guaiacol and at 3.3 µg/l of 4-methylguaiacol. As explained in section 10.1 the actual 'threshold' is likely to be slightly above this value (between 10.3 and 18.2 µg/L). The values calculated here are not dissimilar to some estimated thresholds calculated from data collected in Western Australia using Verdelho of 7.4 and 22.8 µg/L guaiacol (Kennison pers. comm. 2007).

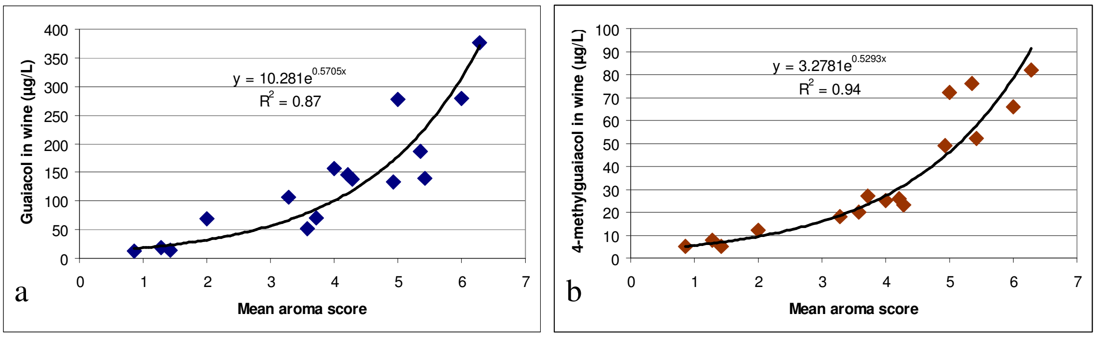


Figure 21. Mean wine aroma score compared with **a)** guaiacol and **b)** 4-methylguaiacol concentration in the wine.

The mean taste score also showed a very good correlation with both guaiacol (r^2 = 0.87) and 4-methylguaiacol (r^2 = 0.88) (Figure 22 a & b). Extrapolating the mean taste score to 0 gives an 'apparent threshold' of smoke taint at 6.0 µg/L of guaiacol and at 2.2 µg/l of 4-methylguaiacol. The lower 'apparent threshold' for smoke taste compared to smoke aroma concurs with the thresholds demonstrated for guaiacol in water and apple juice (Eisele and Semon 2005).

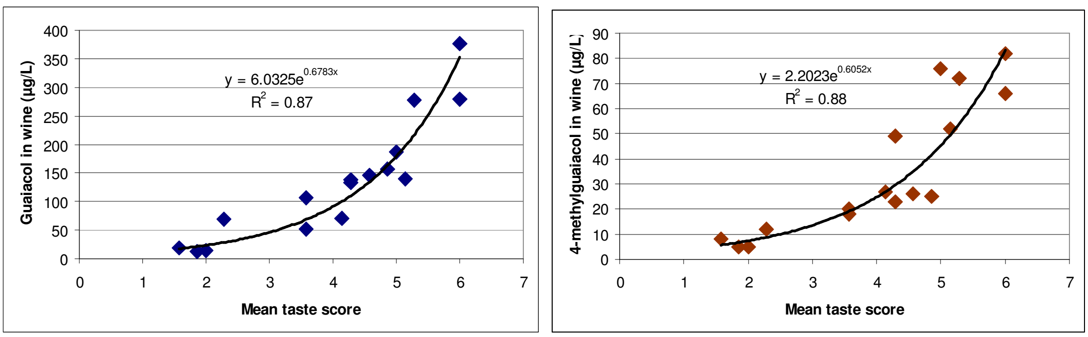


Figure 22. Mean wine taste score compared with **a)** guaiacol and **b)** 4-methylguaiacol concentration in the wine.

Given the 'apparent' smoke taint threshold for wine is equivalent to 10.3 µg/L guaiacol for aroma and 6.0 µg/L guaiacol for taste, and using the relationship between wine and grape guaiacol concentrations established earlier (Figure 14), we can calculate an estimated grape guaiacol concentration of 5.7-5.9 µg/kg as the level at which smoke taint can be perceived in the wine. This value is close to the 6 µg/kg of guaiacol in grapes used to determine at which concentration grapes would not be processed into 'vintage' wines at some north east Victoria wineries in 2007. Please note that this 'threshold' value of guaiacol in grapes varied between wineries typically between 2-6 µg/kg. It should also be noted that this value is a mean of all varieties and under one particular winemaking procedure, and the 'cut-off' value may vary with variety and the way the grapes are processed (e.g. red vs white) and fermented.

<u>Smoke taint descriptors</u>

In a preliminary tasting of the 2007 wines made from grapes in north east Victoria seven local winemakers provided descriptors of the wines. For this preliminary tasting, the panel were asked to be open minded about what descriptors they would use. In total, around 30 descriptors were used for aroma and taste but seven were most commonly used – ash, drying, bitter, smoky, bacon, campfire (recently extinguished) and ashtray. Several others that were less commonly used were toasty/charcoal, dirty, smoked meat and aniseed. The wine tasters remarked that it was difficult to find descriptors that went across all wines and the fruit characters coming through in the wines also made it difficult to discern the aroma and taste characteristics.

With the most commonly used descriptors, Chardonnay wines had the lowest number of mentions for ash and campfire, Merlot had highest number of mentions for ash and lowest for drying, bitter, smoky and ashtray, Shiraz had the highest number of mentions for ash, bitter and bacon, Sangiovese had the highest number of mentions for smoky, campfire and ashtray and lowest for bacon, and Cabernet Sauvignon had the highest number of mentions for drying, smoky and ashtray, and lowest for bitter. As this was only a preliminary screening, further tasting work with a trained panel, agreed descriptors and repeated, randomised wine samples is necessary to provide more definitive comments on the wines.

11. Gaps and opportunities for future R&D

This review and report on recent R&D has highlighted aspects of smoke taint in grapes that need further investigation. These include:

- Investigating relationships between the different methods of preparing berries for analysis, e.g. maceration of juice on skins for several days versus whole berry analysis, frozen versus non-frozen berries, etc., so comparisons can be made between reported results.
- Should the assay procedures also measure bound or potential compounds? Is measuring 'free' compounds adequate if glycosidically bound compounds are present and get released during fermentation? Perhaps there is a need to measure free and total concentrations of the compounds after breaking the glycoside bonds. Acid and enzyme hydrolysis techniques (see Sefton 1998) should be applied to smoke tainted juices to assess the response.

- Pathway of entry of guaiacol into the berry needs to be defined. Direct entry into the berry has been demonstrated and translocation within the vine has been demonstrated, but entry through the leaf needs to be tested. Similarly entry through the roots needs to be tested.
- There is a need to confirm where in the berry (skins, pulp and seeds) the bulk of the smoke taint compounds are present under natural smoke concentrations, whether this varies across varieties, and the best extraction technique to account for this. Do bunch stems provide any contribution?
- How rapidly do smoke taint compounds move into the berry or vine? An understanding of the sorption dynamics is required.
- Investigation into smoke taint compounds being carried through to following seasons within the vine. Anecdotal comments suggest so. If correct, what could growers do to reduce berry uptake?
- Whilst sensitivity to exposure at different growth stages has been determined for one variety in WA, field results in Victoria in 2006-07 didn't always follow the suggested pattern. A broader range of varieties need testing.
- Further clarification of correct sample sizes where the grapes are close to the marginal guaiacol concentration (as an indicator of smoke taint). Only one series of samples on one variety were near the 'cut-off' value and further coefficients of variation data is required.
- Using frozen grapes to make wine is not the usual process. Experiments comparing smoke taint compounds in grapes and wine should be repeated using fresh grapes.
- The greatest prospect for processing smoke tainted grapes is to produce clean juice and not attempt skin contact with whites or red wine production on skins. A focus on improved techniques to minimise smoke taint extract in juice would be beneficial, e.g. a combination of chilled grapes, immediate separation of skins from juice (new grape pressing techniques that squash the berry and remove the skin immediately), separation of solids, potential aeration of juice, etc. needs formulating. This aspect could be assisted by measuring free and total guaiacol (and other compounds) in different press fractions processed into wine.
- Investigate whether guaiacol or other smoke taint compounds are potential precursors for other compounds, e.g. tannins, or potentially bind to certain added tannins, and devise procedures which may reduce the smoke content in juice or wine.
- The wines made from north east Victoria in 2006-07 should undergo more extensive tasting with a trained panel using agreed descriptors. This should be done at progressive stages of development of the wines to assess whether the intensity or type of descriptors change.
- The wines could also be used to determine more precisely the threshold of smoke taint in relation to measured smoke taint compounds using triangular tastings to calculate Best Estimate Thresholds of aroma and taste.

- The estimated guaiacol concentration in grapes at which smoke taint becomes evident, of around 6 µg/kg, was based on data across a number of varieties. This 'apparent threshold' needs to be tested further to ascertain whether there are any varietal differences.
- The reasons for the apparent differences between varieties, e.g. differences in uptake of certain smoke compounds, different extraction rates from grapes to wine, and differences in the ratio of some smoke indicator compounds (e.g. guaiacol:4-methylguaiacol), require further investigation as it may help understand the mechanisms of uptake and lead to mitigation practices.
- The project has demonstrated good agreement between perceived smoke taint in juice and wine and measures of at least two of smoke taint compounds. A process to assess red grapes prior to fermentation needs to be developed, e.g. using homogenised must samples, rather than waiting lengthy periods for results to come back from the laboratory.
- There is an opportunity to develop some smoke taint compound standards, e.g. guaiacol or whole smoke concentrations at around threshold values, as well as up to high levels so tasters can see the range of potential smoke taint.
- The wines from 2006-07 should be assessed for other compounds that may be indicators of smoke taint from forest fires. The University of NSW and Bushfire CRC may be useful collaborators, along with the AWRI.
- A simple indicator of smoke intensity is required for growers and winemakers to decide whether they have a potential issue with smoke taint contamination. Visibility through a smoke haze could be easily undertaken by knowing the distances to various landmarks. There needs to be some connection made between visibility (concentration of smoke) and number of days at certain visibilities (overall smoke intensity index), and concentration of smoke taint compounds in the grapes. Otherwise, a simple and cheap gadget based on smoke detector technology would be useful.
- Investigate other options for assessing smoke taint, e.g. electronic nose, ELISA based tests or a specific probe for smoke related compounds, for more rapid assessment in the vineyard or winery.
- Once a better understanding of the timing and mechanism of uptake of smoke taint compounds into the grapes is achieved, viticultural mitigation practices can be developed. If leaves play a substantial role in uptake, then treatments such as leaf removal or antitranspirants could be tested.
- Another area worthy of investigation is whether hang time plays a role in sequestering smoke taint compounds into the vine and grapes, and the potential for very early or very late harvesting to mitigate smoke taint compounds.
- Answers to many of the questions above would assist in improving the controlled burning practices of the Victorian Department of Sustainability and Environment (DSE). Knowing the timing of uptake more clearly, and the impact of smoke intensity on uptake, would

improve the controlled burn strategies near vineyards and other susceptible crops. This could involve viticultural inputs into models relating to controlled burns and bushfires in sensitive areas.

- Given the activities in Western Australia, the Bushfire CRC and other agencies, it is suggested a National viticulture RD&E group be formed to maintain links with each other and develop a coordinated approach to the issue.

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