School of Science
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Smoke Derived Taint in Grapes and Wine

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DECLARATION

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

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ABSTRACT

Smoke derived taint in grapes and wine is an issue of increasing significance and severity for the wine industry internationally. On commencement of this research, insufficient knowledge existed as to the effects of smoke on grapevines and the development of smoke taint in wine, with no substantial published information. This research was undertaken to investigate the effect of smoke exposure to grapevines on the development of smoke aromas, flavours and compounds in final wines. As such, this study pioneers the purposeful application of smoke to grape bunches and field-grown grapevines to establish the direct link between smoke exposure and the development of smoke taint in wine.

This research identified key periods of grapevine sensitivity to smoke uptake as: (1) from shoots 10 cm in length to full-bloom (low levels of smoke taint); (2) from berries pea size to the onset of veraison (variable levels of smoke taint); and (3) from 7 days post veraison to harvest (high levels of smoke taint). A novel smoke application methodology consisting of a smoke generator and greenhouse-grade tent was developed to facilitate the accurate application of smoke treatments to field-grown grapevines. Smoke treatments were applied to grapevines at key stages during the seasonal growth cycle, on repeated occasions and at a range of densities and durations.

Elevated concentrations of guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, eugenol and furfural were detected, by gas chromatography-mass spectrometry analysis, in wines made from fruit exposed to smoke; whereas these compounds were either not detected or detected in trace concentrations in wines produced from unsmoked (control) fruit. Wine sensory analysis established a difference between smoked and unsmoked wines, with smoked wines exhibiting ‘smoky’, ‘dirty’, ‘earthy’, ‘burnt’ and ‘smoked meat’ aromas. The density and duration of smoke exposure to grapevines was found to affect the chemical composition and sensory properties of wine and repeated smoke applications demonstrated a cumulative effect.

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PUBLICATIONS

Kennison, K.R., Wilkinson, K.L., Williams, H.G., Smith J.H. and Gibberd, M.R. (2007) Smoke derived taint in wine: effect of postharvest smoke exposure of grapes on the chemical composition and sensory characteristics of wine. Journal of Agricultural and Food Chemistry 55, 10897-10901.

Kennison, K.R., Wilkinson, K.L., Pollnitz, A.P., Williams, H.G. and Gibberd, M.R. (2009) Effect of timing and duration of grapevine exposure to smoke on the composition and sensory properties of wine. Australian Journal of Grape and Wine Research 15, 228-237.

Kennison, K.R., Gibberd, M.R., Pollnitz, A.P. and Wilkinson, K.L. (2008) Smoke-derived taint in wine: the release of smoke-derived volatile phenols during fermentation of merlot juice following grapevine exposure to smoke. Journal of Agricultural and Food Chemistry 56, 7379-7383.

Kennison, K.R., Wilkinson, K.L., Pollnitz, A.P., Williams, H.G. and Gibberd, M.R. (2011) The effect of smoke application to field-grown Merlot grapevines at key phenological growth stages on wine sensory and chemical properties. Australian Journal of Grape and Wine Research 17, S5-S12.

Kennison, K.R., Williams, H.G. and Gibberd, M.R. (2011) The density and duration of smoke exposure to grapevines influences the development of smoke-like compounds, flavours and aromas in resultant wine. Manuscript submitted to the Australian Journal of Grape and Wine Research.

CONTRIBUTION OF OTHERS

The contribution of all other authors to papers is detailed in the co-author statements in Appendix I.

ADDITIONAL PUBLICATIONS AND PRESENTATIONS

The following publications and presentations are relevant to this thesis but are not included in it.

Refereed papers

Hayasaka, Y., Dungey, K.A., Baldock, G.A., Kennison, K.R. and Wilkinson, K.L. (2010) Identification of a β-D-glucopyranoside precursor to guaiacol in grape juice following grapevines exposure to smoke. Analytica Chimica Acta 660, 143-148.

Fisher, D., Kennison, K. and Ward, G. (2009) Wine, forests and smoke: land users living in harmony. Extension Farming Systems Journal 5 (2), 201-205.

Papers and poster presentation at conferences

Kennison, K.R., Ward, G. and Gibberd, M.R. (2010) Western Australian research on smoke derived taint in grapes and wine: aiming for a national smoke effect reduction system. Oral presentation at Smoke Effect Workshop: 14th Australian Wine Industry Technical Conference: Eds. R.J. Blair, T.H. Lee and I.S. Pretorius. 3-8 July, 2010, Adelaide.

Kennison, K.R., Wilkinson, K.L., Pollnitz, A.P., Williams, H.G. and Gibberd, M.R. (2010) Grapevine exposure to smoke at key phenological timings effects the chemical composition and sensory characteristics of resultant wines. Poster and abstract in: 14th Australian Wine Industry Technical Conference: Conference Proceedings. Eds. R.J. Blair, T.H. Lee and I.S. Pretorius. 3-8 July, 2010, Adelaide.

Kennison, K.R., Louis, C.D., Renton, M., Fisher, D.L., Shepherd, D.P. and Ward, G. (2010) Reducing the negative effects of smoke on grapes and wine: development of a smoke reduction toolkit. Poster and abstract in: 14th Australian Wine Industry Technical Conference: Conference Proceedings. Eds. R.J. Blair, T.H. Lee and I.S. Pretorius. 3-8 July, 2010, Adelaide.

Kennison, K.R., Ward, G. and Gibberd, M.R. (2009) Western Australian research on smoke derived taint in grapes and wine: aiming for a national smoke effect reduction system. Presentation and abstract in Smoke Taint: Issues in Wine Production, Wednesday Nov 4th 2009, University of California Davis.

Kennison, K.R., Wilkinson, K.L., Ward, G. and Gibberd, M.R. (2008) WA smoke taint program: delivering outcomes for industry. Presentation at Grape and Wine Research and Development Corporation Technical Industry Workshop: Smoke Taint Proceedings, 29 July 2008, Melbourne.

Kennison, K.R., Wilkinson, K.L., Williams, H.G. and Gibberd, M.R. (2007) Sensory characteristics and chemical composition of smoke-tainted wines. Poster and abstract in Blair, R.J., Williams, P.J. & Pretorius, I.S. (Eds) 13th Australian Wine Industry Technical Conference: Conference Proceedings, 28 July to 2 August, 2007, Adelaide.

Kennison, K.R., Wilkinson, K.L., Williams, H.G. and Gibberd, M.R. (2007) The timing and duration of grapevine exposure to smoke effects the chemical composition of wine. Poster and abstract in Blair, R.J., Williams, P.J. & Pretorius, I.S. (Eds) 13th Australian Wine Industry Technical Conference: Conference Proceedings, 28 July to 2 August, 2007, Adelaide.

External presentations

Kennison, K.R., Ward, G., Diggle, A., Saam-Renton, M., Airey, M., Fisher, D., Kelly, D., Haswell, D. and Gillard, J. (2011) Completing the smoke effect picture: systems development to reduce the negative effects of smoke on grapes and wine. Oral presentation at Victorian Smoke Effect Regional Seminars in Myrtleford 17th, Yarra Valley 18th and Ararat 19th May 2011.

Kennison, K.R., Ward, G., Diggle, A., Saam-Renton, M., Airey, M., Fisher, D., Haswell, D., Gillard, J. and Gibberd, M. (2011) Smoke effect systems development. Oral presentation to National Smoke Taint Meeting, Melbourne, Victoria 11th February 2011.

Kennison, K.R. (2011) Western Australian research on smoke derived taint in grapes and wine: smoke effect research update. Oral presentation at Department of Environment and Conservation Bushfire Smoke Meeting, Pemberton, 9th February 2011.

Kennison, K.R., Ward, G. and Gibberd, M.R. (2009) Western Australian research on smoke derived taint in grapes and wine: aiming for a national smoke effect reduction system. Oral presentation to wine industry, faculty and students at Washington State University, Prosser, USA 12th November 2009.

Kennison, K.R., Ward, G. and Gibberd, M.R. (2009) Western Australian research on smoke derived taint in grapes and wine: aiming for a national smoke effect reduction system. Oral presentation to wine industry, faculty and students at Oregon State University, Corvallis, USA 9th November 2009.

Kennison, K.R., Wilkinson, K.L., Williams, H.G., Ward, G. and Gibberd, M.R. (2009) Reducing the risk of smoke effect in grapes and wine. Oral presentation to Grape and Wine Research and Development Corporation Regional Meeting, Mt Barker, 22nd October 2009.

Kennison, K.R., Wilkinson, K.L., Williams, H.G., Ward, G. and Gibberd, M.R. (2009) Reducing the risk of smoke effect in grapes and wine. Department of Agriculture and Food WA Grape and Wine Seminars Western Australia. Margaret River 21st, Manjimup 22nd and Mount Barker 23rd September 2009.

Kennison, K.R. (2009) Smoke model system development. Oral presentation at Viticulture Framework meeting. Perth, WA 4th September 2009.

Kennison, K.R. (2009) Smoke derived taint in grapes and wine: research update. Oral presentation to the Smoke Effect Working Group and industry. Perth, WA 26th February 2009.

Kennison, K.R., Wilkinson, K.L., Williams, H.G. and Gibberd, M.R. (2008) WA smoke taint research program: delivering outcomes for industry. Oral presentation to members of the Smoke Effect Working Group, industry and researchers. Bunbury, WA 25th January and Manjimup, WA 15th August 2008.

Kennison, K.R. (2008) WA smoke taint program: delivering outcomes for industry. Oral presentation at the National Smoke Effect Working Group Technical Workshop. Melbourne, Victoria 29th July 2008.

Kennison, K.R. (2008) Smoke effect research. Oral presentation at the Horticulture Program Forum, Department of Agriculture and Food WA, Mandurah, WA. 9th May 2008.

Kennison, K.R., Wilkinson, K.L., Gibberd, M.R. and Ward, G. (2007) Smoke derived taint in grapes and wine. Oral presentation at Victorian Smoke Effect Regional Seminars in Knoxfield, 11th, Oxley 12th and Nagambie 13th April 2007.

Kennison, K.R. (2007) Smoke derived taint in grapes and wine. Agricultural Research WA Landmark Seminar Series. Cowaramup, WA. 15th June 2007.

Kennison, K.R., Wilkinson, K.L., Gibberd, M.R. and Ward, G. (2007) Smoke derived taint in grapes and wine. Oral presentation at National Smoke Effect Technical Forum. Milawa 12th April 2007.

Kennison, K.R., Wilkinson, K.L., Gibberd, M.R. and Ward, G. (2007) Smoke derived taint in grapes and wine. Oral presentation to members of the Smoke Effect Working

Group and wine industry. Bunbury, WA 16th February and Manjimup, WA 3rd May 2007.

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Kennison, K.R. (2005) Bushfire generated smoke taint in grapes and wine. Oral presentation at the Horticulture Program Forum, Department of Agriculture and Food WA, Mandurah, WA. 23rd September 2005.

Press and media interviews

Kennison, K.R., Wilkinson, K.L. and Gibberd, M.R. (2009) Latest developments in the investigation of smoke effect in grapes and wine. Department of Agriculture and Food WA, Wine Industry Newsletter No. 90.

Jones, L., Krstic, M., Kennison, K. and Simos, C. (2008) Workshop discussion guides the future of smoke taint research. Australian Viticulture Vol. 12 No. 5 pp. 58-59.

Ward, G., Kennison, K. and Gibberd, M. (2008) Decision making enhanced by smoke taint research. Australian Viticulture Vol. 12 No. 3 pp. 98-99.

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Kennison, K.R., Wilkinson, K.L., Pollnitz, A.P. and Gibberd, M.R. (2007) The timing and duration of grapevine exposure to smoke effects the chemical composition of wine. Department of Agriculture and Food WA, Wine Industry Newsletter No. 84.

Kennison, K.R. (2005) Bushfire generated smoke taint in grapes and wine. Department of Agriculture and Food WA, Wine Industry Newsletter No. 77.

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CHAPTER 1 INTRODUCTION AND OVERVIEW

1.1 INTRODUCTION

Smoke derived taint in grapes and wine is an issue of increasing frequency of occurrence and severity for the wine industry internationally. Grapevines exposed to smoke during sensitive periods of growth can result in the occurrence of smoke-like aromas, flavours and smoke derived volatile compounds in the grapes and subsequent wines. Such wines can exhibit 'smoky', 'salami', 'smoked salmon', 'burnt', 'ash' and 'ashtray' sensory characteristics (Høj et al. 2003) and are often unfit for purpose. Wine regions internationally have been adversely affected by smoke taint resulting in a decrease in product quality, damage to wine brands, economic and social costs with Australian, Mediterranean, South African, Californian and British Columbian wine regions adversely impacted by smoke taint during the past decade (Høj et al. 2003, Krstic et al. 2007, Mira de Orduña 2010, Zybach et al. 2009). Due to the warming climate of Earth, an increase in fire-risk weather events is occurring (Hennessy et al. 2005, Pitman et al. 2007, Westerling et al. 2006), resulting in increased smoke exposure to grapevines and frequency of smoke taint in wine (Mira de Orduña 2010).

In the few published reports of smoke taint in wine that exist, a consistent theme of the lack of scientific research on the issue is apparent (Høj et al., Sheppard et al. 2009). When this study commenced, smoke taint was a new phenomenon for the wine industry and rapid research was required to assist with understanding and minimising the taint in wine. This PhD research program was therefore undertaken to investigate the effects of smoke exposure to grapevines on the development of smoke taint in wine.

The published papers compiled in this thesis have greatly contributed to the knowledge of smoke taint in grapes and wine. Published Paper 1 entitled *Kennison, K.R., Wilkinson, K.L., Williams, H.G., Smith J.H. and Gibberd, M.R. (2007) Smoke derived taint in wine: effect of postharvest smoke exposure of grapes on the chemical composition and sensory characteristics of wine. Journal of Agricultural and Food*

Chemistry 55, 10897-10901 presents the first known peer-review scientific paper on the topic of smoke taint in grapes and wine. This paper details the purposeful application of smoke to grape bunches post-harvest and demonstrates the direct link between smoke exposure and the occurrence of smoke taint in wine. Research in Paper 1 established a clear sensory difference between unsmoked (control) wines and wines made from fruit exposed to smoke that exhibited 'smoky', 'dirty', 'earthy', 'burnt' and 'smoked meat' characters. This research also identified the presence of volatile phenols guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol and eugenol in wines made from grapes exposed to smoke; compounds that were not detected in unsmoked wines and were therefore attributed to the application of smoke. Wine sensory analysis was also employed to establish the aroma detection threshold of smoke taint in wine.

The timing and duration of smoke exposure was shown to significantly affect the chemical and sensory properties of resultant wines in Published Paper 2. Published Paper 2 entitled *Kennison, K.R., Wilkinson, K.L., Pollnitz, A.P., Williams, H.G. and Gibberd, M.R. (2009) Effect of timing and duration of grapevine exposure to smoke on the composition and sensory properties of wine. Australian Journal of Grape and Wine Research 15, 228-237* is the first known paper detailing the intentional application of smoke to field-grown grapevines between the grapevine growth periods of veraison to harvest. This paper investigated the effect of both the timing and duration of smoke exposure on the chemical composition and sensory properties of wine. Novel research methodology was designed and successfully implemented to apply smoke to field-grown grapevines. The application of smoke to field-grown grapevines was found to influence the accumulation of volatile phenols and smoke aromas such as 'burnt rubber', 'smoked meat', 'leather' and 'disinfectant' in resultant wines. These characteristics were found to accumulate in wines made from fruit of grapevines exposed to repeated smoke applications. The peak period of vine sensitivity to smoke uptake was determined to be at 7 days post verasion.

The concentration of key smoke marker compounds was found to increase throughout the fermentation process, an occurrence that signalled the potential for underestimation

of smoke taint in fruit and juice samples. Published paper 3 entitled *Kennison, K.R., Gibberd, M.R., Pollnitz, A.P. and Wilkinson, K.L. (2008) Smoke-derived taint in wine: the release of smoke-derived volatile phenols during fermentation of Merlot juice following grapevine exposure to smoke. Journal of Agricultural and Food Chemistry 56, 7379-7383* investigated the evolution of smoke taint during the fermentation process. The concentration of smoke marker compounds guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol and eugenol were found in low or undetectable levels in free-run juice but increased throughout alcoholic and malolactic fermentation. Subsequent investigation demonstrated the smoke marker compounds could be released from free-run juice by strong acid (pH 1.0) and enzyme (ß-glucosidase) hydrolysis, but not by mild acid (pH 3.2 to 3.7) hydrolysis, suggesting the presence of precursor forms.

A breakthrough in knowledge regarding the timing of smoke exposure on grape and wine production was gained in Published Paper 4 entitled *Kennison, K.R., Wilkinson, K.L., Pollnitz, A.P., Williams, H.G. and Gibberd, M.R. (2011). The effect of smoke application to field-grown Merlot grapevines at key phenological growth stages on wine sensory and chemical properties. Australian Journal of Grape and Wine Research 17, S5-S12.* This paper examined grapevine sensitivity to smoke uptake throughout the growing season and identified 3 key periods of sensitivity to smoke uptake by vines and the subsequent development of smoke taint characteristics in wine. Period 1 (from the growth stage of shoots at 10 cm to full bloom) showed low levels of smoke uptake and taint development in wine; Period 2 (from berries at pea size through to the onset of varaison) showed variable (low to medium) levels of smoke taint development in wine with Period 3 (from 7 days post veraison to harvest) being a heightened period of grapevine sensitivity to smoke exposure and the development of smoke taint in wine. Fruit yields were also found to be significantly reduced one season subsequent to repeated smoke exposures, however smoke taint was not detectable in wines made from fruit of the same vines harvested in the year following smoke exposure by either chemical or sensory analysis.

A subsequent chapter (unpublished paper) has been added to this thesis to build on the established information regarding the development of smoke taint in grapes and wine. This paper is titled *Kennison, K.R., Williams, H.G. and Gibberd, M.R. (2011) The density and duration of smoke exposure to grapevines influences the development of smoke-like compounds, flavours and aromas in resultant wine* (Paper submitted to the Australian Journal of Grape and Wine Research). This study determined the minimum amount of smoke exposure to field-grown grapevines (5% obs/m for 20 min, 20 % obs/m for 10 min and 10 % obs for 20 min) required to create smoke taint in resultant wines. This is the first paper to demonstrate that the density and duration of smoke exposure to grapevines affects the chemical composition and sensory properties of wine.

1.2 RESEARCH AIMS

The overall aims of this research were to: (i) characterise the effects of smoke exposure to grapevines on the development of smoke taint in grapes and wine; and (ii) to investigate the impact of timing, density, duration and assimilation of smoke components by grapevines. The overall aims and objectives of this thesis are detailed in the thesis flow chart (Figure 1).

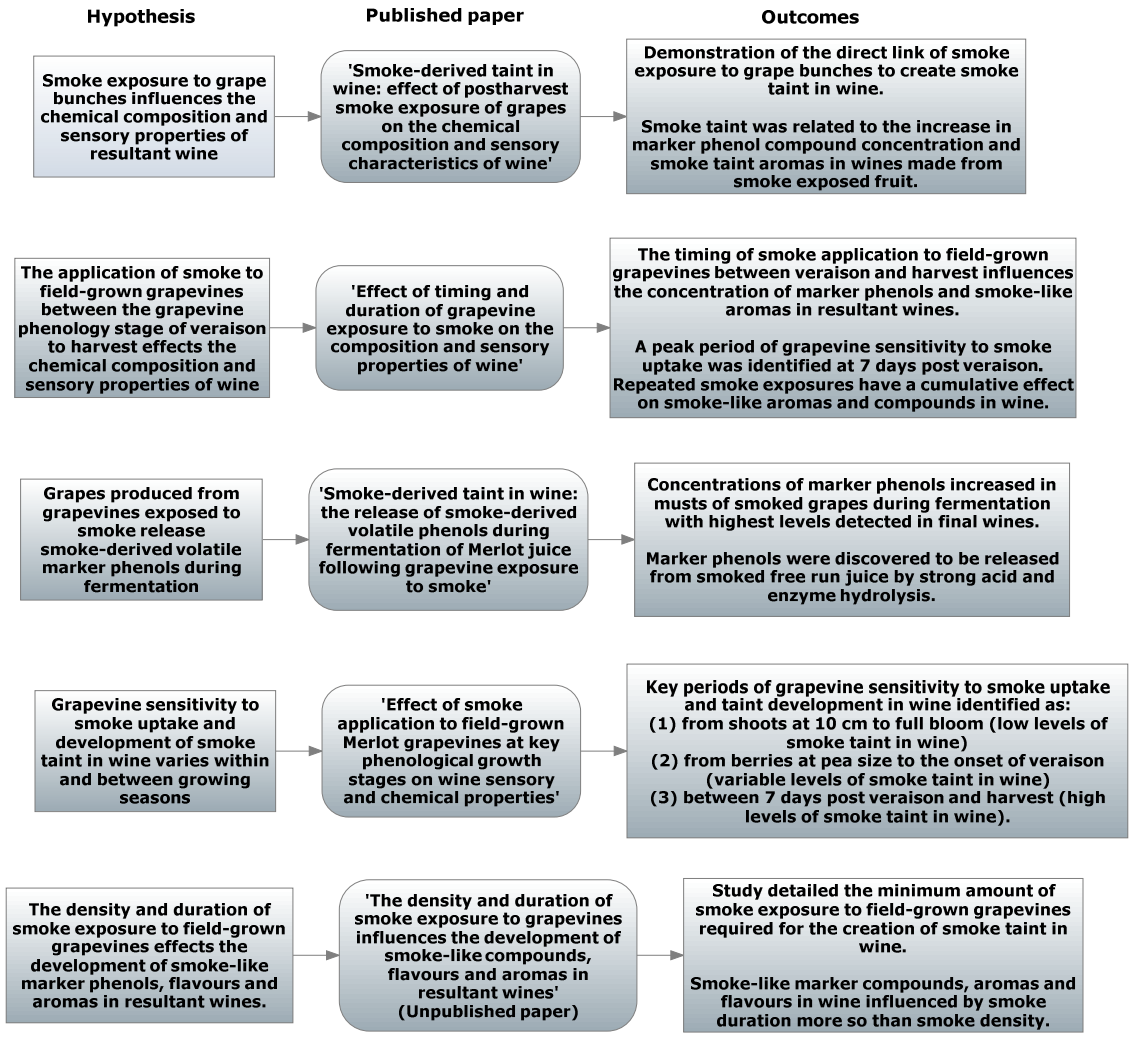

Figure 1. Flow chart of thesis hypotheses, published (and unpublished) papers and direct research outcomes.

The aims and objectives of this study involved the:

- Determination of the effect of smoke exposure to grape bunches on the chemical composition and sensory properties of wine;
- Investigation of the effect of smoke applied to grapevines at various key phenological stages of grapevine growth and development;
- Evaluation of seasonal and yearly effects of smoke exposure on grapevine growth, function and development;

- Identification of specific volatile organic compounds in smoke that contribute smoke taint sensory characteristics in grapes and wine;
- Determination of the effect of variation in smoke concentration and exposure duration on the development of smoke taint in wine;
- Investigation of the effect of fermentation on the development of smoke taint in wine;
- Wine sensory analysis to determine the sensory smoke taint effect on wine made from fruit harvested from grapevines exposed to smoke at key phenological stages of growth and development and with a range of smoke concentrations and durations.

CHAPTER 2 LITERATURE REVIEW

2.1 COMPOSITION OF SMOKE

Smoke contains a multitude of substances that can vary in structure and concentration depending on the origin and composition of the smoke source. Aerosol smoke physically consists of vapour, an aerosol mixture of liquid, gas and solid phases (Simon et al. 2005). Smoke vapour is comprised of inorganic gases such as carbon monoxide, nitrogen dioxide and ozone, as well as volatile and semi-volatile organic compounds (Lee et al. 2005, McKenzie et al. 1994). Particulate matter ($PM_{2.5}$ and PM_{10}), polycyclic aromatic hydrocarbons (PAHs), benzene and aldehydes found to be detrimental to human health are also present in smoke (Austin et al. 2001, Naeher et al. 2007). The emission of these substances in smoke from fires is highly variable. Research has shown volatile organic compounds, particulate matter and CO to be present in a wide range of concentrations in smoke from prescribed fires (De Vos et al. 2009). Furthermore, pyrolytic conditions (including combustion temperature, oxygen availability and fuel moisture content) have been shown to influence the composition of smoke (Baltes et al. 1981, Maga 1988a, Simoneit et al. 1993).

Fuel composition also has an influence on the concentration of smoke components. The variety and composition of fuel as the source for smoke generation is dependent on the plant species and the environment. The chemical composition of wood can not be defined precisely for a plant species due to variation in plant components (root, stem, branch, leaves, bark), type of wood (normal, compression, tension) and growing conditions (Pettersen 1984). However, wood produced from vegetative biomass, such as forested environments, is predominantly comprised of lignin (18-35%), cellulose (40-45%) and hemicellulose (20-35%) (Maga 1989). Other minor compound classes can also be present in wood; for example volatile oils, terpenes (0 to 5%), aliphatic fatty acids (0 to 5%), proteins and phenolic compounds (Maga 1988b). The composition and concentration of such compounds varies depending on fuel type and composition.

Previous studies have investigated the concentrations of smoke-derived volatile compounds emitted during the pyrolysis of major wood components such as cellulose, hemicellulose and lignin (Mohan et al. 2006). These studies have shown the wood component of lignin, and to a lesser extent, polysaccharides to be of interest in the emissivity of major volatile components during pyrolysis (McKenzie et al. 1994, Wittkowski et al. 1992). The pyrolysis products of lignin included phenols and phenol derivatives such as guaiacol, 4-methylguaiacol, 4-ethylguaiacol and syringol (Edye and Richards 1991).

2.2 EFFECT OF SMOKE COMPOUNDS ON GRAPES AND WINES

Smoke has been used for centuries to impart pleasing organoleptic properties to food and for food preservation (Holley and Patel 2005). Food processing technology has advanced to not only include smoking of foods but also the manufacture of smoke flavourings (both in the aqueous phase and as tar residues) that can be added to foods to impart smoke-like sensory and anti-microbial attributes (Bortolomeazzi et al. 2007, Kostyra and Barylko-Pikielna 2006). Numerous studies have investigated the composition of smoke and liquid smoke flavourings in order to identify the compounds responsible for the smoke-like sensory characteristics. These studies have found smoke and smoke flavourings to be comprised of key volatile compounds including phenols, lactones, pyrazines, furans, pyrans, acids and carbonyls (Guillén et al. 1995, Guillén and Ibargoitia 1998, Maga 1988a, Wittkowski et al. 1990, Wittkowski et al. 1992). Of these volatiles the key smoke compounds of interest are guaiacol and 4-methylguaiacol (Baltes at al. 1981, Wittkowski et al. 1992). Guaiacol is known to exhibit ‘smoky’, ‘phenolic’ and ‘chemical’ aromas (Boidron et al. 1988, Eisele and Semon 2005) and 4-methylguaiacol is reported to have ‘toasted’ and ‘ash’ aromas (Boidron et al. 1988).

Guaiacol and 4-methylguaiacol are often detectable in wines that are fermented or matured in toasted oak barrels (Boidron et al. 1988, Maga 1989, Swan 2004). Such compounds are released from barrels during the toasting process and extracted into wine during fermentation and/or storage (Maga 1989). Guaiacol has been detected in wine at

concentrations from 0 to 100 µg/L (Pollnitz et al. 2000), with an aroma detection threshold of 20 µg/L in white wine (Simpson et al. 1986) and 75 µg/L in red wine (Boidron et al. 1988). Whilst 4-methylguaiacol has been detected in wines at lower concentrations, being 0 to 20 µg/L (Pollnitz 2000), with an aroma detection threshold of 65 µg/L in white and red wine (Boidron et al. 1988).

In smoke tainted wine, guaiacol and 4-methylguaiacol have been identified as the most important volatile phenol compounds contributing to the sensory smoke taint (Høj et al. 2003, Sheppard et al. 2009). Sensory analysis of smoke tainted wine has identified 'smoky', 'burnt', 'earthy', 'ashtray', 'dirty' and general 'smoked meat/food' aromas and flavours (Høj et al. 2003, Whiting and Krstic 2007). In studies undertaken in British Columbia, Sheppard et al. (2009) purposefully applied smoke to grapevines and measured guaiacol and 4-methylguaiacol in grapes as key analytes of interest for the detection of smoke taint. Guaiacol and 4-methylguaiacol were detected at elevated concentrations in grapes exposed to smoke (average 10.4 µg/kg and 2 µg/kg respectively) in comparison to control grapes (average 1.2 and 0.7 µg/kg respectively) (Sheppard et al. 2009). Guaiacol and 4-methylguaiacol were positively correlated and elevated concentrations of these compounds, ranging from 2 to 26 µg/L, were detected in the more mature grapes (Sheppard et al. 2009). The sensory characteristics of smoke tainted fruit were not evaluated and wines were not made in this study however authors speculated that the guaiacol concentrations were high enough to warrant sensory detection in final wines.

Studies investigating the effect of smoke on grapes and wine have been limited in number and involved grape analysis only. Sheppard et al. (2009) stated that the observed concentrations of guaiacol (average 10.4 µg/kg) and 4-methylguaiacol (average 2 µg/kg) in smoke tainted grapes would result in subsequent wines being impacted by smoke taint (Sheppard et al. 2009) however as wine was not produced from grapes in this study with the true chemical and sensory smoke taint effects unknown.

Besides guaiacol and 4-methylguaicol, other compounds are known to be present in smoke and to exhibit aromas that could be considered smoke-like and therefore contribute to smoke taint in wine. 4-Ethylguaiacol has been described as having a 'smoky', 'spicy' and 'toasted bread' aroma and 4-ethylphenol a 'horsy', 'stable' and 'phenolic' aroma (Boidron et al. 1988, López et al. 1999). Other research has attributed the smoky aroma of some wines to other compounds, such as benzenemethanethiol, although these wines were not thought to be smoke tainted (Tominaga et al. 2003). Smoke and smoke flavouring contain numerous compounds (Guillén et al. 1995, Guillén and Ibargoitia 1998, Maga 1988a, Wittkowski et al. 1990, Wittkowski et al. 1992) that could potentially contribute to the smoke taint in wine. Presently, guaiacol and 4-methylguaiacol are measured as analytes of interest in grapes and wine as putative markers for the presence of smoke taint to the isolation of numerous other compounds. Researchers are endeavouring to discover additional compounds and their precursors detectable in both smoke exposed grapevine organs and smoke tainted wine that may contribute to the smoke taint (Dungey et al. 2011, Hayasaka et al. 2010b, 2010c). Guaiacol and 4-methylguaiacol are currently the most effective indicators of smoke taint in wine although the full implication and extent of the level of smoke taint in wine is unlikely to be provided by volatile phenol analysis alone.

A number of winemaking and amelioration techniques to reduce negative smoke taint characteristics in wine have been trialled. Ristic et al. (2011) demonstrated reduced skin contact time during fermentation could reduce smoke taint in wine. This resulted in decreased 'smoke' aromas and flavours, whilst 'fruit' aromas and flavour were concurrently enhanced. Interestingly, smoke taint characteristics in wine could be further reduced by the addition of oak chips or tannin during the fermentation process (Ristic et al. 2011). Limited techniques are available for the amelioration of smoke taint in wine. Fudge et al. (2011) investigated the use of solid phase adsorption treatment and reverse osmosis to reduce guaiacol and smoke-like aroma and flavour characteristics in wine. These techniques were found to be successful immediately post amelioration treatment, however the smoke taint was found to slowly return with aging.

2.3 SMOKE EFFECTS ON PLANTS

Studies have investigated the effect of pollution, including chemical and tobacco smoke, on plants (Schaeffer et al. 1987) however information does not exist as to the effect of smoke on fruit, grapes and grapevines. As previously discussed, smoke contains a multitude of components including, but not limited to, carbon monoxide, nitrogen oxides, carbon dioxide, ozone, sulphur oxides and particulate matter (Goode et al. 1999, Radojevic 2003). Many of these smoke components are known to damage plant biochemical processes; for instance ozone has been reported to reduce plant growth; sulfur dioxide can damage plant surfaces resulting in necrotic lesions (Schempp et al. 2005); nitrogen (as NO, NH_3 and NO_2) has been discovered to affect both cellular and whole-plant metabolism (Stulen et al. 1998); and elevated CO_2 has led to the increase of total biomass production including the increase in yield of fruit bearing plants and grapevines (Bindi et al. 2001). Also, heavy smoke exposure with a high concentration of particulate matter can affect the amount of photosynthetically active radiation available to the plant (Yamascoe et al. 2006).

Smoke has been used successfully to enhance the germination response of seeds from numerous plant species (Brown and van Staden 1997). As such, smoke acts as a germination cue, indicating that conditions are favourable for germination and plant production (Brown and van Staden 1997). The mechanism of action of smoke on seeds has been investigated, concentrating on smoke temperature effects (Tieu et al. 2001), aqueous smoke extracts of smoke dissolved in water (Baxter et al. 1994) and desiccating effects (Brown and van Staden 1997). A compound in smoke that promotes the germination of fire dependent species was identified as butenolide 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one (Flematti et al. 2004). Recently, Flematti et al. (2009) have discovered 5 additional germination promoting compounds that are all analogues of the butenolide compound.

Few studies have investigated the direct effect of smoke on plants. Davies and Unam (1999) showed the photosynthetic rates of some tree species (including *Dryobalanops*

rappa, Durio zibethinus and Gonystylus bancanus) to be reduced subsequent to prolonged exposure to smoke produced from Indonesian forest fires. Gilbert and Ripley (2002) investigated the effect of smoke exposure on *Chrysanthemoides monilifera* and discovered a 1 min smoke exposure to reduce stomatal conductance, intercellular CO_2 concentration and CO_2 assimilation rate for 5 hours post-smoke exposure. Calder et al. (2010) applied smoke to 3 deciduous angiosperms and 3 evergreen conifers for 20 min and found, for the majority of species, more than a 50% reduction in photosynthetic capacity.

2.4 SMOKE EFFECTS ON THE GRAPEVINE

Documented research that investigates the occurrence of smoke exposure to grapevines, the effects of this smoke exposure on the function of the grapevine and subsequent development of taint in grapes and wine is limited. Initial investigations into the nature and amelioration of smoke taint in grapes and wine were conducted by The Australian Wine Research Institute (Høj et al. 2003) subsequent to a significant bushfire event in south-eastern Australia. This study predominantly concentrated on the taint characteristics that smoke had created in the grape berries, grape juice and wine with little understanding of the impact of smoke on the grapevine. Høj et al. (2003) investigated the location of smoke taint within the grape berries and discovered the smoke-derived compounds guaiacol and 4-methylguaiacol to be detected in the skins of berries and not in grape berry pulp. It is unknown how the smoke taint compounds came to reside within the skin of the berry, although Hayasaka et al. (2010a) reported guaiacol glycoconjugates to be translocated between grapevine leaves and berries to a limited extent.

The effect of smoke timing, duration and density to field-grown grapevines is currently unknown which has industrial implications for management of the issue. The grapevine phenological growth stages, when smoke exposure is detrimental to fruit quality, and the effects of smoke duration and density have a direct effect on wine grape management and wine production. For instance, knowledge of the effect of smoke exposure to key

phenological growth stages would determine when a risk of smoke taint existed. With knowledge of this risk, wine producers would therefore be able to adjust harvesting and winemaking techniques to minimise the smoke taint. Information is not available on the effect of timing of smoke exposure to grapevines in the literature. This could result in prescribed fires adjacent to vineyards scheduled for ignition at times which could conflict with wine production.

The grapevine (*Vitis vinifera*) is a deciduous perennial plant that has a bi-annual growth cycle comprising phenological growth stages that include bud-burst, fruit initiation, fruit development, harvest and dormancy. Each phenological stage is unique and has been characterised by an identification system referred to as the Modified Eichhorn-Lorenz System (E-L system) (Coombe 1995). The E-L system is an important tool for classification of the grapevine stage of production and is applicable to commercial viticulture regardless of grapevine variety, clone, production system and region. The smoke taint study by Høj et al. (2003) did not convey the stage of grapevine development at which smoke exposure occurred. Sheppard et al. (2009) applied smoke to grapevines at preveraison, postveraison and maturity but unfortunately did not use the E-L system instead recording the number of days post flowering to indicate their smoke exposure timing. Sheppard et al. (2009) numbering system is unable to be applied to other grape producing regions as the number of days for grapevine production can differ depending on the environmental and seasonal conditions (Coombe 1988). Sheppard et al. (2009) found the concentration of guaiacol and 4-methylguaiacol to increase as the grapes matured, with concentrations generally higher in mature grapes. However, the timing and number of smoke exposures were not described and it is therefore difficult to correspond these timings to an exact stage of vine development. Furthermore, the Sheppard et al. (2009) study is inconclusive as it details the effects of smoke exposure to grapes only, ignoring the chemical and sensory effects on the wine product. It is therefore unknown as to which phenological stages of grapevine growth and development are susceptible to smoke uptake and taint development in wine.

Effects of smoke composition on taint development in grapes are unknown. Research documenting the implications of smoke exposure conditions on the development of smoke taint is lacking. Sheppard et al. (2009) applied smoke to grapevines generated from the pyrolysis of 500 g of Ponderosa pine which produced smoke for approximately 1 hour. Whilst the fuel weight is known for this study, the exact density and duration of smoke exposure to the grapevine is unknown. As previously described, smoke is a complex substance that is influenced by combustion conditions. Smoke density, duration, temperature and humidity have been found to influence the chemical composition and sensory properties of smoked foods (Tóth and Potthast 1984, Boyle and Schmidt 1999, Ogbadu 2000) however studies have concentrated on the antimicrobial effect of smoke rather than the sensory effect (Fellows 2009). The effect of smoke duration and density on the development of smoke taint in wine is therefore unknown.

Recent studies have alluded to the mechanism of metabolism of guaiacol within the grapevine. Hayasaka et al. (2010c) found guaiacol to be accumulated in glycosylated forms, i.e. as ß-D-glucopyranoside and was discovered in grape juice samples prepared from grapes exposed to smoke. It was furthermore discovered that additional volatile phenols discovered in bushfire smoke, such as phenol, *p*-, *m*- and *o*-cresols, 4-methylguaiacol, syringol and 4-methylsyringol, could also be detected in glyconconjugate forms in juice of grapes exposed to smoke (Dungey et al. 2011, Hayasaka et al. 2010b). These discoveries endeavour to provide a more accurate estimation of the development of volatile phenols during winemaking and storage, and may be used as a predictive tool to determining the level of smoke taint in grapes though this has yet to be confirmed by wine sensory analysis.

Of the few studies that investigate the effect of smoke on grapes and wine, the majority of these studies have been conducted subsequent to fire events with limited understanding of the smoke conditions that actually existed at such fire events. That is, research has been conducted following fire and smoke events that have occurred in proximity to vineyards and has investigated the chemical and sensory smoke taint effects that have resulted from the smoke exposure to grapevines (Høj et al. 2003, Whiting and

Krstic 2007). However, the research has been unable to quantitatively determine the smoke and grapevine conditions at the timing of smoke exposure. Aspects such as the smoke density and duration are unknown, the grapevine phenological timing of smoke application is unknown and it is even difficult to ascertain whether grapes were actually sampled from grapevines exposed to smoke. These studies have additionally made comparisons of smoke taint between varieties without considering the experimentation variance of grapevine phenological stage or the quantification of smoke exposure (Whiting and Krstic 2007). As such, the investigations are limited in their application to the understanding of the effects of smoke exposure to grapevines.

CHAPTER 3 RESEARCH DESIGN

The research design for each experiment has been well documented in the attached papers. However, additional information detailing specific aspects of the overall research design is presented below.

3.1 SMOKE APPLICATION METHODOLOGY

Initial smoke treatments, for the application of smoke to grape bunches post-harvest, were performed in a purpose built smoke facility located at Kings Park and Botanic Gardens (Perth, Western Australia). This facility was purposely designed for smoke application to native seeds and as such comprised a small metal 'smoke house' structure (3 m L x 3 m H x 3m W) containing racks for the purpose of holding seed trays. This facility was similar to that described by Dixon et al. (1995), with smoke produced within a metal drum (50 L) located adjacent to the facility. Smoke was generated by the combustion of dry fuel and forced, by air, through metal pipes into the smoke house.

For the purpose of applying smoke to potted grapevines and field-grown grapevines the research included the development of a smoke application apparatus. This apparatus was developed utilising two major components including (a) a smoke generator and (b) a tent structure. The smoke generator comprised a galvanised metal drum (50 L) and was

based on the model described by Dixon et al. (1995). The smoke generator had an inlet pipe for air induction and an outlet pipe for smoke expiration. The drum was lidded (galvanised metal) to contain fuel, fire and smoke and mounted on a steel trolley structure for easy manoeuvrability. A remote control variable speed air pump (12 volt) was used to control the amount of air intake, and therefore smoke output from the drum.

The tent structure, used to enclose grape bunches, potted grapevines and field-grown grapevines during the smoke application only was constructed from a galvanised metal frame. The size of the frame varied depending whether the research was for smoke application to field-grown grapevines (6 m L x 2.5 m H x 2 m W) or to potted vines (0.8 m L x 0.9 m H x 1.5 m W). Frames were covered with a greenhouse grade Solarweave plastic (Gale Pacific, Australia) which enabled the containment of smoke and diffusion of sunlight for plant photosynthesis without causing high temperatures. Control (unsmoked) grapevines were similarly enclosed within tents that were considered to be clean and free of smoke, i.e. had not been previously used for smoke treatments. Each tent contained 2 fans to circulate air and smoke around the vines. A generator was employed as a power source to run the air pump and tent fans. Smoke was applied during the morning prior to high temperatures.

In this study, the fuel used for the production of smoke was kept consistent throughout all smoke experimentation. The pyrolysis of fuel can generate a multitude of compounds which is influenced by the fuel species (Maga 1988b). Previous studies investigating the effect of smoke on the germination of native seeds utilised a variety of fuels for smoke production. Many South African studies of smoke and seed germination have utilised smoke produced from native grasses (Baxter et al. 1994, Brown and van Staden 1997, Sparg et al. 2005). Groundbreaking smoke studies conducted in Western Australia that identified compounds in smoke responsible for the germination of native seeds, utilised a basic cellulose and lignin composition fuel including filter paper (Flematti et al. 2004) and straw (Stevens et al. 2007, Flematti et al. 2009) for the production of smoke and smoke water. For the purpose of our experiment, the choice of fuel was based on these previous studies and considered the requirement for the fuel to

be an economical, readily available source. For this purpose dry barley straw was selected as a model fuel. The straw was obtained at the commencement of the project and stored, under dry conditions, for utilisation throughout the duration of the project.

Determining the consistency of smoke in all smoke applications was an important aspect of this research. Many tools are available for the measurement of smoke with varying capabilities and reliabilities (Chow et al. 2008). Typically, these tools include instruments that measure particle concentration and size, carbon concentration, gas emissions (CO_2, CO, NO_x, SO_4) and chemical compounds (Chow 1995). In this study, laser photometry was initially employed for the measurement of smoke particulate matter. A DustTrack laser photometer (TSI Model 8520, TSI Inc., St. Paul, Minnesota, USA) that measured particulate matter ($PM_{10} \leq 10$ µm in diameter) was employed in early experimentation. This equipment was reliable to measure the reproducibility of smoke however did not contain sufficient filters to protect the equipment from smoke damage after repeated use. Further investigation identified more reliable and sophisticated smoke detection equipment, i.e. a nephelometer (Adam et al. 2004). The nephelometer employed in this study (VESDA Laser FOCUS^{TM} VLF-250, Victoria, Australia) was portable and able to measure smoke density and duration within smoke tents. Smoke density was recorded as the percentage of visual obscuration over a distance of one meter and recorded in measurements of % obs/m.

3.1.2 Smoke treatments on field-grown grapevines

Due to the lack of information on smoke exposure to grapevines and the development of smoke taint in wine, the concentration and duration of smoke exposure required to effect smoke taint in wine was unknown. The duration of smoke application to grape bunches in the purpose-built smoke house relied on previous smoke experiments involving smoke application to native seeds using the same facility (Tieu et al. 2001) that resulted in the smoke application for 1 hr. This smoke application was found to be successful in our experiments in creating smoke taint in wine however the concentration of the smoke taint was considered to be high. Subsequent to smoke application to grape bunches in the purpose built smokehouse, applications of smoke to field-grown grapevines were

initiated. Due to the high smoke levels experience from the smoke-bunch experiment, the smoke application to field grown grapevines was reduced to 30 min and maintained at a high smoke exposure of 30% obs/m. The 30 % obs/m smoke exposure for 30 min was found to be effective in creating smoke taint in wine and was therefore maintained for field studies.

Smoke application to field-grown grapevines investigated the effect of the timing of smoke application, throughout the grapevine growth season, on the development of smoke taint in wine. Smoke was applied to *Vitis vinifera* cv. Merlot grapevines once at key Eichhorn-Lorenz (E-L) growth stages (Coombe 1995) from shoots at 10 cm (E-L 12), full bloom (E-L 23), berries pea size (E-L 31), bunch closure (E-L 32), onset of veraison (E-L 35), veraison + 3 days (E-L 35 + 3 d), veraison + 7 days (E-L 35 + 7 d), veraison + 10 days (E-L 35 + 10 d), berries at intermediate sugar content (E-L 36), berries at intermediate sugar content + 3 days (E-L 36 + 3 d), berries not quite ripe (E-L 37) and harvest (E-L 38). This research was conducted over 3 seasons to account for climate variation and to test smoke exposure at all key grapevine growth stages. In order to test the effect of repeated smoke applications, 8 smoke applications were also applied to Merlot grapevines (at an average of 3 day intervals) from veraison to harvest.

As only one smoke density and duration (30% obs/m for 30 min) had been trialled, this study was continued to investigate the effect of lower smoke densities over a range of durations. Smoke was applied to field-grown grapevines at high densities (5, 10 and 20% obs/m) for short durations 5, 10 and 20 min then for one low density (2.5% obs/m) for longer durations (10, 20, 40 and 80 min).

3.2 DETECTION OF SMOKE TAINT IN WINE

Of importance to identifying any issue of taint in grapes and wine is the ability to be able to quantify the presence and intensity of the taint. As the phenomenon of smoke taint in wine is a recent occurrence, the measurement of smoke taint has been limited to the analysis of key marker compounds. Two compounds, guaiacol and 4-methylguaiacol,

were identified as key compounds responsible for the smoke taint in wine (Høj et al. 2003). Guaiacol and 4-methylguaiacol are present in smoke (Baltes at al. 1981, Wittkowski et al. 1992), are known to contribute to the smoke aromas and flavours in wine and to contribute 'smoky', 'phenolic', 'chemical' toasted' and 'ash' aromas and flavours (Boidron et al. 1988, Eisele and Semon 2005). However, smoke contains numerous additional compounds identified as being smoke-like in aroma and flavour (Boidron et al. 1988, López et al. 1999) that could therefore also contribute to smoke taint in wine. Researchers are currently endeavouring to elucidate these compounds and to discover additional compounds that contribute to smoke taint (Dungey et al. 2011, Hayasaka et al. 2010b). Guaiacol and 4-methylguaiacol are effective indicators of smoke taint in wine although are limited in their ability to quantify the entire intensity of the taint. Therefore, in order to fully quantify the intensity of smoke taint in wine, this research has employed both chemical and sensory analysis techniques.

3.2.1 Chemical analysis

In order to quantitatively measure guaiacol and 4-methylguaicol in grapes and wine, gas chromatography-mass spectrometry (GC-MS) analysis has routinely been employed throughout this research. In all research, quantitative analysis was performed by the Australian Wine Research Institute's Analytical Services Laboratory (Adelaide, Australia). Analysis was conducted using an Agilent 6890N gas chromatograph coupled to a 5975 inert source mass spectrometer. The key compounds of interest for analysis in this study were guaiacol and 4-methylguaiacol however analysis of additional compounds, including 4-ethylguaiacol, 4-ethylphenol, eugenol and furfural, were conducted to determine their relative contributions. Analysis was conducted using stable isotope dilution assay methods reported previously (Pollnitz 2000, Pollnitz et al. 2000, Pollnitz et al. 2004).

3.2.2 Organoleptic perceptions of smoke taint in wine

Wine sensory analysis techniques were employed throughout this study to describe and quantify the effects of smoke taint in wine. A combination of sensory methods were

utilised depending on the research objective. Sensory analysis techniques included threshold determination, quantitative descriptive analysis and difference tests (Meilgaard et al. 2007, Lawless and Heymann 1998a and 1998b).

3.2.2.1 Threshold determination

Of initial interest in this study was the determination of the aroma detection threshold of smoke taint in wine. Aroma detection threshold testing was utilised to establish the minimum amount of smoke taint aroma that could be detectable in wine by regular wine consumers. For each wine tested, the aroma detection threshold was represented as the concentration of guaiacol and 4-methylguaiacol in wine that corresponded to the smoke taint character. The aroma detection threshold was determined as per the American Society for Testing and Materials (ASTM) method 679E. This method employed 33 regular wine consumers, aged between 18 and 55 years with similar numbers of males and females. The wines were presented in ascending order of concentration i.e. as dilutions of smoked wines in control wine (0.11, 0.33, 1.0, 3.0, 9.0, 27.0 and 81.0 ml). The wines were presented as part of a triangle test where consumers were asked to detect the sample that was different.

Threshold determination testing was effective to determine the concentration of smoke taint in wine yet throughout this study it was revealed that additional compounds, other than guaiacol and 4-methylguaiacol, were likely to be responsible for the taint. Utilising the aroma detection threshold method was useful in studies of a single compound however a multitude of compounds results in difficulty gaining a true result (Lawless and Heymann 1998a). With smoke taint, this is further compounded by the lack of understanding of the precise compounds in smoke, and therefore smoke tainted wine, contributing to the taint. Additional sensory techniques, such as quantitative descriptive analysis, were therefore employed to gain a greater understanding of the extent of smoke taint in wine.

3.2.2.2 Quantitative descriptive analysis

It was furthermore imperative in this study to employ effective methods of wine sensory analysis that would identify and quantify the intensity of smoke taint. A wine sensory evaluation technique known as Quantitative Descriptive Analysis (QDA) was employed (Meilgaard et al. 2007, Lawless and Heymann 1998b). QDA is a process that involves a small number of participants that generate the language of product attributes to be tested and are trained to rank the intensity of such attributes (Stone and Siddell 2004). In this study, QDA was conducted on two occasions with panels of up to 11 educated wine consumers aged between 21 and 50 years. Panellists were selected based on a number of criteria including their availability, interest, experience (i.e. at least 100 hours of wine education), non-smokers, regular wine consumers, in good health and able to detect the aroma of smoke at a pre-determined threshold. The panellists were trained during 8 training sessions, 2 per week, in preparation for the formal QDA. Wines were assessed for intensity of aroma and flavour attributes. During these training sessions, the panellists isolated and agreed on descriptive terms to be used in the formal QDA. Panellists were further trained to rank the presence and intensity of the descriptive terms on an unstructured 100 point line scale. During formal QDA each panellist received 20 mL of wine in 3 digit coded ISO standard wine tasting glasses. All glasses were lidded to avoid contamination of other wines and the tasting environment. No one panellist received the same sample at the same time as the wine presentation was randomised, with the coding system being unique to each individual panellist. No more than 6 wine samples were evaluated at any one time with panellists leaving the tasting area to an external environment for 10 min between each sample to avoid sensory fatigue.

3.2.2.3 Difference test

Additional wine sensory methods were undertaken to provide a greater understanding of the effect of smoke density and duration on wine. For this purpose, difference testing was employed utilising regular wine consumers (Meilgaard et al. 2007) as per the Australian Standard 2542.2.2 (2005). Due to the low level of smoke exposure to vines in this study, the difference test included tasting of wines. Panellists were pre-screened

to ensure they were regular wine consumers, of good health, non-smokers, interested in wine tasting, available and over the age of 21 years. Each wine was tested a total of 30 times with a total of 130 panellists utilised in this study. During the difference test panellists were presented with 3 wines in a randomised block design of smoke (A) and control (B) wines in a balanced reference (ABB, ABA, AAB, BAA, BAB, BBA). Any one panellist did not receive the same wine sample at the same time. Panellists were required to smell and taste the wines, indicate the sample that was different and to describe why they thought the sample was different.

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CHAPTER 4 PUBLISHED PAPERS

4.1 PUBLISHED PAPER 1

Kennison, K.R., Wilkinson, K.L., Williams, H.G., Smith J.H. and Gibberd, M.R. (2007) Smoke derived taint in wine: effect of postharvest smoke exposure of grapes on the chemical composition and sensory characteristics of wine. Journal of Agricultural and Food Chemistry 55, 10897-10901.

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Smoke-derived Taint in Wine: Effect of Postharvest Smoke Exposure of Grapes on the Chemical Composition and Sensory Characteristics of Wine

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Although smoke exposure has been associated with the development of smoke taint in grapes and subsequently in wine, to date there have been no studies that have demonstrated a direct link. In this study, postharvest smoke exposure of grapes was utilized to demonstrate that smoke significantly influences the chemical composition and sensory characteristics of wine and causes an apparent 'smoke taint'. Verdelho grapes were exposed to straw-derived smoke for 1 h and then fermented according to two different winemaking treatments. Control wines were made by fermenting unsmoked grapes. Sensory studies established a perceivable difference between smoked and unsmoked wines; smoked wines were described as exhibiting 'smoky', 'dirty', 'earthy', 'burnt' and 'smoked meat' characters. Quantitative analysis, by means of gas chromatography–mass spectrometry, identified guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, eugenol, and furfural in each of the wines made from smoked grapes. However, these compounds were not detected in the unsmoked wines, and their origin is therefore attributed to the application of smoke. Increased ethanol concentrations and browning were also observed in wines made from grapes exposed to smoke.

KEYWORDS: Gas chromatography–mass spectrometry; grapes; guaiacol; smoke; taint; *Vitis vinifera*; wine

INTRODUCTION

Taint in grapes and wine as a consequence of grapevine exposure to smoke has resulted in a decline in product quality and significant financial losses for wine producers throughout the world. To date, the effects of smoke on either grapevine physiology or the organoleptic properties of grapes and wine have not been reported in the literature. However, some preliminary investigations have been carried out by the Australian Wine Research Institute (*1*). The role of smoke in stimulating the germination of dormant seeds of some native plant species has been well documented (for example refs *2*–*4*), and the effect of smoke on the photosynthetic gas exchange of *Chrysanthemoides monilifera* has been reported (*5*), but largely, research relating the effect of smoke on plant physiology and on the composition of fruit and fruit-derived products is limited.

Smoke and liquid smoke flavoring preparations have long been employed by the food industry to enhance the aroma, flavor, and color characteristics of foodstuffs, in particular, meat, fish, and cheese (*6*–*8*). Consequently, considerable research has been undertaken to establish the chemical composition of such preparations. Smoke is generated during the pyrolysis (combustion) of wood, with the composition dependent upon the fuel composition, particle size, moisture content, combustion temperature, and availability of oxygen (*9, 10*). Wood is primarily composed of cellulose, hemicellulose, and lignin, contributing 40−45%, 20−35%, and 18−35% of total dry weight, respectively (*11*). During the pyrolytic process, thermal degradation of wood components generates a complex mix of volatile organic compounds. Numerous volatile compounds have been reported in smoke, smoke flavoring preparations, and smoked food products, including phenol derivatives, carbonyls, organic acids and their esters, lactones, pyrazines, and furan and pyran derivatives (*6, 10, 12, 13*). Of these, smoke aroma has primarily been attributed to the phenol derivatives (*7, 10*); in particular, guaiacol (2-methoxyphenol) and 4-methylguaiacol, which exhibit 'smoky', 'phenolish', 'burning wood', 'ash', 'sharp', 'sweet', 'burnt' and 'smoked bacon' aroma characters (*7, 14, 15*).

Guaiacol and 4-methylguaiacol are routinely identified in wines matured in oak barrels, at concentrations between 10 and

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Table 1. Aroma Detection Thresholds for Guaiacol and 4-Methylguaiacol in Water, Model Wine, White Wine, and Red Wine[a]

	aroma detection threshold (μg/L) in			
	water	model wine	white wine	red wine
guaiacol	5.5	20	95	75
4-methylguaiacol	10	30	65	65

[a] Reference *15*.

100 μg/L for guaiacol and between 1 and 20 μg/L for 4-methylguaiacol (*16*), both of which are derived from lignin degradation (*7*) during the toasting process of cooperage. The aroma detection thresholds of guaiacol and 4-methylguaiacol in water and wine are given in **Table 1** (*15*).

The contribution of oak-derived guaiacol to wine aroma has been previously reported. In an oak-aged Chardonnay, the concentration of guaiacol has been shown to positively correlate to the perceived intensity of the smoky aroma (*17*), whereas Rapp and Versini (*18*) found guaiacol to have a negative effect on wine aroma at concentrations exceeding 80 μg/L. Simpson et al. (*19*) found guaiacol to be responsible for an off-flavor in wine; the taint, originating from contaminated corks, was attributed to guaiacol levels ranging from 0.07 to 2.63 mg/L; a detection threshold of 20 μg/L was also reported in this study.

It is therefore conceivable that guaiacol and other phenol derivatives could accumulate in grapes as a result of smoke exposure, and at elevated concentrations, they could lead to an apparent taint. This study was undertaken to test this hypothesis and to demonstrate that smoke exposure of grapes can influence the chemical composition and sensory characteristics of wine.

MATERIALS AND METHODS

Smoke Treatment. Smoke treatments were performed in a purpose-built smoke house (3 × 3 × 3 m) located at the Kings Park and Botanic Gardens (Perth, Western Australia), similar to that described by Dixon et al. (*20*). Whole bunches of grapes were placed on wire racks within the smoke house and exposed to smoke generated by the combustion of dry straw in a metal drum (50 L), for one hour at ambient temperature (25 °C). Following smoke exposure, bunches were randomly mixed to reduce variation in smoke exposure.

Winemaking. Verdelho grapes (350 kg) were harvested when the total soluble solids (TSS) of the grapes reached 24 ± 0.5 °Brix, and a portion (130 kg) of the fruit was separated and exposed to smoke, as described above. The fruit was divided into parcels (approximately 60 kg each), two smoked fruit parcels and two unsmoked fruit parcels. Each fruit parcel was (separately) stored overnight in cool rooms (5 °C) and allowed to warm to ambient temperature (18 °C) before being crushed and destemmed. The fruit parcels were then processed and fermented to produce four experimental wines: (i) a wine made from free run juice of unsmoked grapes, the 'unsmoked free run' treatment; (ii) a wine made from free run juice of smoked grapes, the 'smoked free run' treatment; (iii) a wine made from free run juice fermented on skins from unsmoked grapes, the 'unsmoked free run on skins' treatment; and (iv) a wine made from free run juice fermented on skins of smoked grapes, the 'smoked free run on skins' treatment. These winemaking methods were specifically chosen to reflect commercial white and red wine production, that is, clarification and primary fermentation only for white wine production, and oxidative primary fermentation with skin contact, followed by malolactic fermentation, for red wine production. For free run wines, must was pressed immediately, the juice and pressings were combined, and tartaric acid was added to adjust the pH to 3.4 prior to settling (3 days at 5 °C). The clarified juice was then separated into 15 L demijohns (three replicates per treatment) and inoculated with EC1118 yeast (Lallemand Inc., Montreal, Canada). Following primary fermentation, the wines were racked and free SO_2 adjusted (to 30 ppm) before being cold stabilized (−2 °C for 7 days), filtered, and bottled. For free run on skins wines, must was separated into 30 L fermentation vessels (three replicates per treatment), tartaric acid added to adjust the pH to 3.4, and the samples were inoculated with EC1118 yeast. The fermenting musts were plunged twice per day, and the wine was pressed at a total soluble solids level of 3.6 °Brix. The wines were transferred to 15 L demijohns and held at 25 °C until the residual sugar approached 0 g/L. The wines were then racked from gross lees and inoculated with OENOS culture (Chr. Hansen, Hoersholm, Denmark). Following malolactic fermentation, the wines were again racked and free SO_2 adjusted (to 30 ppm) before being cold stabilized (−2 °C for 7 days), filtered, and bottled. The remaining unsmoked fruit (100 kg) was fermented as above to produce a base free run wine and a base free run on skins wine, for the purpose of blending for sensory trials. Ethanol concentrations were determined by distillation, alcohol hydrometry, and spectral measurements according to the method described by Iland et al. (*21*).

Gas Chromatography–Mass Spectrometry Analysis. Quantitative analyses were performed by the Australian Wine Research Institute's Analytical Services Laboratory (Adelaide, Australia), using an Agilent 6890N gas chromatograph coupled to a 5975 inert source mass spectrometer. Guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, eugenol, and furfural were quantified by the stable isotope dilution assay methods reported previously (*16, 22–24*).

Difference Testing of Smoked and Unsmoked Wines. Difference tests were conducted using the triangle test method described by Meilgaard et al. (*25*), using a panel of 24 members. Panelists were of European origin, aged between 18 and 55, with similar numbers of males and females. Wines were presented to the panel using a balanced, randomized presentation order where all possible configurations (ABB, BAA, AAB, BBA, ABA, BAB, where A denotes unsmoked wine and B denotes smoked wine) were presented an equal number of times. Panelists assessed two sets of wine; one set comprised of wines made from free run and one set comprised of wines made from free run on skins. Panellists smelt, but did not taste the samples, and were asked to identify the sample within each set that was different.

Aroma Detection Thresholds of Taint in Smoked Wines. The detection threshold of smoke taint in free run wine was determined according to the American Society for Testing and Materials (ASTM) method 679E, using 33 judges. Judges were of European origin, aged between 18 and 55, with similar numbers of males and females. Wines were presented (as part of a triangle test) in ascending order of concentration spaced by a factor of 3, with the smoked free run wine (0.11, 0.33, 1.0, 3.0, 9.0, 27.0, and 81.0 mL) diluted with base free run wine to 250 mL. Panellists smelt, but did not taste the samples. Those panellists who could detect the spike at all of these concentrations were then tested at lower concentrations; conversely, those who could not detect the spike at any of the concentrations were tested at higher concentrations. The detection threshold of smoke taint in free run on skins wine was determined in the same manner.

Statistical Methods. Data were analyzed by two-way analysis of variance (ANOVA) using GenStat (9th Edition, VSN International Limited, Herts, UK). Mean comparisons were performed by least significant difference (LSD) multiple comparison tests at $P < 0.05$.

RESULTS AND DISCUSSION

Postharvest smoke exposure by grapes resulted in detectable differences in the chemical composition and sensory characteristics of wine. Difference tests (*25*) established a clearly perceivable difference in the aroma profile of smoked and unsmoked wines. The sensory panel, comprising 24 judges, scored 22 correct responses for the free run wine set and 24 correct responses for the free run on skins wine set. These results indicate smoked wines and unsmoked wines are significantly different at the 99.9% confidence level; hence, smoke exposure of grapes prior to vinification alters wine quality.

The detection thresholds of smoke taint were then determined to evaluate the intensity of the taint and the potential for its reduction through blending. Thresholds (*25*) are reported as the volume of smoked wine (free run or free run on skins) diluted

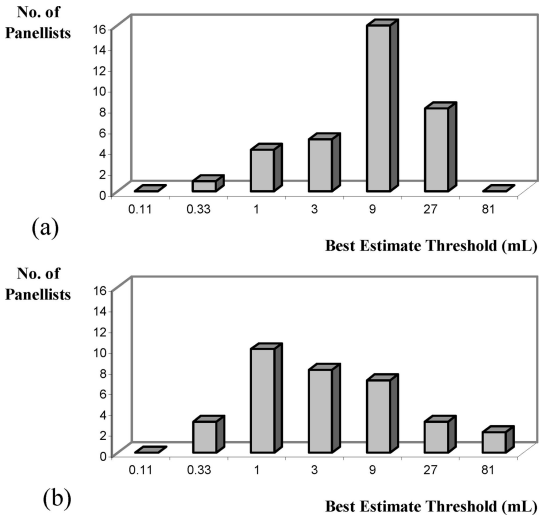

Figure 1. Histograms showing best-estimate threshold distributions for (**a**) smoke taint in smoked free run wine, and (**b**) smoke taint in smoked free run on skins wine.

with base wine (to 250 mL). For each smoked wine, a group threshold was calculated as the geometric mean of each panelist's individual best-estimate threshold, which was the geometric mean of the highest concentration missed and the next highest concentration tested. The aroma thresholds were calculated to be 3.9 mL for the smoked free run wine and 1.9 mL for the smoked free run on skins wine, corresponding to dilutions of 1.6 and 0.8% of original concentrations, respectively. The distributions of best-estimate thresholds for individual panelists are shown in **Figure 1**. The difference and detection threshold tests indicate that smoke exposure has a significant effect on the sensory characteristics of wine. Furthermore, in this study, the taint persisted even with high levels of dilution (by more than 98%), thus limiting options for blending.

The process by which smoke is generated involves the pyrolysis of wood (or other plant material) and is reminiscent of the toasting process of barrel cooperage. Both involve the thermal degradation of structural components, cellulose, hemicellulose, and lignin, resulting in the generation of volatile organic compounds. Stable isotope dilution assays have been developed to quantify oak-derived flavor compounds of organoleptic significance (including guaiacol and 4-methylguaiacol) in oak extracts and barrel-aged wines (*16, 22–24*). These assays were employed to ascertain the composition of smoked and unsmoked wines, and the results obtained indicate a significant treatment effect due to smoke exposure. Guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, eugenol, and furfural were detected in wines made from smoked grapes, but not in wines made from unsmoked grapes, irrespective of the winemaking methods employed (**Table 2**).

The smoked wines, both free run and free run on skins, contained unusually high levels of guaiacol (1470 and 969 μg/L, respectively) and 4-methylguaiacol (326 and 250 μg/L, respectively). Typically, wines aged in oak (and not affected by smoke exposure) contain guaiacol and 4-methylguaiacol at concentrations of between 10 and 100 μg/L and between 1 and 20 μg/L, respectively (*16*). It should be noted that guaiacol has also been identified as a component of acid and enzyme hydrolysates prepared from Merlot and Shiraz juice, at concentrations up to 50 μg/L, apparently deriving from grape

Table 2. Concentrations of Guaiacol, 4-Methylguaiacol, 4-Ethylguaiacol, 4-Ethylphenol, Eugenol, Furfural, 5-Methylfurfural, and Vanillin Present in Smoked and Unsmoked Wines

	concentration[a] (μg/L) in			
	smoked free run	unsmoked free run	smoked free run on skins	unsmoked free run on skins
guaiacol	1470 a	n.d.	969 b	n.d.
4-methylguaiacol	326 a	n.d.	250 b	n.d.
4-ethylguaiacol	128 a	n.d.	111 b	n.d.
4-ethylphenol	59 a	n.d.	67 b	n.d.
eugenol	20 a	n.d.	26 b	n.d.
furfural	16 a	n.d.	13 b	n.d.
5-methylfurfural	n.d.	n.d.	n.d.	n.d.
vanillin	n.d.	n.d.	n.d.	n.d.

[a] Values followed by a different letter within rows are significantly different; n.d.) not detected. Mean values from three replicates. Values were in agreement to ca. 5%.

shikimic acids (*26, 27*). However, in the present study, guaiacol could not be detected in unsmoked wines, and its origin is therefore attributed to the application of smoke. Previous studies (*1*) have indicated that smoke-derived guaiacol and 4-methylguaiacol preferentially accumulate in the skins of grapes, so in our study, they were expected to occur at higher concentrations in the wines made from smoked grapes fermented on skins. That higher concentrations were instead observed in the smoked free run wines suggests permeation of smoke into the grape berry. The lower levels of guaiacol derivatives in smoked free run on skins wines might also be attributed to winemaking conditions, that is, the loss of volatile compounds through either volatilization due to the higher fermentation temperatures and oxidative conditions or adsorption by grape skins. It is important to note that, in this study, permeation may reflect a relatively high intensity of smoke exposure and the fact that smoke was applied postharvest to bunches, whereas previous studies were based on field applications. Indeed, postharvest application was selected as a treatment to minimize potential confounding effects of field exposure (such as time, intensity, and smoke type). Regardless, the guaiacol and 4-methylguaiacol concentrations measured far exceed both detection threshold concentrations and concentrations typically reported in barrel-aged wines. Consequently, at these levels, both compounds would undoubtedly contribute to the intense smoky character evident in the smoked wines.

4-Ethylguaiacol, 4-ethylphenol, eugenol, and furfural were also detected in the smoked wines, albeit within concentration ranges previously reported in wine (*15, 22*). Therefore, although the presence of these compounds is attributed to postharvest smoke exposure, they are unlikely to be key contributors to smoke taint. Interestingly, in wine, 4-ethylguaiacol and 4-ethylphenol are typically formed from grape-derived *p*-coumaric acid and ferulic acid (respectively) through the action of *Brettanomyces/Dekkera* yeast (*15, 28*). In this study, the absence of these compounds in the unsmoked wines instead supports a formation pathway involving the thermal degradation of lignin, such as proposed by Fiddler et al. (*29*). 5-Methylfurfural and vanillin were not detected in any of the wines made from smoked grapes. These compounds were either not formed at detectable levels under the conditions employed in this experiment or they experienced further degradation. Vanillin has been reported as an intermediate in the thermal degradation of lignin, with its decomposition yielding vanillic acid and guaiacol (*29*).

Quantitative GC-MS analysis established that the detection thresholds of smoke taint correspond to guaiacol and 4-methylguaiacol concentrations of 23 and 5 μg/L, respectively, for the

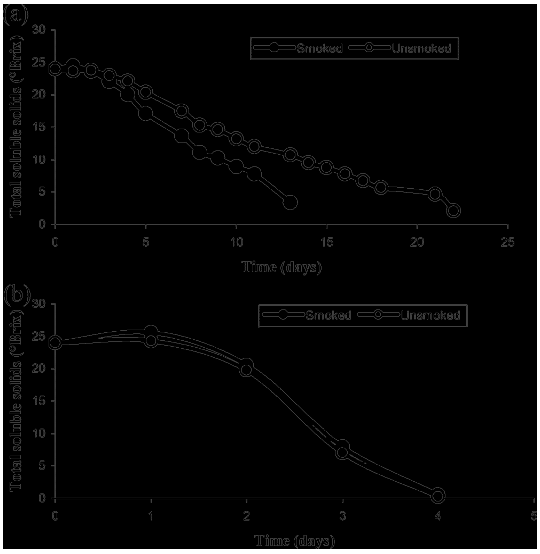

Figure 2. Fermentation curves for (**a**) smoked and unsmoked free run wines, and (**b**) smoked and unsmoked free run on skins wines. Mean values from three replicates; standard errors are obscured by symbols, so they are not shown, but they were <0.5 in all cases.

smoked free run wine and 7 and 2 μg/L, respectively, for the smoked free run on skins wine. Because these concentrations are near or below the detection thresholds reported for guaiacol and 4-methylguaiacol (*15, 19*), we therefore conclude that neither is solely responsible for the perception of smoke taint. The smoke taint threshold concentrations are also strongly supportive of this, with an increased threshold observed for the smoked free run wine relative to the smoked free run on skins wine; that is, threshold concentrations did not correlate with guaiacol and 4-methylguaiacol concentrations. It is quite likely that additional smoke-derived volatile compounds contribute to the taint observed in wines made from smoked grapes, and identification of these compounds is the subject of ongoing research. Nevertheless, guaiacol and 4-methylguaiacol are useful as indicators of smoke taint.

To investigate the development of smoke taint during the winemaking process, grapes were vinified according to two different winemaking methods, reflecting commercial white and red wine production. Free run wines were clarified (3 days at 5 °C) prior to fermentation, and free run on skins wines were fermented in open vessels with skin contact followed by malolactic fermentation. In the case of free run wines, postharvest smoke exposure resulted in an increased fermentation rate (completing fermentation 9 days earlier), but showed no effect on the fermentation rate of free run on skins wines (**Figure 2**).

Significant differences in ethanol concentrations and wine color were also observed between smoked and unsmoked wines (**Table 3**). Smoked wines had higher alcohol contents than their corresponding unsmoked wines, indicating a higher attenuation of sugars to ethanol during the fermentation process. Wines fermented on skins showed increased levels of brown pigments as compared with free run wines; this is not unexpected, because of the oxidative nature of this winemaking method. However, smoked wines also exhibited increased browning as compared with their corresponding unsmoked wines, irrespective of the winemaking methods employed. The effect of smoke exposure on both fermentation rate and development of brown pigments

Table 3. Ethanol Content and Color Analysis of Smoked and Unsmoked Wines[a]

	smoked free run	unsmoked free run	smoked free run on skins	unsmoked free run on skins
ethanol content (% v/v)[b]	14.3 a	14.1 b	14.7 c	13.8 d
estimated brown pigments (au)[c]	0.097 a	0.060 b	0.203 c	0.142 d

[a] Values followed by a different letter within rows are significantly different. [b] Mean values from three replicates; values were in agreement to ca. 0.5%. [c] Mean values from three replicates; values were in agreement to ca. 10%.

in white wine is the subject of ongoing further study. We anticipate that these observations may be explained by the effect of smoke compounds on membrane integrity within the grape berries and skins. Smoke exposure is likely to damage membrane-bound processes and, as such, may possibly lead to the release of proteases and other cellular enzymes associated with injury response. This response to smoke may then have the potential to considerably alter berry chemistry prior to fermentation, an effect which may have been exacerbated by our postharvest treatment.

In this trial, dry straw was chosen as a model fuel for the application of a cold smoke treatment. Like wood, straw comprises cellulose, hemicellulose, and lignin, and its pyrolysis was therefore expected to generate smoke of similar composition to wood-derived smoke. The use of dry straw also enables the reproducible generation of smoke, as employed in current field trials involving the application of smoke to grapevines. Although it is recognized that forest fuels may contribute a broader range of potential smoke taint compounds the complexity of fuel composition, burn rates, combustion temperatures, and environmental conditions are confounding influences and are the subject of further studies.

This study has demonstrated a direct link between the smoke exposure of grapes and the development of smoke taint in subsequent wines. Smoke taint was readily perceived by sensory analysis, with the sensory panel able to detect the taint at dilutions of less than 2% of the original concentration. Further studies involving field exposure of grapevines to smoke are currently underway.

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Effect of timing and duration of grapevine exposure to smoke on the composition and sensory properties of wine

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Abstract

Background and Aims: Grapevine smoke exposure has been reported to produce smoke aromas in wine, resulting in 'smoke taint'. This study describes the application of smoke to field-grown grapevines between veraison and harvest to investigate the effect of timing and duration of smoke exposure on wine composition and sensory attributes.

Methods and Results: Smoke was applied to grapevines as either a single smoke exposure to different vines at veraison or at 3, 7, 10, 15, 18 or 21 days post-veraison or repeated smoke exposures to the same vines at veraison and then at 3, 7, 10, 15, 18 and 21 days post-veraison. Gas chromatography-mass spectrometry analysis of guaiacol, 4-methylguaiacol, 4-ethylguaiacol and 4-ethylphenol showed elevated levels in all wines produced from fruit from smoked grapevines. Repeated smoke exposures had a cumulative effect on the concentration of these compounds. A trained sensory panel identified the aromas of 'burnt rubber', 'smoked meat', 'leather' and 'disinfectant' in all wines derived from smoke-exposed grapevines but not in control wines.

Conclusions: Smoke application to field-grown grapevines between veraison and harvest can influence the accumulation of volatile phenols and intensity of smoke aromas in resultant wines. A peak period of vine sensitivity to smoke at 7 days post-veraison is identified. Repeated smoke exposures have a cumulative effect.

Significance of the Study: This is the first study to report the deliberate and controlled smoke application to field-grown grapevines demonstrating the timing and duration of smoke exposure to significantly affect wine chemical and sensory characters.

Abbreviations

BET best estimate threshold; **FAN** free amino nitrogen; **GC-MS** gas chromatography-mass spectrometry; **PCA** principal component analysis; **TSS** total soluble solids.

Keywords: *gas chromatography-mass spectrometry, grapevines, guaiacol, smoke taint*, Vitis vinifera, *volatile phenols*

Introduction

Postharvest smoke exposure of grapes has been shown to influence the chemical composition and sensory characteristics of wine with the potential to cause an apparent 'smoke taint' (Kennison et al. 2007). However, to date, smoke has not been deliberately applied to grapevines in a field situation to determine the impact on grape and wine composition under controlled conditions. Furthermore, the effect of timing and duration of grapevine smoke exposure on the development of smoke taint in wine has not been previously investigated. As such, scientific literature relating to the in-field exposure of grapevines to smoke in the development of smoke taint is scant despite the issue's high relevance to viticulture in Australia and overseas.

Smoke is a highly complex substance, comprising particulate matter, carbon monoxide, carbon dioxide, polycyclic aromatic hydrocarbons, ozone (O_3), various oxides of nitrogen and sulfur as well as a multitude of volatile and semi-volatile organic compounds (McKenzie et al. 1994, Nolte et al. 2001, Radojevic 2003, Reisen and Brown 2006). The composition of smoke can vary greatly depending on both the fuel source and pyrolytic conditions, in particular combustion temperature, oxygen

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availability, and moisture content (Baltes et al. 1981, Maga 1988, Simoneit et al. 1993).

Smoke can impart desirable organoleptic properties to foods. These are largely attributed to the presence of smoke-derived volatile compounds including phenols, carbonyls, acids, esters, lactones, pyrazines, furan and pyran derivatives (Maga 1988, Wittkowski et al. 1990, McKenzie et al. 1994, Guillén et al. 1995, Guillén and Ibargoitia 1998, Fine et al. 2001). Of these volatiles, guaiacol and 4-methylguaiacol are considered to be key smoke components (Baltes et al. 1981, Wittkowski et al. 1992). They are derived from the thermal degradation of wood lignin during combustion and exhibit 'smoky', 'musty', 'caramel', 'burning', 'sweet', 'phenolic', 'sharp', and 'smoked sausage' aromas and flavours (Baltes et al. 1981, Boidron et al. 1988, Wittkowski et al. 1992, Rocha et al. 2004).

In wine, guaiacol and 4-methylguaiacol typically originate from oak barrel fermentation and/or maturation (Boidron et al. 1988, Maga 1989, Swan 2004) at concentrations of up to 100 and 20 mg/L for guaiacol and 4-methylguaiacol, respectively (Pollnitz et al. 2004). However, a range of volatile phenols, including guaiacol, 4-methylguaiacol, 4-ethylguaiacol and 4-ethylphenol, have recently been identified in juice, unwooded wine, acid and enzyme hydrolysates prepared from smoke-affected grapes (Kennison et al. 2007, 2008). Because these compounds were absent from the corresponding control samples (i.e., unsmoked grapevines), their origin was attributed directly to smoke exposure.

The aroma descriptors, aroma detection thresholds and wine concentrations reported for guaiacol, 4-methylguaiacol, 4-ethylguaiacol, and 4-ethylphenol are shown in Table 1. Because guaiacol exhibits the lowest aroma detection threshold (Boidron et al. 1988) and was the most abundant volatile phenol detected in smoke-tainted wines (Kennison et al. 2007, 2008), it is considered to be of greatest importance. Boidron et al. (1988) reported aroma thresholds for guaiacol in various media – 5.5 mg/L in water, 20 mg/L in model wine, 95 mg/L in white wine and 75 mg/L in red wine – whereas Simpson et al. (1986) reported a lower detection threshold, just 20 mg/L, for guaiacol in a dry white table wine. However, the detection thresholds for guaiacol and 4-methylguaiacol may in fact be even lower than these earlier data. Indeed, Eisele and Semon (2005) suggest that guaiacol is by far more potent. They determined the best estimate threshold for guaiacol to be 0.48 mg/L in water and 0.91 mg/L in apple juice, with even lower taste detection thresholds reported at 0.17 mg/L and 0.24 mg/L in water and apple juice, respectively. In a previous study involving the postharvest application of smoke to grape bunches, a perceptible 'smoke taint' was still evident after considerable blending to achieve sub-threshold concentrations of guaiacol and 4-methylguaiacol (Kennison et al. 2007). This suggests that guaiacol and 4-methylguaiacol might not be solely responsible for smoke taint in wine (Kennison et al. 2007).

The effects of grapevine smoke exposure on the composition and sensory properties of wine are currently not well understood. This study was therefore undertaken to address this knowledge gap, and investigates the effect of both timing and duration of grapevine smoke exposure on wine quality.

Materials and methods

Trial establishment and smoke application

The trial was sited in the locality of Capel in the Geographe region of Western Australia. The site was selected based on an infrequent history of smoke exposure and location away from forested areas. No incidences of externally derived smoke were observed at this site throughout the duration of the experimental period.

Purpose-built greenhouse-type tents measuring 6 m long ¥ 2.5 m high ¥ 2 m wide were constructed from galvanised steel framing to enclose grapevines for the application of smoke. The tents were covered with a greenhouse-grade Solarweave plastic (Gale Pacific, Australia) designed to enable plant photosynthesis and productivity. Smoke was generated by the combustion of dry barley straw in a (50 L) lidded metal drum for 30 min. A remotely controlled variable speed air pump was used

Table 1. Aroma descriptors, aroma detection thresholds and wine concentrations reported for guaiacol, 4-methylguaiacol, 4-ethylguaiacol and 4-ethylphenol.

Compound	Aroma descriptors	Aroma detection threshold (mg/L)				Wine concentration (mg/L)
		Water	Model wine	White wine	Red wine	
Guaiacol	Smoky†, phenolic§, chemical§	0.48§§ 5.5†	20†	95† 20‡	75†	0–100¶
4-methylguaiacol	Toasted†, ash†	10†	30†	65†	65†	0–20††
4-ethylguaiacol	Smoky†, spicy†, toasted, bread§	25†	47†	70†	150† 110‡‡	2–437¶
4-ethylphenol	Horsy†, stable†, phenolic§	130†	440†	1100†	1200† 605‡‡	2–2200¶

†Boidron et al. (1988), ‡Simpson et al. (1986), §López et al. (1999), ¶Pollnitz et al. (2000), ††Pollnitz (2000), ‡‡Chatonnet et al. (1992), §§Eisele and Semon (2005).

to force air through an inlet pipe into the lidded drum, which subsequently forced smoke through an outlet tubing into the tent. Particulate matter (PM_{10}, i.e., :::10 mm in diameter) within the tent was monitored using a DustTrack laser photometer (TSI Model 8520; TSI Inc., St. Paul, Minnesota, USA) to maintain a maximum PM_{10} level of 200 mg/m^3 for the duration of each smoke treatment, a level considered comparative to a high-pollution incident (Reisen and Brown 2006).

Treatments

Two independent smoke experiments were conducted using *Vitis vinifera* cv. Merlot grown in a commercial vineyard. These were a single smoke exposure (for 30 min) of field-grown grapevines that was applied at either veraison or 3, 7, 10, 15, 18, 21 or 24 days post-veraison. Alternatively, field-grown grapevines received eight consecutive smoke exposures (for 30 min each) applied to the same vines at the beginning of veraison then at 3, 7, 10, 15, 18, 21 and 24 days post-veraison. For each smoke experiment, a control (i.e., unsmoked) treatment was also established, where grapevines were enclosed in tents (as earlier) but without the addition of smoke. Each treatment was replicated three times.

Winemaking

At harvest, the three replicates per treatment (approximately 15 kg each) were harvested on the same day for fruit analysis and wine production. Samples of smoked and control grape juice were analysed for total soluble solids (TSS) by refractometry (Iland et al. 2004) and for free amino nitrogen (FAN) by methods described by Dukes and Butzke (1998). Each fruit replicate was harvested at an average TSS of 21.6 ± 1.8 °Brix, crushed, destemmed inoculated with *Saccharomyces cerevisiae* EC1118 yeast (200 mg/L) (Lallemand Inc., Montreal, Canada) and fermented in 15-L fermentation vessels. Fermenting musts were plunged twice daily and the wine was pressed from the skins when the TSS approached 0 °Baumé. All wines were stored in 4.6 L enclosed glass fermenters at 15°C until the residual sugar was below 2 g/L. After the wines were racked from gross lees, they were inoculated with *Leuconostoc oenos* (10 mg/L) (Vinaflora Oenos, Chr. Hansen, Denmark) for malolactic fermentation. On the completion of malolactic fermentation, as determined by quantitative malic acid analysis, wines were racked from lees, free SO_2 was adjusted to 30 ppm and the wines were cold stabilised (28 d at 2°C). Wines were then filtered (5 mm) and bottled. The alcohol content of final wines was measured by ebulliometry (Iland et al. 2004).

Quantitative determination of guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, furfural, 5-methylfurfural, eugenol and vanillin

Guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, furfural, 5-methylfurfural, eugenol and vanillin were quantified by gas chromatography-mass spectrometry (GC-MS) using methods reported previously (Spillman et al. 1997, Pollnitz 2000, Pollnitz et al. 2000, 2004, Kennison et al. 2008).

Sensory analysis

Sensory analysis of experimental wines (smoked and control) was conducted by a panel of eight trained judges comprising four males and four females aged between 21 and 30 years. Panellists were selected on the basis of interest and availability, having experienced at least 100 h of tertiary wine sensory education and being regular wine consumers, non-smokers, of good health, and able to detect smoke aroma of red and white smoked wines at predetermined thresholds ascertained by Kennison et al. (2007). Wines were assessed for aroma only (not tasted on the palate) so as to reduce any potential negative health impacts associated with the tasting of smoke-tainted wine and in accordance with ethical requirements for conducting sensory experiments (Meilgaard et al. 2007).

Panellists underwent eight quantitative descriptive analysis training sessions (two per week) prior to formal evaluation (Meilgaard et al. 2007). Descriptive aroma terms, based on wines used in the study, were generated by panellists with the panel consensus on six descriptive terms. Utilising experimental wines as references, panellists were trained to measure the smoke aroma presence and intensity on an unstructured 100-point line scale.

Formal evaluation of two wine replicates from both the single and repeated smoke experiments (i.e., 22 wines in total) was conducted over four sessions, each held at the same time on different days. Wine samples (20 mL) were presented to panellists at room temperature in three digit coded ISO standard tasting glasses in a randomised order. All glasses were covered with glass covers to avoid contamination of the testing area and other samples. To avoid sensory fatigue, panellists were required to leave the testing area to an external environment (for 10 min) after evaluating each sample.

Statistical methods

All data were analysed using SPSS version 14.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Analysis of variance (ANOVA) was used to analyse chemical data at the 5% level of significance ($P < 0.05$). Wine sensory data were analysed by ANOVA and principal component analysis (PCA).

Results

Effect of smoke exposure on grapes and wine

Smoke was applied to field-grown grapevines as either a single smoke exposure applied at either veraison or at 3, 7, 10, 15, 18, 21 or 24 days post-veraison; or as eight smoke exposures applied to the same vines at veraison and then at 3, 7, 10, 15, 18, 21 and 24 days post-veraison. Fruit from unsmoked (control) vines obtained a higher average fruit TSS level (22.3 °Brix) than fruit from vines subjected to smoke application (Table 2). The TSS was lowest in grapes from vines that had received repeated smoke exposures (19.3 °Brix). These vines also produced

Table 2. Yield, total soluble solids (TSS) and free amino nitrogen (FAN) of grapes and alcohol content of wine derived from smoked and control (unsmoked) grapevines.

Treatment†		Fruit			Wine
		Yield (kg/vine)	TSS (°Brix)	FAN (mg/L)	Alcohol (% v/v)
Control		17.0^{a}	22.33^{a}	87.2^{f}	12.80^{a}
Single smoke exposure at:	0 day post veraison	12.1^{b}	20.93^{b}	96.0^{ef}	12.47^{ab}
	3 days post veraison	16.1^{a}	19.73^{cd}	112.8^{bcd}	11.23^{c}
	7 days post veraison	15.9^{a}	21.07^{b}	117.3^{b}	12.13^{b}
	10 days post veraison	15.7^{a}	19.40^{d}	114.0^{bc}	10.93^{cd}
	15 days post veraison	15.9^{a}	21.03^{b}	102.8^{cde}	12.07^{b}
	18 days post veraison	14.8^{a}	21.07^{b}	100.1^{def}	12.00^{b}
	21 days post veraison	16.1^{a}	20.67^{bc}	108.6^{bcde}	11.93^{b}
	24 days post veraison	15.2^{a}	21.23^{b}	101.7^{cde}	12.53^{ab}
Repeated smoke exposure		11.0^{b}	19.33^{d}	134.4^{a}	10.57^{d}
LSD (5%)		2.36	1.018	13.3	0.6478

†Smoked grapevines were subjected to either a single smoke application or eight repeated smoke applications between veraison and harvest (i.e., at 0, 3, 7, 10, 15, 18, 21 and 24 days post-veraison). Means followed by the same letter within columns are not significantly different at $P ::: 0.05$, $n = 3$.

the lowest fruit yields (11 kg/vine) as compared with the mean fresh fruit weight of 15.3 kg/vine for all other treatments (Table 2). Repeated smoke exposure to vines resulted in the development of necrotic lesions on leaves, effects that were not seen on control grapevines or grapevines exposed to single smoke applications (not shown).

FAN in grapes at harvest was significantly higher in fruit from vines subjected to repeated smoke exposures (134.4 mg/L, $P < 0.05$) compared with all other treatments, with the unsmoked treatments lowest in FAN (87.2 mg/L) (Table 2). Fruit from vines subject to repeated smoke exposures also had higher total SO_2 (18 mg/L) and pH (3.8) values in grape juice at harvest compared with all other single smoke and control (unsmoked) treatments.

The fermentation rate of must was faster for grapes from vines exposed to repeated smoke exposures. In comparison with wines from the unsmoked (control) treatment that completed fermentation after 12 days, wines from the repeated smoke exposure treatment took only 8 days to complete fermentation (Figure 1). Fermentation rates of musts from all other smoke treatments were also faster (10–12 days). Ethanol content was up to 17% lower in wines made from fruit from grapevines subject to repeated smoke exposures (10.6% v/v) than in the other wines, and the highest ethanol content was in the wines from non-smoke-exposed control vines (12.8% v/v) (Table 2). Similarly, all wines vinified from grapes from vines exposed to a single smoke exposure contained intermediate ethanol concentrations between 10.9 and 12.5% v/v.

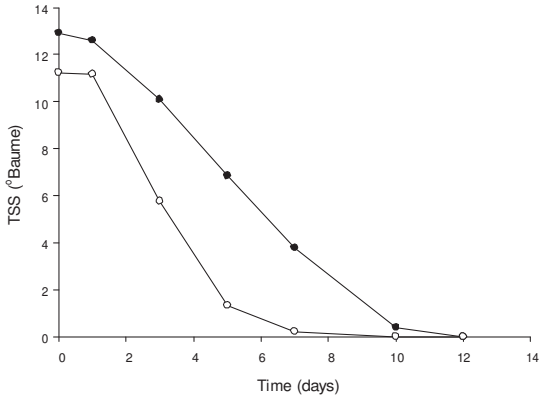

Figure 1. Fermentation curves for fruit harvested from grapevines exposed to repeated smoke applications (O) from veraison to harvest (i.e., at 0, 3, 7, 10, 15, 18, 21 and 24 days post-veraison) and control (unsmoked) grapevines (e). Mean values from three replicates; standard errors are obscured by symbols and so are not shown but are <0.5 in all cases.

Quantitative determination of smoke-derived volatiles in grapes and wine

The volatile phenols guaiacol, 4-methylguaiacol, 4-ethylguaiacol and 4-ethylphenol were selected as analytes of interest based on their reported contribution to the aroma and flavour of smoked food products (Baltes et al. 1981) and provenance in smoke-tainted wines (Kennison et al. 2007, 2008). Vanillin, eugenol, furfural and 5-methylfurfural were included in the quantitative GC-MS analysis but because of being detected at levels that have a negligible effect on aroma properties (i.e., :::5 mg/L for vanillin, eugenol, and 5-methylfurfural and :::40 mg/L for furfural) are not discussed further.

GC-MS analysis detected large and significant differences in volatile phenol composition between control wines and wines derived from smoked grapevines (Table 3 and Figure 2). The highest levels of smoke-derived volatile phenols occurred in wines made from

Table 3. Mean concentrations and standard errors of guaiacol, 4-methylguaiacol, 4-ethylguaiacol and 4-ethylphenol in wines made from fruit harvested from smoked and control (unsmoked) grapevines.

Treatment†	Concentration (mg/L)‡							
	Guaiacol		4-methylguaiacol		4-ethylguaiacol		4-ethylphenol	
	Mean	SE	Mean	SE	mean	SE	Mean	SE
Control	4[b]	1.4	n.d.[b]	n/a	tr.[b]	n/a	tr.[b]	n/a
Smoked	388[a]	26.3	93[a]	7.3	16[a]	1.3	58[a]	2.9

†Smoked grapevines were subjected to eight repeated smoke exposures applied between veraison and harvest (i.e., at 0, 3, 7, 10, 15, 18, 21 and 24 days post-veraison).
‡For each analyte, means followed by the same letter are not significantly different at $P ::: 0.05$, $n = 3$.
n.d., not detected; tr., trace (i.e., positive identification but <1 mg/L); SE, standard error.

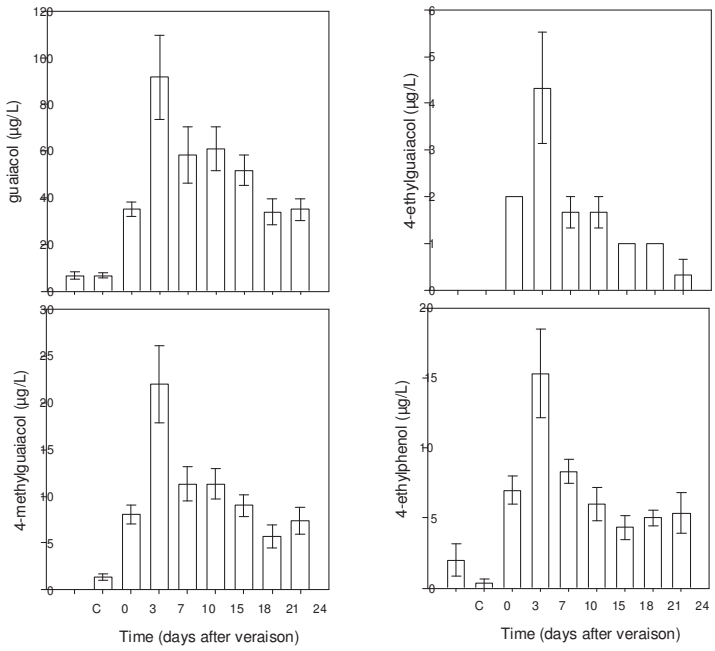

Figure 2. Guaiacol, 4-methylguaiacol, 4-ethylguaiacol and 4-ethylphenol concentrations in wine made from grapes harvested from grapevines exposed to single smoke applications between veraison and harvest (i.e., at 0, 3, 7, 10, 15, 18, 21 or 24 days post-veraison); C, control. Data are means ($n = 3$). Error bars show two standard errors of the mean.

vines repeatedly exposed to smoke, i.e., 388 mg/L of guaiacol, 93 mg/L of 4-methylguaiacol, 16 mg/L of 4-ethylguaiacol and 58 mg/L of 4-ethylphenol. In contrast, control wine contained between 4 mg/L of guaiacol and non-detectable (<1 mg/L) levels of the other phenols. Wine derived from repeatedly smoked grapevines contained at least four-fold higher volatile phenol concentrations than any of the wines derived from grapevines that received a single smoke treatment.

Importantly, the timing of grapevine smoke exposure was found to influence the concentration of smoke-derived volatile phenols in wine. For the experiments involving single smoke applications, the highest concentrations of guaiacol, 4-methylguaiacol, 4-ethyphenol and 4-ethylguaiacol corresponded to wine derived from grapevines exposed to smoke 7 days post-veraison (Figure 2). Guaiacol concentration increased from 7 mg/L for smoke exposure at the initial onset of veraison, peaked at 92 mg/L for smoke exposure at 7 days post-veraison, then decreased to between 34 and 61 mg/L for each subsequent smoke exposure until harvest. Similar compositional trends were observed for 4-methylguaiacol, 4-ethylguaiacol and 4-ethylphenol.

Sensory analysis of experimental wines

Quantitative descriptive analysis of wines made from fruit of grapevines exposed to smoke identified aromas of 'burnt rubber', 'smoked meat', 'leather', and 'disinfectant and hospital' as being associated with smoke taint (Boidron et al. 1988, López et al. 1999). Aromas of 'red berry fruits' and 'confection' were identified as wine char-

Table 4. Analysis of variance for wine sensory attribute ratings of 'burnt rubber', 'smoked meat', 'leather', 'disinfectant'/'hospital', 'red berry fruits' and 'confection' for wine (W), panellist (P), replicate (R), wine by panellist (W ¥ P), panellist by replicate (P ¥ R) and wine by replicate (W ¥ R).

Aroma descriptor	Wine (W)	Panellist (P)	Replicate (R)	W ¥ P	P ¥ R	W ¥ R
Burnt rubber	14.34***	3.71***	0.13	1.69*	0.44	0.65
Smoked meat	21.56***	3.13***	0.02	0.97	0.65	0.52
Leather	11.55***	4.02***	0.98	0.99	0.83	0.30
Disinfectant/hospital	8.06***	5.14***	0.84	1.31	0.58	0.72
Red berry fruits	9.65***	6.19***	1.37	2.18***	0.35	0.41
Confection	8.63***	4.55***	0.46	2.58***	0.17	0.44

F ratios are shown as sources of variation. Significance indicated by $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

acters common in distinguishing control wines from unsmoked vines. ANOVA showed both the wines and the panellists to be sources of wine sensory variation for all aroma attributes ($P < 0.001$) (Table 4). However, there was no significant variation among the replicates (i.e., wine by replication and panellist by replication), indicating consistency in panellist rating between sessions and wines. Other sources of variation resulted from the wine by panellist interactions for the aroma descriptors of burnt rubber ($P < 0.05$), red berry fruits ($P < 0.001$) and confection ($P < 0.001$), indicating that individual panellists have different thresholds or levels of sensitivity to the aromas of these compounds.

Wines vinified from grapes of vines receiving repeated smoke exposures attained higher scores for off-aromas ('burnt rubber', 'smoked meat', 'leather', and 'disinfectant and hospital') compared with all other wines (Figure 3). Compared with wines made from fruit of vines exposed to eight smoke applications, wines made from fruit produced from unsmoked (control) vines exhibited significantly higher scores for 'confection' and 'red berry fruits' aromas ($P < 0.001$). Wines from single smoke exposure experiments revealed the full range of aroma characters from 'red berry fruits' and 'confection' through to 'smoked meat', 'burnt rubber', 'leather', and 'disinfectant and hospital' as displayed in the PCA biplot (Figure 4). The PCA of mean aroma results from single-smoked wines showed that principal component 1 (PC1) accounted for 93% of the overall variation and principal component 2 (PC2) accounted for 4% of the variation (Figure 4). PC1 is largely characterised by the contrast of positive loadings on smoke-like aromas ('leather', 'burnt rubber', 'smoked meat', and 'disinfectant and hospital' aromas) and negative loadings on fruit and wine aromas ('red berry fruit' and 'confection' aromas) (Table 5). PC2 is further defined with a positive loading for the 'disinfectant and hospital' aroma and with negative loadings for smoke and fruit aroma descriptors. The smoke-like aroma descriptors of 'leather', 'burnt rubber' and 'smoked meat' were highly correlated with each other (r = 0.74 to 0.79). Likewise, there was a high correlation between Merlot wine character aroma descriptors of 'red berry fruits' and 'confection' (r = 0.87). The aroma descriptor of

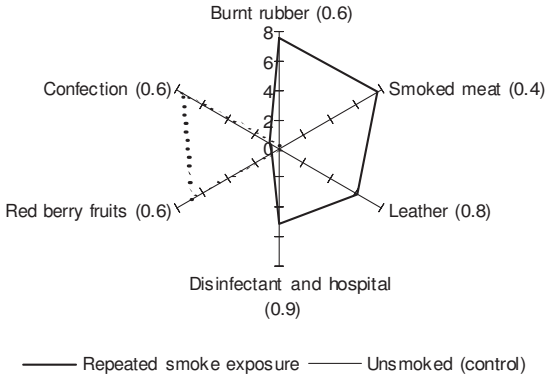

Figure 3. Polar coordinate (cobweb) graph of mean aroma intensity ratings of 'burnt rubber', 'smoked meat', 'leather', 'disinfectant and hospital', 'red berry fruits' and 'confection' for wines made from grapes from grapevines exposed to eight smoke applications (—) (applied at veraison and then at 3, 7, 10, 15, 18, 21 and 24 days post-veraison) and control (unsmoked) treatments (----) (least significant difference (LSD)), $P < 0.001$ are indicated in parenthesis for each term). Aroma descriptor values for unsmoked (control) wines range from 0 ('smoked meat') to 0.3 ('burnt rubber').

'disinfectant and hospital' was negatively correlated with wine character aromas (r = –0.55 to –0.59) and weakly correlated with smoke-like aromas (r = 0.48 to 0.52).

The sensory properties of each experimental wine varied depending on the timing of smoke application. Single smoke exposure to grapevines at either 7 or 10 days post-veraison led to more intense 'leather', 'smoked meat', 'burnt rubber', and 'disinfectant and hospital' aromas in resultant wines at higher levels than other timings of smoke exposure (Figure 4). Single smoke exposure to grapevines at veraison and at 18, 21, and 24 days post-veraison subsequently produced wines with high aromas of 'red berry fruits' and 'confection' and low smoke aroma characteristics.

Discussion

Previous research has shown that the postharvest smoke exposure of grapes affects the chemical composition and aroma of wine, leading to the development of perceivable

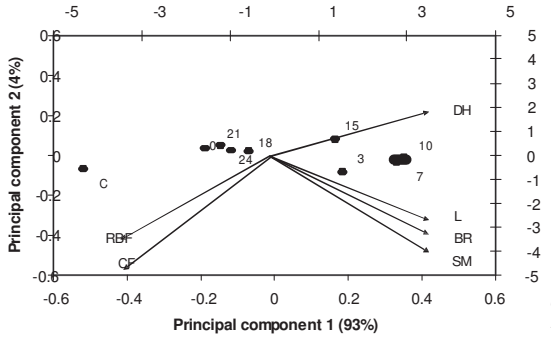

Figure 4. Principal component analysis (PCA) biplot of mean wine sensory scores from experiments that applied a single smoke exposure to field-grown grapevines between veraison and harvest (i.e., at 0, 3, 7, 10, 15, 18, 21 or 24 days post-veraison) and wine derived from control (unsmoked) vines (C). Aroma descriptors are indicated by arrows labelled DH ('disinfectant and hospital'), L ('leather'), BR ('burnt rubber'), SM ('smoked meat'), CF ('confection') and RBF ('red berry fruits').

Table 5. Factor loadings on principal component 1 (PC1) and principal component 2 (PC2) for aroma descriptors of 'burnt rubber', 'smoked meat', 'leather', 'disinfectant'/'hospital', 'red berry fruits' and 'confection' for wines derived from single smoke exposure applied to grapevines at veraison or at 3, 7, 10, 15, 18, 21 or 24 days post-veraison.

Aroma descriptor	PC1	PC2
Burnt rubber	0.41	−0.38
Smoked meat	0.41	−0.48
Leather	0.42	−0.31
Disinfectant/hospital	0.40	0.20
Red berry fruits	−0.41	−0.42
Confection	−0.40	−0.56

smoke taint aromas (Kennison et al. 2007). The current study demonstrates that field exposure of grapevines to smoke can lead to the development of smoke taint in wine, the timing of smoke exposure to grapevines can influence the chemical and sensory properties of resultant wine, and repeated smoke exposure has a cumulative effect on the concentration of smoke-derived volatile compounds in resultant wines.

The concentration of smoke taint indicator compounds (guaiacol, 4-methylguaiacol, 4-ethyphenol and 4-ethylguaiacol) measured in wine varied depending on the timing and number of smoke exposures that the vines received. Levels of these compounds in wines from grapevines subjected to a single smoke exposure at the onset of veraison were low, with guaiacol levels comparable to those found in control treatments (6.7 mg/L). However, smoke exposure at 7 days post-veraison resulted in higher levels of guaiacol (92 mg/L). Later smoke exposures resulted in significant levels of taint, but the concentrations of the indicator compounds were always less (63–93%) than wine corresponding to smoke exposure at 7 days post-veraison. The reasons for variation in sensitivity to smoke exposure during the post-veraison period are currently unclear. During veraison, changes occur in assimilate partitioning of sugar uptake and metabolism (Conde et al. 2007) and in phloem unloading from symplastic to apoplastic pathways (Zhang et al. 2006). The chemical and structural characteristics of grape cell walls also change during this period (Nunan et al. 1998, Mullins et al. 2000). The peak uptake of volatile smoke components by fruit following smoke exposure at 7 days post-veraison may be related to these changes in berry physiology. An alternative hypothesis is that the peak period of uptake identified in this experiment may be independent of ontogeny and could also relate to changes in sensitivity to uptake of the compounds at the leaf level associated with short-term environmental effects on vine physiology such as vine water status. These hypotheses are the subject of an ongoing study.

Repeated smoke exposures to field-grown grapevines led to accumulation of smoke compounds to high levels. Irrespective of when smoke exposures were applied to vines, from the period of veraison to harvest, the effects on the levels of guaiacol, 4-methylguaiacol, 4-ethylguaiacol and 4-ethylphenol in wines were additive. Indeed, the sum of smoke taint-related compounds detected in wines generated from single smoke exposure treatments closely approximates the compound levels detected in wines generated from repeated smoke exposures (Figure 5). These results imply that repeated or prolonged vineyard smoke exposure, which can occur from frequent fire events, over the post-veraison period will potentially have a cumulative negative effect on resultant wine quality and value.

Quantitative descriptive wine aroma analysis demonstrated an increase in smoke-related aromas described as 'burnt rubber', 'smoked meat', 'leather', and 'disinfectant and hospital' in wines from the repeated smoke exposure experiment. These aromas clearly dominated the wine's sensory profile, overpowering any 'confection' and 'red berry fruits' aromas (Figure 3). Smoked wine from the repeated smoke exposure experiment contained guaiacol and 4-methylguaiacol at levels well in excess of the highest published aroma detection thresholds for red wine (of 75 and 65 mg/L, respectively), although 4-ethylguaiacol and 4-ethylphenol were present at subthreshold concentrations (Boidron et al. 1988). In contrast, the volatile phenols were either not detected or detected at trace levels only in control wines. Therefore, as in previous studies, the source of volatile phenols can be attributed directly to smoke (Kennison et al. 2007, 2008). Furthermore, the accentuation of smoke taint in wine derived from the repeatedly smoked grapevines is correlated with the increased levels of guaiacol and 4-methylguaiacol, with little or no contribution from 4-ethylguaiacol or 4-ethylphenol.

Smoke-like aromas were also present to various degrees in all wines vinified from the single smoke exposure experiment regardless of smoke application timing

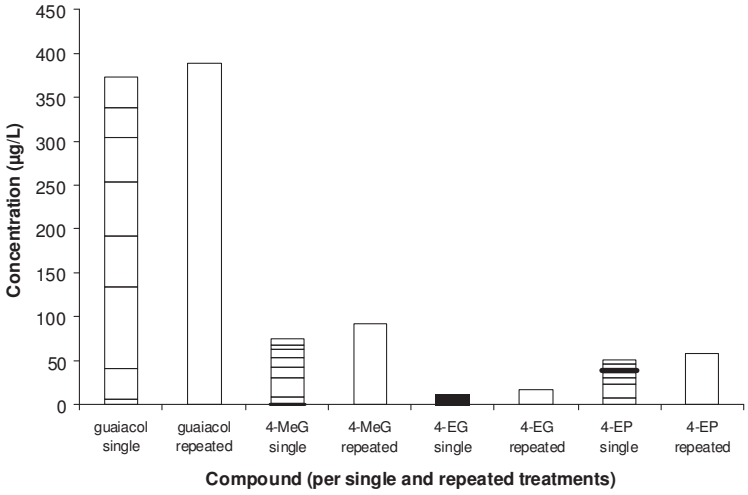

Figure 5. Sum of guaiacol, 4-methylguaiacol (4-MeG), 4-ethylguaiacol (4-EG) and 4-ethylphenol (4-EP) concentration in eight wines made from Merlot grapes from vines that each received a single field-based smoke exposure (applied at veraison or at 3, 7, 10, 15, 18, 21 or 24 days post-veraison as indicated by bands) versus compound levels detected in wine from Merlot grapes from vines that received repeated smoke exposures (applied at veraison then again at 3, 7, 10, 15, 18, 21 and 24 days post-veraison).

and resultant compound level in wine. The levels of guaiacol, 4-methylguaiacol, 4-ethylguaiacol and 4-ethylphenol were detected by the panellists in all these wines below reported aroma thresholds for red wine (Boidron et al. 1988, Chatonnet et al. 1992) except for smoke application at 7 days post-veraison that produced wine with guaiacol above the reported aforementioned aroma detection threshold in red wine of 75 mg/L. Panellists detected elevated smoke aromas in the 7 days post-veraison wines, although smoke aromas were also detected in all other wines made from fruit of vines exposed to single smoke applications – even though their compound levels were below published aroma thresholds. It should be noted that there is some conjecture regarding the detection threshold for guaiacol; certainly there is disagreement between published thresholds, i.e., 75 mg/L reported by Boidron et al. (1988) and 20 mg/L reported by Simpson et al. (1986). A recent workshop demonstrated that all 60 delegates presented with 70 unidentified wines including a control and a wine spiked with guaiacol (20 mg/L) were able to discern the wines, giving descriptors for the latter such as 'smoky' and 'burnt bacon' (Mark Sefton, pers. comm., 2007). Given the intricate and complex nature of smoke, indicator compounds play an important role in assessing the extent and impact of smoke taint on wine quality. In the present study, the volatile phenols, together with sensory analysis, have been proven as effective smoke taint markers. However, it is acknowledged that with time, additional volatile compounds will likely be identified as components of smoke, which are also responsible for the discernable aroma attributes of smoke-tainted wine.

Grapevine smoke exposure leads to increased levels of FAN in grapes, an effect most evident following repeated smoke applications. Interestingly, grapes harvested from repeatedly smoked grapevines also fermented the most rapidly, in agreement with previous studies (Kennison et al. 2007). While the increase in ferment rate may be associated with the increased FAN in must (Henschke and Jiranek 1993, Bell and Henschke 2005), the basis for this increase is unclear. Some direct contributions from nitrogenous smoke compounds is possible as research has demonstrated the uptake and assimilation, by nitrite reductase, of nitrogenous compounds (NO and NO_2) by plants (Hosker and Lindberg 1982, Nussbaum et al. 1993, Stulen et al. 1998, Takahashi et al. 2001); however, it is likely that any such contribution would be very minor based on simple mass balance. Increased FAN may also be linked to the injury response of grapes following high levels of smoke exposure, for example, a biochemical response to necrotic lesion that developed on laminae following repeated smoke treatment (Heath 1980).

Field-based smoke exposure to grapevines showed an adverse effect on grape ripening (e.g., sugar accumulation) irrespective of the timing and duration of smoke application. In a recent study, the stomatal conductance, CO_2 assimilation rate and intercellular CO_2 levels of *Chrysanthemoides monilifera* were reduced for 5 h following smoke exposure for 1 min, with 24 h required to achieve physiological recovery to control levels (Gilbert and Ripley 2002). Additionally, the presence of SO_2 and O_3 in smoke has been shown to induce stomatal closure in grapevines (Rosen et al. 1978). It is therefore conceivable that the photosynthetic capacity of grapevines decreases following smoke exposure, which in turn inhibits grape maturation and ripening. Furthermore, these physiological effects would be further exacerbated by the loss of photosynthetically active leaf area because of the formation of necrotic lesions on laminae (Heath 1980), as occurred in the current study with grapevines subjected to repeated smoke treatments.

In summary, the deliberate application of smoke to field-grown grapevines between veraison and harvest affected yield, grape composition (sugar accumulation and FAN), wine composition, wine sensory properties, and most importantly, wine quality. The volatile phenol levels and intensity of smoke taint in wine was influenced by both the timing and the duration of grapevine

exposure to smoke. For single smoke treatments, the highest levels of volatile phenols were observed in wines corresponding to smoke exposure 7 days post-veraison. For repeated smoke treatments, a cumulative effect on smoke-derived volatile phenol concentrations was observed.

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Smoke-Derived Taint in Wine: The Release of Smoke-Derived Volatile Phenols during Fermentation of Merlot Juice following Grapevine Exposure to Smoke

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The release of smoke-derived volatile phenols during the fermentation of Merlot grapes, following grapevine exposure to smoke, has been investigated. The concentrations of guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, and eugenol were determined by gas chromatography–mass spectrometry and found to increase throughout the winemaking process. Only trace levels ($\leq$1 μg/L) of guaiacol and 4-methylguaiacol could be detected in free run juice derived from the fruit of smoked vines; the highest levels, 388 μg/L and 93 μg/L, respectively, were observed in the finished wine. Control wine (derived from fruit of unsmoked vines) contained 4 μg/L guaiacol, with the volatile phenols either not detected or detected at only trace levels ($\leq$1 μg/L) throughout fermentation. The role of enzyme and acid catalyzed hydrolysis reactions in releasing smoke-derived volatile compounds was also investigated. The volatile phenols were released from smoked free run juice by strong acid hydrolysis (pH 1.0) and enzyme (*β*-glucosidase) hydrolysis, but not mild acid hydrolysis (juice pH 3.2–3.7). Guaiacol was again the most abundant smoke-derived phenol, present at 431 μg/L and 325 μg/L in strong acid and enzyme hydrolysates, respectively. Only trace levels of each phenol could be detected in each control hydrolysate. This study demonstrates the potential for underestimation of smoke taint in fruit and juice samples; the implications for the assessment of smoke taint and quantification of volatile phenols are discussed.

KEYWORDS: ***P*-Glucosidase; fermentation; grapes; grapevines; guaiacol; hydrolysis; 4-methylguaiacol; smoke exposure; smoke taint; volatile phenols; wine**

INTRODUCTION

In recent years, significant forest fires have occurred in Asia, Africa, Europe, North America, South America, and Australia, and the incidence of such fires is expected to escalate as a result of climate-induced changes to weather, particularly increased temperature, drought, wind and natural ignition sources (*1*). In some cases, fires have occurred in close proximity to wine regions resulting in vineyard smoke exposure and smoke tainted wines. The taint, characterized by objectionable 'smoky', 'dirty' and 'burnt' aromas and a lingering retro-nasal 'ash' character on the palate (*2*), has caused significant financial loss for grape and wine producers and is therefore an issue of increasing concern.

Grape and grapevine exposure to smoke has been shown to affect the chemical composition and sensory properties of wine (*2, 3*). A number of volatile phenols including guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethlyphenol and eugenol (4-allylguaiacol) were detected in wines made from grapes which had received a postharvest exposure to smoke. Since these compounds were not present in wines made from unsmoked grapes, their origin was attributed to smoke exposure (*3*).

In wine, guaiacol, 4-methylguaiacol and eugenol are typically associated with oak barrel maturation (*4–6*), derived predominantly from the thermal degradation of oak lignin during the

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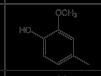
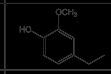
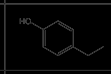
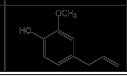
toasting process of cooperage (*7*), although there are significant levels of eugenol present in untoasted oak (*5*). In contrast, 4-ethylguaiacol and 4-ethylphenol are bacterial in origin, arising from the action of *Brettanomyces*/*Dekkera* yeast on grape-derived *p*-coumaric acid and *p*-ferulic acid (*6, 8*). Numerous studies have identified these phenols as components of smoke and liquid smoke preparations (e.g, refs *9–12*), with guaiacol and 4-methylguaiacol reported as two of the most abundant phenols occurring in wood smoke, in both the vapor phase and aqueous extracts (*13*). Additionally, these volatile phenols are associated with smoke-like aromas (**Table 1**); guaiacol and 4-methylguaiacol in particular, which impart 'smoky', 'phenolish', 'aromatic', 'sharp' and 'sweet' aroma characters (*6, 10, 11*). Although guaiacol and 4-methylguaiacol are not considered solely responsible for smoke taint (*3*), they nevertheless represent useful marker compounds, with levels of guaiacol and 4-methylguaiacol indicative of levels of smoke taint.

In previous studies in this laboratory, the intensity of smoke taint has been observed to increase during the fermentation of smoke affected grapes. This is consistent with anecdotal evidence from industry that smoky characters either appeared during fermentation of grapes which had not previously exhibited smoke taint, or increased throughout the winemaking process. The release of volatile secondary metabolites from grape and oak-derived flavor precursors via enzyme and acid catalyzed hydrolysis has been previously demonstrated (e.g., refs *14–17*). Accordingly, the hydrolytic release of smoke-derived volatiles from involatile precursors, such as glycoconjugates, could be responsible for the intensification of smoke aroma during fermentation. Indeed guaiacol has been previously reported as a component of acid and enzyme hydrolysates prepared from Merlot and Shiraz juices (*14, 16*), presumably deriving from glycoconjugate precursor forms. To date, the assessment of smoke taint relies on either sensory evaluation or quantification of guaiacol and 4-methylguaiacol. The presence of conjugated precursors is therefore problematic for both sensory and chemical analysis. This study was undertaken to investigate (i) the evolution of smoke-derived volatile phenols during fermentation, following grapevine exposure to smoke; (ii) the release of volatile phenols under acid and enzyme catalyzed reaction conditions; and (iii) the implications of the results for carrying out analysis of smoke affected grapes and juice.

Table 1. Structures and Aroma Descriptors of Smoke-Derived Volatile Phenols

compound	structure	aroma descriptors
guaiacol		smoky, phenolish, aromatic, sharp, sweet (*6,10,11*)
4-methylguaiacol		smoky, toasted, ash, vanilla-like, sweet, phenolish, fruity, sharp (*6,10,11*)
4-ethylguaiacol		smoky, sweet, spicy, clove-like (*6,8,10*)
4-ethylphenol		horsy, leather, medicinal, smoky, barnyard, animal, stable, sweaty saddle (*6,8*)
eugenol		clove, vanilla-like, phenolish (*5,11*)

MATERIALS AND METHODS

Field Application of Smoke to Grapevines. Merlot grapevines within a vineyard located in Capel, Western Australia were exposed to eight successive smoke applications (30 min each) between veraison and harvest, i.e., at 0, 3, 7, 10, 15, 18, 21 and 24 days post-veraison. Smoke applications were performed (in triplicate) using a purpose built smoke tent similar to that described in seed germination experiments by Dixon et al. (*18*), constructed from galvanized steel and greenhouse film (Solarweave). Smoke was generated in a metal drum (50 L) by combustion of dry straw and pumped into the smoke tent with the grapevines (3 per replicate) enclosed. Dry barley straw was selected as a fuel source to minimize variation in combustion conditions, enabling reproducible smoke application. Control grapevines were similarly enclosed in identical (smoke-free) tents for the duration of each smoke treatment to minimize differences in environmental conditions (such as humidity, temperature and light exposure). Tents were removed following each experimental treatment.

Winemaking. Grapes (three fruit replicates of approximately 16 kg each) were harvested from control (unsmoked) and smoked grapevines on the same day, corresponding to total soluble solids (TSS) contents of 22 °Brix and 19 °Brix, respectively. For each treatment, the fruit was processed to produce three replicate wines, according to standard small-lot winemaking procedures. The fruit was crushed, destemmed and fermented in 15 L fermentation vessels with EC1118 *Saccharomyces cereVisiae* yeast (Lallemand Inc., Montreal, Canada). The fermenting musts were plunged twice per day and the wine was pressed from the skins at a TSS level of 3.6 °Brix. Wines were transferred to 15 L demijohns and held at 15 °C until the residual sugar approached 0 g/L. The wines were then racked from gross lees and inoculated with *Leuconostoc oenos* (Vinaflora Oenos, Chr. Hansen, Denmark). On completion of malolactic fermentation, wines were again racked and free SO_2 adjusted (to 30 ppm) before being cold stabilized (2 °C for 28 days), filtered and bottled.

Sampling. Samples (approximately 50 mL aliquots) were collected from each smoked and control fermentation replicate, throughout the winemaking process. For smoked ferments, the sampling times were: after crushing (i.e., free run juice), after 1, 3, 5 and 7 days of maceration, at the end of alcoholic fermentation and after bottling (i.e., finished wine). The same sampling times were employed for control ferments, but with the inclusion of a sampling point after 10 days of maceration. The additional control sampling point was necessitated by differences in fermentation rates between smoked and control ferments. As in previous studies (*3*), smoke exposure increased fermentation rates, with smoked ferments completing alcoholic fermentation 3 days earlier than control ferments. Each ferment was also sampled immediately before and after pressing (i.e., at 7 and 10 days of maceration for smoked and control ferments, respectively); grape marc samples (approximately 50 g) were also collected after pressing. Finished wines were reanalyzed approximately 12 months post-bottling. Prior to analysis, must and wine samples were clarified by centrifugation and grape marc samples were crushed in liquid nitrogen.

Preparation of Acid and Enzyme Hydrolysates. Acid and enzyme hydrolysis experiments were conducted (in duplicate) using control and smoked free run juice, based on methodology described elsewhere (*17, 19*). Chemicals and enzymes were purchased from Sigma-Aldrich. Mild acid hydrolysates (i.e., juice pH: 3.2 for control juice and 3.7 for smoked juice) were prepared by heating grape juice (10 mL) for 1 h at 100 °C. Strong acid hydrolysates (i.e., pH 1.0, achieved by addition of concentrated sulfuric acid) were prepared by heating grape juice (10 mL) for 1 h at 100 °C. Enzyme hydrolysates were prepared by treating grape juice (10 mL) with almond emulsion /*i*-glucosidase enzyme (25 mg) for 24 h at 30 °C.

Quantitative Gas Chromatography–Mass Spectrometry Analysis. Guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethyphenol, eugenol, furfural and 5-methylfurfural were quantified by the stable isotope dilution assay methods reported previously (*20–23*). These publications include details of the syntheses of the internal standards used herein. For all analytes: the linear dynamic range was 0, and 1–1000 μg/L; the limit of detection was 1 μg/L; and the precision was <5% relative standard deviation. The purity of all standards was verified by GC-MS.

Preparation of Must and Wine Samples for Analysis. A deuterated internal standards (I.S.) solution of d_4-furfural (1.06 μg), d_3-guaiacol

(1.13 μg), d_3-4-methylguaiacol (0.840 μg) and d_4-4-ethylphenol (0.721 μg), in ethanol (100 μL) was added to the sample (5 mL) in a screw cap vial using a glass syringe (100 μL Hamilton). The organic solvent (diethyl ether/n-pentane 1:2 (v/v) ca. 3 mL) was added, and the mixture was shaken briefly. A portion of the organic layer (ca. 2 mL) was then placed in a vial ready for instrumental analysis.

Preparation of Marc Samples for Analysis. For each marc sample, a 4.0 g subsample was accurately weighed into a screw cap vial. The I.S. solution as above (100 μL) and the organic solvent as above (10 mL) were added (to immerse the marc sample) and the lid screwed on. After 24 h at room temperature the vial was swirled briefly and then a portion of the organic layer (2 mL) was then placed in a vial ready for instrumental analysis.

Reference Standards. Reference standards containing 100 μL of deuterated internal standards ethanolic solution (as described above) and 100 μL of normal unlabeled analytes ethanolic solution (furfural (1.674 μg), 5-methylfurfural (2.073 μg), guaiacol (4.646 μg), 4-methylguaiacol (1.536 μg), 4-ethylguaiacol (2.025 μg), eugenol (2.108 μg) and 4-ethylphenol (1.798 μg)) in diethyl ether/*n*-pentane (1:2 (v/v), approximately 2 mL) were used.

Gas Chromatography–Mass Spectrometry Analysis. An Agilent Technologies 6890 gas chromatograph (GC) was equipped with a Gerstel MPS2 multipurpose sampler and coupled to an Agilent 5973N mass selective detector. The gas chromatograph was fitted with an approximately 30 m × 0.25 mm, 0.25 μm J&W DB-Wax fused silica capillary column. The carrier gas was helium (BOC Gases, high purity), linear velocity 50 cm/sec; flow rate 1.2 mL/min. vacuum compensated at the mass spectrometer interface. The oven temperature was started at 50 °C, held at this temperature for 1 min, increased to 240 at 10 °C/min, and held at this temperature for 20 min. The injector temperature was 200 °C and the transfer line was held at 240 °C. The sample volume injected was 2 μL. The splitter, at 30:1, was opened after 36 s, and the liner used was resilanized borosilicate glass, tapered, with a plug (2–4 mm) of resilanized glass wool at the column interface. The instrument was controlled with Agilent G1701CA ChemStation software in conjunction with the Gerstel MASter software (version 1.81). For quantification of the smoke volatiles, positive ion electron impact mass spectra at 70 eV were recorded in Selective Ion Monitoring (SIM) mode. The ions monitored were *m/z* 98, *100* for d_4-furfural (dwell 50 ms); *m/z* 95, *96* for furfural (dwell 50 ms); *m/z* 95, 97, *112* for 5-methylfurfural (dwell 50 ms); and *m/z* 77, 131, 149, *164* for eugenol (dwell 25 ms). The italicized ions were the ones used for quantitation (by peak area). 5-Methylfurfural was quantified versus d_4-furfural as internal standard (IS). Eugenol was quantified versus d_4-4-ethylphenol as IS. Other SIM conditions have been published previously (*20, 21*). The data was analyzed with Agilent MSD ChemStation software (Build 75).

Statistical Methods. Data were analyzed by two-way analysis of variance (ANOVA) using GenStat (8th Edition, VSN International Limited, Herts, UK). Mean comparisons were performed by least significant difference (LSD) multiple comparison tests at $P < 0.05$.

RESULTS AND DISCUSSION

The volatile phenols, guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol and eugenol, were either not detected or detected at only trace levels (≤1 μg/L) in free run juice derived from fruit of smoke-exposed grapevines. However, the concentration of each compound increased dramatically and progressively throughout fermentation, with the highest levels observed in finished wine (**Table 2**). The corresponding finished control wine (derived from fruit of unsmoked grapevines) contained 4 μg/L guaiacol, but 1 μg/L or less of the other phenols of interest. As in previous studies (*3*), the absence of these compounds (at significant concentrations) in control wines indicates that in smoked samples they derive almost exclusively from the application of smoke to grapevines. Of the smoke-derived volatile phenols measured, guaiacol and 4-methylguaiacol were the most abundant, present in the finished wine at 388 μg/L and 93 μg/L, respectively. This is consistent with previous studies which reported guaiacol and 4-methylguaiacol as the most abundant phenolic components occurring in smoke (*13*). Eugenol was the least abundant phenol measured, with just 3 μg/L detected in the finished wine.

Table 2. Concentrations of Guaiacol, 4-Methylguaiacol, 4-Ethylguaiacol, 4-Ethylphenol, and Eugenol in Ferments Derived from the Fruit of Smoked and Unsmoked Grapevines Throughout the Winemaking Process

	concentration[a] (μg/L)				
sample	guaiacol	4-methyl guaiacol	4-ethyl guaiacol	4-ethyl phenol	eugenol
		unsmoked			
free run juice	n.d.	n.d.	n.d.	n.d.	n.d.
after 1 day maceration	tr.	tr.	n.d.	n.d.	tr.
after 3 days maceration	tr.	tr.	n.d.	n.d.	tr.
after 5 days maceration	tr.	tr.	n.d.	n.d.	tr.
after 7 days maceration	tr.	tr.	n.d.	n.d.	tr.
after 10 days maceration	1	tr.	n.d.	n.d.	tr.
after alcoholic fermentation	1	tr.	n.d.	n.d.	tr.
finished wine	4	n.d.	tr.	tr.	tr.
12 months post-bottling	3	tr.	tr.	tr.	n.d.
		smoked			
free run juice	1 a	tr.	n.d.	n.d.	n.d.
after 1 days maceration	68 b	11 a	10 a	5 a	2 ab
after 3 days maceration	168 c	26 b	8 a	5 a	1 a
after 5 days maceration	203 cd	32 bc	9 a	15 b	2 a
after 7 days maceration	249 d	42 c	9 a	17 b	2 a
after alcoholic fermentation	249 d	43 c	8 a	23 c	1 a
finished wine	388 e	93 d	16 b	58 d	3 b
12 months post-bottling	371 e	124 e	29 c	94 e	4 c

[a] Values are the means from three replicates and were in agreement to ca. 10%. Values followed by a different letter within columns are significantly different ($P < 0.05$). n.d. = not detected; tr. = trace (i.e., positive identification but <1 μg/L).

Preliminary studies conducted by the Australian Wine Research Institute showed guaiacol and 4-methylguaiacol accumulated in skins, rather than pulp, of smoke affected grapes (*2*). As such, the increase in volatile phenol concentrations throughout winemaking could be attributed to ongoing extraction from skin tissues; except that phenol concentrations continued to increase during malolactic fermentation (i.e., after skins were pressed from the wine). The increased phenol content following pressing instead implies the presence of one or more precursor compounds.

Pressing itself had no apparent effect on the composition of wine, with very similar phenol concentrations observed in wine immediately before and after pressing (**Table 3**). Comparable levels of guaiacol and 4-methylguaiacol were observed in both marc and wine derived from smoke-exposed vines; but the marc retained approximately 2.5 times as much 4-ethylguaiacol and 4-ethylphenol than found in the wine. Small amounts of guaiacol and 4-methylguaiacol (6 μg/L and 2 μg/L, respectively) were observed in control grape marc, but only traces were detected in control wine, immediately before or after pressing.

Hydrolytic studies confirmed the release of smoked-derived volatile phenols under acid and enzyme catalyzed reaction conditions (**Table 4**), further supporting their accumulation in smoke affected grapes in conjugated precursor forms. The evolution of phenols through /*i*-glucosidase activity alludes to glycoconjugate precursors, such as /*i*-D-glucopyranosides. Guaiacol, 4-methylguaiacol, 4-ethylguaiacol, and 4-ethylphenol were identified as components of both strong acid and enzyme hydrolysates of smoked free run juice. These hydrolysates smelled strongly of 'smoke' and 'smoked meat', respectively, by informal sensory evaluation. In contrast, the mild acid hydrolysates and each of the control hydrolysates exhibited

Table 3. Concentrations of Guaiacol, 4-Methylguaiacol, 4-Ethylguaiacol, 4-Ethylphenol, and Eugenol in Ferments (Pre- and Post-Pressing) and Grape Marc Derived from the Fruit of Smoked and Unsmoked Grapevines

	concentration[1] (μg/L or μg/kg)				
sample	guaiacol	4-methyl guaiacol	4-ethyl guaiacol	4-ethyl phenol	eugenol
		unsmoked			
wine (pre-pressing)	1	tr.	n.d.	n.d.	tr.
wine (post-pressing)	tr.	tr.	n.d.	n.d.	tr.
grape marc	6	2	n.d.	n.d.	n.d.
		smoked			
wine (pre-pressing)	249 a	42 a	9 a	17 a	2 a
wine (post-pressing)	246 a	41 a	9 a	15 a	1 a
grape marc	251 a	38 a	22 b	52 b	6 b

[1] Values are the means from three replicates and were in agreement to ca. 10%. Values followed by a different letter within columns are significantly different ($P < 0.05$). n.d. ❯ not detected; tr. ❯ trace (i.e., positive identification but <1 μg/L).

'berry', 'fruit' and 'jammy' aromas, with the volatile phenols detectable at only trace levels (<1 μg/L). Guaiacol and 4-methylguaiacol were again the most abundant smoke-derived phenols; present in the strong acid hydrolysate at 431 μg/L and 162 μg/L, respectively, and in the enzyme hydrolysate at 325 μg/L and 82 μg/L, respectively. Eugenol was again the least abundant phenol measured, with small quantities (5 μg/L or less) detected in strong acid hydrolysates of both smoked and control free run juice, respectively.

Guaiacyl /*i*-D-glucopyranoside has been previously isolated from the fruit of anise (*Pimpinella anisum* L.) (*24*) and guaiacol has been identified in enzyme hydrolysates of several fruits, including tomato, mango and badea (*25–27*). This further supports our hypothesis of naturally occurring guaiacyl /*i*-D-glucopyranoside. It is possible that plants (including grapevines) may glycosylate some volatile compounds in order to minimize toxic effects to cells, or to increase their solubility to facilitate cellular transportation. Certainly, there is literature precedence for the glycosylation of phenol in cultured plant cells (*28*). The provenance of glycosylated volatile phenols in smoke affected grapes and wine is therefore the subject of ongoing research.

Higher levels of guaiacol, 4-methylguaiacol and 4-ethylguaiacol were observed in the (smoked) strong acid hydrolysate compared to the (smoked) finished wine, suggesting incomplete hydrolysis of putative precursor compounds during fermentation. When the smoked wine was reanalyzed 12 months post-bottling, similar guaiacol levels were observed, but 4-methylguaiacol, 4-ethylguaiacol and 4-ethylphenol levels increased (**Table 2**), suggesting further hydrolysis of soluble precursors with bottle age. This is akin to the accumulation of (toasted) oak derived volatile compounds in wine to different extents during aging, even with no further contact with the oak (*29*). The nature and concentration of smoke taint precursor compounds may influence the release of their free volatile aglycones under various conditions; certainly, the evolution of phenol derivatives post-bottling supports the presence of precursors in addition to simple /*i*-D-glucopyranosides.

At juice pH and in the absence of glycosidase activity, glycoconjugates are relatively stable toward chemical hydrolysis (*30*), except where the carbohydrate unit is bonded to an activated hydroxyl group (*15*), which could explain the absence of smoke-derived phenols in the mild acid hydrolysate. Furfural and 5-methylfurfural were identified as acid hydrolysate components in both smoked and control samples, but their origin is likely attributable to acid catalyzed thermal degradation of carbohydrates, and not grapevine smoke exposure. The higher levels observed in the strong acid hydrolysates simply reflect the more aggressive hydrolysis conditions.

Significant quantities of guaiacol or 4-methylguaiacol would not be expected to form through hydrolysis of glycoconjugate precursors at juice pH. However, micro-organisms with /*i*-glucosidase activity could certainly liberate these compounds during fermentation. The enzymatic release of smoked-derived volatile phenols therefore provides a plausible explanation for the observed intensification of smoke taint during fermentation. Most importantly, it should be recognized that if smoke-derived volatile compounds do indeed accumulate in grapes as odorless glycoconjugates following grapevine exposure to smoke, there may well be no apparent smoke taint at the time of harvest. However, the hydrolytic release of such volatiles could lead to the development of smoke aromas during fermentation, and subsequently smoke tainted wine.

For assessment of smoke taint contingent on guaiacol and 4-methylguaiacol determination, we recommend sample preparation be taken into consideration to ensure hydrolysis of any glycoconjugate precursors which might be present. In the current trial, where grapevines were deliberately exposed to repeated and relatively high intensity smoke applications, strong acid hydrolysis yielded higher levels of smoke-derived volatile phenols than enzyme hydrolysis. The strong acid hydrolysis conditions used in this study, i.e., pH 1.0 for 1 h at 100 °C, are those employed in the glycosyl-glucose assay for the quantification of glycosides in

Table 4. Concentrations of Guaiacol, 4-Methylguaiacol, 4-Ethylguaiacol, 4-Ethylphenol, Eugenol, Furfural and 5-Methylfurfural in Free Run Juice, and Acid and Enzyme Hydrolysates of Juice Derived from Fruit of Smoked and Unsmoked Grapevines

	concentration[a] (μg/L)						
sample	guaiacol	4-methyl guaiacol	4-ethyl guaiacol	4-ethyl phenol	eugenol	furfural	5-methyl furfural
			unsmoked				
free run juice	n.d.	n.d.	n.d.	n.d.	n.d.	2	tr.
mild acid hydrolysate	tr.	tr.	tr.	tr.	n.d.	76	2
strong acid hydrolysate	tr.	tr.	tr.	tr.	2	15150	640
enzyme hydrolysate	tr.	tr.	tr.	tr.	n.d.	7	2
			smoked				
free run juice	1	tr.	n.d.	n.d.	n.d.	2	tr.
mild acid hydrolysate	tr.	tr.	tr.	tr.	n.d.	40	2
strong acid hydrolysate	431	162	31	48	5	12800	860
enzyme hydrolysate	325	82	13	27	n.d.	8	2

[a] Values are the means from three replicates for juice samples and two replicates for hydrolysate samples. Values were in agreement to ca. 10%. n.d. ❯ not detected; tr. ❯ trace (i.e., positive identification but <1 μg/L).

grapes, juice and wine (*19*). However, since these reaction conditions could also catalyze various aglycone side reactions (for example aglycone degradation), enzyme hydrolysis may be more appropriate for commercial samples, where less intense smoke exposure would likely give lower volatile phenol levels. Accordingly, the potential under-estimation of smoke taint can be reduced.

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Effect of smoke application to field-grown Merlot grapevines at key phenological growth stages on wine sensory and chemical properties

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Abstract

Background and Aims: Smoke exposure of grapevines and development of smoke taint in wine are issues of increasing incidence and severity. There is limited understanding of the effect of phenological stage at the time of smoke exposure on taint development. The aim of this study was to demonstrate the variation in smoke uptake and taint development between and within seasons.

Methods and Results: Smoke was applied to field-grown Merlot grapevines at 12 stages of vine development over three growing seasons. Key periods of vine sensitivity to smoke taint in wine were (i) from shoots at 10 cm to full bloom (low levels of smoke taint); (ii) from berries at pea size to the onset of veraison (variable levels of smoke taint); and (iii) between 7 days post-veraison and harvest (high levels of smoke taint).

Conclusions: The severity of taint in wine varied depending on the phenological timing of grapevine smoke exposure. Taint was elevated when exposure occurred between 7 days post-veraison and harvest. The carry-over of smoke constituents the following season was not detectable in wine but yields were reduced.

Significance of the Study: This is the first study to demonstrate the timing of smoke exposure to critically affect wine chemical and sensory characters. These effects were consistent and reproducible over three seasons.

Keywords: *gas chromatography-mass spectrometry*, *grapevine*, *guaiacol*, *phenology*, *smoke taint*, Vitis vinifera, *volatile phenol*

Introduction

The exposure of grapevines to smoke and the subsequent development of smoke taint in wine is an important issue for wine producers globally. Increases in the incidence of wildfires and fire-risk weather (actual and predicted) in Australia, Canada and the USA have been attributed to climate change (Gillett et al. 2004, Hennessy et al. 2005, Westerling et al. 2006). As such, the presence of smoke in viticultural areas is likely to occur more frequently, resulting in the increased occurrence of smoke taint for wine producers.

Smoke produced from the combustion of vegetative biomass contains numerous substances including inorganic gases (carbon monoxide, ozone, nitrogen dioxide), polycyclic aromatic hydrocarbons, volatile and semi-volatile organic compounds, particulate matter ($PM_{2.5}$ and PM_{10}) and oxygenated organics (Schauer et al. 2001, Lee et al. 2005, Naeher et al. 2007). The production of these substances varies with the combustion conditions such as moisture and oxygen availability, temperature and fuel composition (Maga 1988b, Hays et al. 2002, Simon et al. 2005). Fuel composition can also vary depending on the fuel type and source and it is generally comprised of lignin (18–35%), cellulose (40–45%) and hemicellulose (20–35%; Maga 1989). To date, there are no published reports on the effect of different fuels and fuel pyrolysis conditions on the development of smoke taint in wine.

The presence of smoke taint in wine can result in the wine being unacceptable for consumption with tainted wines exhibiting 'burnt rubber', 'smoked meat', 'leather', 'disinfectant', 'ash', 'smoked salmon' and 'salami' characters (Høj et al. 2003, Kennison et al. 2009). However, smoking of foods is one of the oldest methods of food processing and has traditionally been used to impart flavours, aromas, colours and for food preservation by reducing antimicrobial spoilage (Wittkowski et al. 1992, Fellows 2000). Smoke and liquid smoke flavourings utilised in food processing have been shown to contain compounds including carbonyls, aldehydes, lactones, ketones, furans, pyrans and phenols (Maga 1988a, Guillén et al. 1995, Guillén and Manzanos 1996). The volatile phenols guaiacol and 4-methylguaiacol, which derive from the thermal degradation of lignin, are present in smoke and have been reported to exhibit 'smoky', 'phenolic', 'sharp', 'smoked meat' and

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'burning' aromas and flavours (Baltes et al. 1981, Boidron et al. 1988, Maga 1988a, Rocha et al. 2004). Guaiacol and 4-methylguaiacol are routinely detected in wines aged in toasted oak barrels at concentrations up to 100 and 20 mg/L, respectively (Pollnitz et al. 2004).

Research on the effects of smoke exposure at a range of grapevine growth stages and the subsequent development of smoke taint in wine is limited. A direct link between smoke exposure to grapes and the development of smoke taint in wine has been established for both grapes exposed to smoke postharvest (Kennison et al. 2007) and field-grown grapevines exposed to smoke between veraison and harvest (Kennison et al. 2009). The later study identified a peak period of grapevine sensitivity to smoke uptake at 'veraison + 7d'. Furthermore, Kennison et al. (2009) also utilised repeated smoke exposures to grapevines to demonstrate an accumulation effect of volatile phenols in the final wine product (388 mg/L guaiacol, 93 mg/L 4-methylguaiacol) with these wines demonstrating elevated smoke-like aromas of 'burnt rubber', 'smoked meat' and 'leather'. In another study conducted in British Columbia, application of smoke to field-grown grapevines pre-veraison, post-veraison and at maturity resulted in the detection of guaiacol (2 to 26 mg/L) in grapes; however, the study did not investigate the effects of smoke exposure on resultant wines (Sheppard et al. 2009). Therefore, previous studies, to date, have not considered the implication of smoke exposure across the key phenological stages of an entire growing season nor between subsequent growing seasons.

The current study builds on previous research to investigate the effect of smoke exposure of grapevines over three growing seasons. Smoke applications were made over a comprehensive range of phenological stages and the development of volatile phenols and sensory smoke aromas in resultant wines were investigated. This enabled the assessment of treatment effects both within and between seasons.

Materials and methods

Trial establishment

The trial was established on a commercial vineyard (*Vitis vinifera* cv. Merlot) located at Capel (33.575°S, 115.577°E) in the Geographe region of Western Australia. The trial site was selected because of its remote location from forested areas and infrequent history of smoke exposure. During the 3-year period of the trial, no incidences of external smoke generation and exposure were observed.

Smoke generation and application

Proven methodology previously employed for the application of smoke to field-grown grapevines (Kennison et al. 2009) was utilised in this study. In brief, smoke was produced from the combustion of dry barley straw in a 50-L lidded drum and pumped through outlet piping into tents (6 m long ¥ 2.5 m high ¥ 2 m wide) which enclosed field-grown grapevines (three vines per replicate). Tents were constructed from galvanised steel and greenhouse-grade plastic (Solaweave, Gale Pacific, Braeside, Vic., Australia) that enabled light transmission. The density (30% obscuration/m) and duration (min) of smoke applications were measured using portable nephelometer equipment (VESDA Laser FOCUS™ VLF-250, Mount Waverly, Vic., Australia).

In order to assess smoke application to a range of grapevine growth stages and the reproducibility of this application between seasons, smoke treatments were applied to field-grown grapevines over three growing seasons (2006/2007, 2007/2008 and 2008/2009). In each season, different grapevines were selected for experimental treatments in order to minimise any potential carry-over effects. Experimental treatments comprised the application of smoke to grapevines (in triplicate, three vines per replicate) for 30 min. Smoke was applied to different grapevines at various phenological stages of grapevine growth as designated by the modified Eichhorn-Lorenz (E-L) system (Coombe 1995). The E-L system was further modified for this study to incorporate additional smoke application timings (Table 1). Smoke was applied to field-grown grapevines at least once ($n = 1$ to 3) at the growth stages corresponding to E-L: 12 (10-cm shoots); 23 (full bloom); 31 (pea-sized berries); 32 (bunch closure); 35(a) (onset of veraison); 35(b) (veraison + 3d); 35(c) (veraison + 7d); 35(d) (veraison + 10d); 36(a) (intermediate total soluble solids (TSS)); 36(b) (intermediate TSS + 3d); 37 (berries not quite ripe); and 38 (harvest).

Separate smoke treatments were performed to investigate: the potential sequestration of smoke constituents within the grapevine; the phenological carry-over potential of smoke constituents' compounds; and the recovery of grapevines from one season to another. In order to ensure a heavy smoke application, repeated smoke treatments ($n = 8$) were applied to the same vines from the onset of veraison to harvest with wine made from fruit of these vines for sensory and chemical analysis. Wine was also made from fruit produced by the same vines in the following grapegrowing season to investigate grapevine recovery and the potential carry-over of smoke aromas and flavours between years.

Winemaking

Wine was produced from all smoke and control treatments in each year of the trial when grapes reached TSS of 21.4 ± 2.9°Brix as measured by refractometry. Fruit (approximately 15 kg) from each replicate ($n = 3$) of smoke and control (unsmoked) treatments was processed separately to avoid contamination. Fruit was crushed, destemmed and inoculated with *Saccharomyces cerevisiae* EC1118 yeast (Lallemand Inc., Montreal, Canada) at a rate of 200 mg/L and fermented in 15-L stainless steel fermentation vessels. On average, fermenting musts were plunged twice daily until the wine approached a TSS of 0°Brix before being pressed off skins. Wines were then inoculated for malolactic fermentation with *Oenococcus oeni* (Viniflora Oenos, Chr. Hansen, Hørsholm, Denmark) and were stored in 4.6-L glass fermenters at 15°C until completion of malolactic fermentation as determined by quantitative malic acid analysis (Iland et al. 2004). Post-malolactic fermentation, wine sulphur dioxide (SO_2) concentrations were measured by aspiration (Iland et al. 2004) and adjusted to 30 ppm. Wines were subsequently cold stabilised for 28 days at 2°C. Wines were subsequently filtered (5 mm) and bottled.

Quantitative analysis of guaiacol and 4-methylguaiacol

Gas chromatography-mass spectrometry (GC-MS) was utilised to determine guaiacol and 4-methylguaiacol concentrations in both grape and wine samples using previously reported methodology (Spillman et al. 1997, Pollnitz et al. 2004, Kennison et al. 2008). Guaiacol and 4-methylguaicol were selected as analytes of interest as they have previously been used as indicators of smoke taint (Kennison et al. 2009).

Sensory analysis of wine aroma

Sensory analysis of all wines in this study was conducted by quantitative descriptive analysis (Meilgaard et al. 2007) using a trained panel of eight people (four males and four females). Panellists were pre-screened and selected for experience with

Table 1. Key grapevine growth stages from the modified E-L system and additional growth stages used for reference in this study.

Modified E-L grapevine growth stage†		Alternative interpretation of modified E-L growth stages for reference in this study	
Stage number	**Description**	**Stage number**	**Designation**
12	5 leaves separated; shoots approximately 10 cm in length; inflorescence clear	12	10-cm shoots
23	17–20 leaves separated; 50% caps off; full bloom	23	Full bloom
31	Pea-sized berries (7 mm diameter)	31	Pea-sized berries
32	Beginning of bunch closure; berries touching (if bunches are tight)	32	Bunch closure
35	Berry colouring and softening begins; berries begin to enlarge	35(a)	Onset of veraison
		35(b)	Veraison + 3d
		35(c)	Veraison + 7d
		35(d)	Veraison + 10d
36	Berries with intermediate TSS values	36(a)	Intermediate TSS
		36(b)	Intermediate TSS + 3d
37	Berries not quite ripe	37	Berries not quite ripe
38	Berries ripe for harvest	38	Harvest

†Adapted from Coombe 1995. TSS, total soluble solids.

wine sensory education (i.e. >100 h), availability, interest in the study, being non-smokers, regular wine drinkers and of good health. Panellists' ages ranged between 21 and 30 years and all were able to detect the aroma of smoke taint in red wines at a predetermined threshold ascertained by Kennison et al. (2007).

Prior to formal wine evaluation, all panellists participated in eight training sessions where they identified and agreed on six descriptive aroma terms and learnt to measure the intensity of smoke aroma on an unstructured 100-point line scale. Formal wine aroma evaluation was conducted on 30 wines (i.e. two replicates of 15 wine treatments) over six sessions each held at the same time of day on different week days. Samples (20 mL) were presented in three-digit coded ISO standard wine tasting glasses that were lidded with glass covers to avoid contamination of other samples and the testing area. Sample order was completely randomised with each panellist receiving a different sample at any one time in order to avoid bias. In order to avoid sensory fatigue, each panellist left the testing area for approximately 10 min to an outdoor environment after evaluating each sample.

Statistical methods

All data was analysed using Genstat 11th Edition (VSN International Limited, Hemel Hempstead, UK). Analysis of variance (ANOVA) was utilised to analyse wine sensory and chemical data at the 5% level of significance ($P < 0.05$). Further wine sensory data was analysed by Principal Component Analysis (PCA). Chemical data from the phenological smoke application experiments was analysed by the residual maximum likelihood (REML) procedure that was utilised to fit a linear mixed model over 3 years (fixed effect = smoke treatment).

Results

Effects of smoke exposure on chemical properties of wine

The degree of smoke taint, according to guaiacol and 4-methylguaiacol content of wine, varied considerably depending on the phenological timing of grapevine exposure to smoke. Over the three growing seasons, the concentration of guaiacol and 4-methylguaiacol in wines ranged from a low of 0.6 and 0.3 mg/L, respectively, for wines corresponding to grapevine smoke exposure at '10-cm shoots', to a high of 60.7 and 14.1 mg/L, respectively, for wines made from vines exposed to smoke at 'veraison + 7d' (Figure 1). Control wines, i.e. wines produced from fruit harvested from unsmoked vines, contained either no detectable or trace concentrations of guaiacol (i.e. <2 mg/L) and 4-methylguaiacol (i.e. <0.5 mg/L). As in previous studies (Kennison et al. 2007, 2008), 4-methylguaiacol occurred at lower concentrations than guaiacol in all wines, however, followed similar trends across the three growing seasons.

On the basis of guaiacol and 4-methylguaiacol concentration in wine, three key periods of susceptibility to smoke exposure were identified within the annual cycle of active grapevine growth. These were period 1 (P1), defined as the period from '10-cm shoots' to 'full bloom' when exposure to smoke resulted in relatively low concentrations of volatile phenols observed in wine; period 2 (P2) defined from the stage of 'berries of pea size' to the 'onset of veraison' during which moderate but variable concentrations of volatile phenols were observed in wine; and period 3 (P3) defined from the stage of 'veraison + 7d' to 'harvest' during which the highest concentrations of volatile phenols were observed in wine (Figure 1). Average guaiacol and 4-methylguaiacol concentrations in wine were 1.0 and 0.5 mg/L, respectively, for P1; 21.4 and 5.0 mg/L, respectively, for P2; and 48.9 and 8.9 mg/L, respectively, for P3.

Effect of smoke exposure on sensory properties of wine

Trained panellists rated the intensity of smoke-like aromas such as 'burnt rubber', 'smoked meat', 'leather' and 'disinfectant/hospital' to quantify the degree of taint present in wine, together with desired wine attributes such as 'red berry fruits' and 'confection' (Table 2). An ANOVA of sensory data showed the wine treatments to be a source of variation ($P < 0.001$ to $P < 0.05$) for all aroma attributes except for the wine aroma

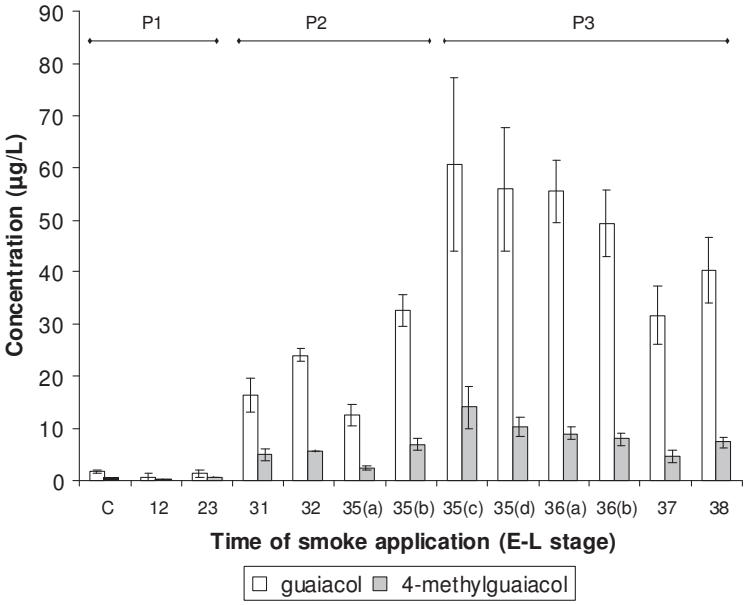

Figure 1. Concentration of guaiacol and 4-methylguaiacol in wine made from fruit of vines exposed to a single smoke application at either E-L 12 (10-cm shoots), 23 (full bloom), 31 (pea-sized berries), 32 (bunch closure), 35(a) (onset of veraison), 35(b) (veraison + 3d), 35(c) (veraison + 7d), 35(d) (veraison + 10d), 36(a) (intermediate TSS), 36(b) (intermediate TSS + 3d), 37 (berries not quite ripe) or 38 (harvest). Three separate periods of vine sensitivity to smoke taint uptake are represented by P1 (low uptake), P2 (variable uptake) and P3 (high uptake). Data is from 2006 to 2008, analysed by REML to produce predicted means and standard errors. C, control; $n = 3$ to 9; error bars show two standard errors of the mean.

Table 2. Analysis of variance for wine sensory attribute ratings of 'burnt rubber', 'smoked meat', 'leather', 'disinfectant/hospital', 'red berry fruits' and 'confection'.

Aroma descriptor	Wine (W)	Panellist (P)	Rep (R)	W ¥ P	P ¥ R	W ¥ R
Burnt rubber	4.922***	4.000***	2.133	2.21**	0.81	1.15
Smoked meat	5.844***	2.994***	0.08	1.36	0.65	1.84
Leather	2.342*	3.747***	1.052	1.78*	0.33	1.31
Disinfectant/hospital	2.455*	9.265***	0.881	1.89*	0.66	0.53
Red berry fruits	2.399*	7.236***	1.813	0.97	1.35	1.45
Confection	1.812	8.161***	3.358	0.73	1.20	2.12*

F ratios are shown as sources of variation. Significance indicated by $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.
Variance is represented for wine (W), panellist (P), replicate (R), wine by panellist (W ¥ P), panellist by replicate (P ¥ R) and wine by replicate (W ¥ R).

attribute of 'confection'. Panellists were also a source of variation ($P < 0.001$) in wine sensory data, however, were consistent in their evaluation between sessions. A wine by panellist interaction in the aroma of 'burnt rubber' ($P < 0.01$), 'leather' ($P < 0.05$) and 'disinfectant/hospital' ($P < 0.05$) was also present. Wine replicates showed reproducibility as not being a source of variation except for the wine by replicate aroma attribute of 'confection'.

Panellists determined all aroma characters to be present in the 3-year wine set with wine-like aromas of 'red berry fruits' and 'confection' in higher mean intensities (44.6 to 44.9) than smoke-like aromas (15 to 18.1; Figure 2). However, while the intensity of smoke-like aromas was more subtle the wines that exhibited higher expression of smoke-like aromas generally also exhibited low wine-like aromas and vice versa. PCA of mean aroma results accounted for 94.4% of overall variation as being comprised of principal component 1 (88.5%) and 2 (5.9%) (Figure 3). Principal component 1 consists of positive loadings on smoke-like aromas of 'burnt rubber' (0.42), 'smoked meat' (0.41), 'leather' (0.41), 'disinfectant/hospital' contrasted with negative loadings on wine-like aromas of 'red berry fruits' (–0.41) and 'confection' (–0.4). Principal component 2 is dominated by negative loadings on all aroma attributes (–0.19 to –0.55) except for the attribute of 'disinfectant/hospital' that has a positive loading (0.15).

The intensity of specific wine aromas was found to vary depending on the phenology at the time of smoke application to grapevines (Figure 3). Wines produced from fruit of unsmoked (control) grapevines and fruit produced from vines smoked in P1 contained dominant wine-like aromas of 'red berry fruits' and 'confection'. Conversely, wines produced from grapevines exposed to smoke at E-L stage 35(b) (veraison + 3d), 35(c) (veraison + 7d), 35(d) (veraison + 10d) and 36(a) (onset of veraison) in P3 were dominated by smoke-like aromas of 'disinfectant/hospital', 'burnt rubber', 'smoked meat' and 'leather'. Interestingly, wine made from fruit of grapevines exposed to smoke at E-L 31 (pea-sized berries) from P2 had elevated smoke-like aromas, levels similar to those detected in wines made from fruit of vines exposed to smoke at E-L 38 (harvest; Figure 2).

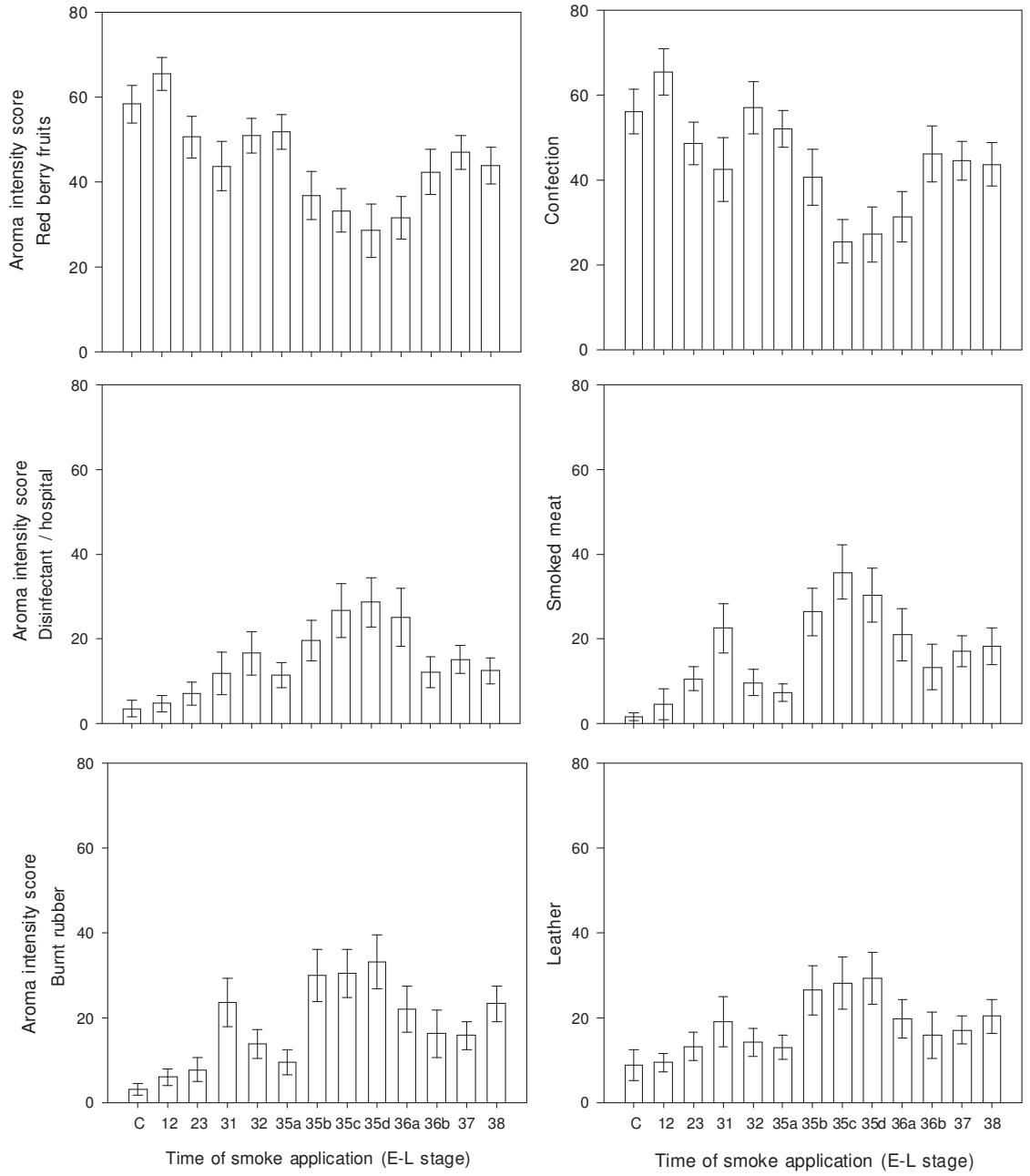

Figure 2. Mean aroma intensity descriptor scores of 'burnt rubber', 'smoked meat', 'leather', 'disinfectant/hospital', 'red berry fruits' and 'confection' detected in wines made from grapes of vines exposed to smoke at either E-L stage 12 (10-cm shoots), 23 (full bloom), 31 (pea-sized berries), 32 (bunch closure), 35(a) (onset of veraison), 35(b) (veraison + 3d), 35(c) (veraison + 7d), 35(d) (veraison + 10d), 36(a) (intermediate TSS), 36(b) (intermediate TSS + 3d), 37 (berries not quite ripe) or 38 (harvest). Error bars indicate two standard errors of the mean.

A relationship was also evident between the concentration of guaiacol and 4-methylguaiacol determined by GC-MS and that of the smoke and wine-like aromas in wine determined by sensory analysis. Regression analysis (not shown) revealed a strong positive linear correlation between guaiacol and the smoke-like aroma characters of 'burnt rubber' ($r = 0.8$), 'smoked meat' ($r = 0.78$), 'leather' ($r = 0.79$) and 'disinfectant/hospital' ($r = 0.87$) and a strong negative linear correlation between guaiacol and the wine-like aroma descriptors of 'confection' ($r = -0.84$) and 'red berry fruits' ($r = -0.88$). Guaiacol

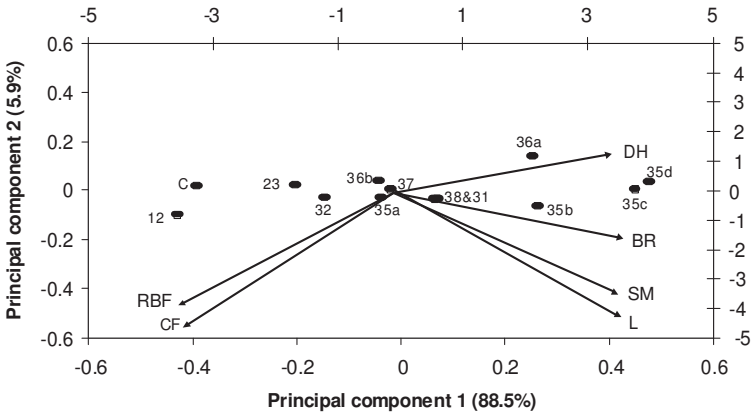

Figure 3. PCA biplot of mean wine sensory scores (■) made from fruit of vines exposed to smoke application at E-L stage 12 (10-cm shoots), 23 (full bloom), 31 (pea-sized berries), 32 (bunch closure), 35(a) (onset of veraison), 35(b) (veraison + 3d), 35(c) (veraison + 7d), 35(d) (veraison + 10d), 36(a) (intermediate TSS), 36(b) (intermediate TSS + 3d), 37 (berries not quite ripe) and 38 (harvest). Aroma descriptors are indicated by arrows labelled BR (burnt rubber), SM (smoked meat), L (leather), DH (disinfectant/hospital), RBF (red berry fruits) and CF (confection). C, control (unsmoked) wine.

and 4-methylguaiacol were therefore adequate indicators of the intensity of smoke-like aromas in wine.

Seasonal effect of smoke exposure on grapes and wine

In order to investigate grapevine recovery and the potential for carry-over of smoke constituents from one season to the next, repeated smoke applications ($n = 8$) were applied to the same vines in a single growing season. The most notable carry-over effect was decreased fruit yield of grapevines in the subsequent season. In the season of repeated smoke application (i.e. year 1), smoked grapevines yielded an average of 11 kg per vine of fruit at harvest, in comparison to control vines, which yielded an average of 15.8 kg per vine (Table 3). In the subsequent season (i.e. year 2), the same vines were not exposed to smoke but continued to produce reduced yields (6.4 kg) and bunch numbers (73) in comparison to the unsmoked (control) vines, which yielded 12.9 kg from 115 bunches.

However, from a chemical and sensorial perspective, no carry-over effect from repeated smoke exposure was observed in the subsequent season. Sensory analysis of wines made from grapes harvested one season after smoke exposure (i.e. year 2) indicated a low level (not significant at $P < 0.05$) of 'smoked meat' aroma, but these wines also exhibited intense 'red berry fruits' and 'confection' aromas, i.e. at similar levels to those of the control wine (Figure 4). In the same wines, the concentration of guaiacol (2 mg/L) and 4-methylguaiacol (0 mg/L) also showed no chemical carry-over effect (Table 3).

Discussion

This study builds on past research (Kennison et al. 2009) and demonstrates that the development of smoke taint depends greatly on the phenological timing of grapevine smoke exposure. In particular, the timing of peak periods of smoke uptake was consistent over the three growing seasons. Furthermore, this study has demonstrated that the carry-over effect of smoke exposure between seasons is limited to physiological responses (i.e. yield and bunch number) and there was no evidence of sequestration of smoke constituents by grapevines. To our knowledge, this is the first study concerning the development of smoke taint as a function of phenology for any crop.

Table 3. Concentration of guaiacol and 4-methylguaiacol in wines made from fruit of vines exposed to eight repeated smoke applications from the period of veraison to harvest (2006), in comparison to wines made from fruit of the same vines 1 year post-repeated smoke exposure (2007) and wines from fruit of control (unsmoked) vines.

	Compound concentration (mg/L) in wine		Yield components (average per vine)	
	Guaiacol	4-methylguaiacol	Fruit yield (kg)	Bunch no.
Year 1: smoke applied to vines†				
Smoke	388.3[a]	93	11.0[a]	129.3[ab]
Control	4.3[b]	n.d.	15.8[b]	148.3[a]
Year 2: 1 year post-smoke application				
Smoke	2[b]	n.d.	6.4[c]	73.7[c]
Control	0[b]	n.d.	12.9[d]	115.3[b]

Means followed by the same letter within columns are not significantly different at $P ::: 0.05$, results are per treatment replicate (three vines), mean values are from three replicates. Fruit yield and average bunch number produced from the same grapevines (i.e. for the 2006 and 2007 seasons) are also represented. †Data from Kennison et al. 2009. n.d. = not detected.

The demonstrable link between phenology and the intensity of smoke taint in wine enabled the identification of three periods of susceptibility to smoke taint. The first period (P1) corresponded to a low level of smoke taint susceptibility and wine produced from fruit of grapevines exposed to smoke during this period contained trace levels of guaiacol (<1 mg/L) and 4-methylguaiacol (<0.5 mg/L) only (Figure 1). The dominant sensory attributes of these wines were 'red berry fruits' and 'confection'. The sensory panel gave low scores for 'burnt rubber', 'smoked meat', 'leather' and 'disinfectant/hospital' aromas (Figure 2). The possible reasons for the low levels of taint following exposure during P1 are most likely related to the lack of fruit because of the early stage of fruit development

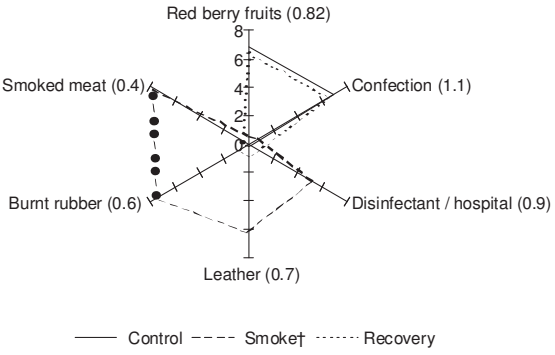

Figure 4. Mean intensity ratings of smoke-like aromas of 'burnt rubber', 'smoked meat', 'leather', 'disinfectant/hospital' and wine-like aromas of 'confection' and 'red berry fruits' in smoke tainted wines, recovery effects (1 year post-smoke exposure) and wines made from fruit of control (unsmoked) grapevines. Scale represents 0 = non-detectable aroma to 8 = highly detectable aroma; †data from Kennison et al. 2009.

rather than the lack of uptake by the vine *per se*. As P1 occurred prior to fruitset, there was minimal surface area for direct uptake by the fruit. Furthermore, translocation of smoke compounds taken up by the vine would have been limited by the lack of a strong source-sink relationship between the leaves and fruit (Ollat and Gaudillère 2000, Ollat et al. 2002). Likewise, compounds taken up during this time would be expected to be diluted through subsequent vine and fruit growth and may also potentially be lost through volatilisation or degradation in a similar manner to that reported for pesticides (Cabras and Angioni 2000).

During P2, when fruit was present on the vine, increased concentrations of taint were observed in wines, i.e. 21.4 mg/L for guaiacol and 5 mg/L for 4-methylguaiacol. Compared with P1, phenol levels were higher, but absolute levels were variable between seasons and exposure timings. P2 wines exhibited enhanced 'red berry fruits' and 'confection' characters, but often also showed enhanced 'burnt rubber', 'leather', 'smoked meat' and 'disinfectant/hospital' characters. This period comprises the initial period of rapid berry growth via cell division, followed by a lag phase of slowed growth and seed maturation (Coombe 1992, Mullins et al. 2000). During this phase, bunches are a comparatively weak sink for photosynthates until the onset of veraison (Hale and Weaver 1962, Ollat et al. 2002). P2 concludes with a heightened level of smoke taint for wines made from fruit of vines exposed to smoke 3 days after veraison. This may represent a transition from P1 to P3 or even a distinct period in itself.

The highest risk period (P3) for the development of smoke taint corresponded to smoke exposure between 'veraison + 7d' and harvest. Elevated concentrations of volatile phenols were measured in wines, being on average, 60.7 mg/L for guaiacol and 14.1 mg/L for 4-methylguaiacol. Wines also exhibited intense 'leather', 'smoked meat' and 'burnt rubber' aromas and were generally disagreeable to panellists. The timing of P3 smoke applications corresponded with the grape berry ripening, i.e. when bunches represent major carbohydrate sinks (Coombe 1992, Ollat et al. 2002). The development of smoke taint during P3 is therefore likely to indicate both direct berry uptake and translocation from leaves to the berry.

Hayasaka et al. (2010a) demonstrated that the marker compound, guaiacol, was assimilated by leaves, conjugated and translocated between leaves and berries. In their study, which incorporated glasshouse-grown vines, the rates of translocation of the extrogenous source of labelled guaiacol were considered to be slow. If translocation is an important contributor to taint accumulation and if the rate is limited, then it is logical that it will be time dependent. Smoke exposure in the late stages of P3 resulted in markedly lower levels of taint (40.3 mg/L guaiacol and 7.3 mg/L 4-methylguaiacol) than smoke exposure in the earlier stages of P3 (60.6 mg/L guaiacol and 14.1 mg/L 4-methylguaiacol). In the same period (P3), there was a strong negative correlation between the timing of smoke application to vines and concentration of both guaiacol ($r = -0.866$) and 4-methylguaiacol ($r = -0.882$) in the wine. This indicates that there is likely to be a rate limitation in the translocation of taint compounds to the fruit and this has implications for the timing of harvest relative to the timing of smoke exposure.

Smoke taint was not found to carry-over in wine in the season that followed high levels of smoke exposure although grapevine yield was reduced. The reduced yield from smoke-exposed vines (6.4 kg) was found to be substantially lower in relation to those vines that were not exposed to smoke (12.9 kg/vine). The reduction in vine yield may be related to the negative impact that smoke could have on the photosynthetic capacity of the vine. A short duration (1 min) of smoke has been documented to reduce the stomatal conductance, CO_2 assimilation rate and intercellular CO_2 levels of *Chrysanthemoides monilifera* with full plant recovery not achieved for 24 h (Gilbert and Ripley 2002). In grapevines, smoke exposure has been shown to decrease the grapevines' ability to accumulate sugar in grape berries and has produced damage on leaf surfaces as evidenced by necrotic lesions (Kennison et al. 2009). The reduction in grapevine yield may therefore likely be a consequence of the effect that smoke may have on the physiological functioning of the vine.

The current study raises several important implications for the wine industry. Firstly, if grapevine smoke exposure occurs early in the grapevine growth cycle, i.e. prior to flowering, then the intensity of smoke taint in resulting wines is likely to be relatively low. However, if smoke exposure occurs between fruit set and harvest, then there is far greater potential for the development of smoke taint in wine, so the presence of marker compounds should be further investigated in smoke-exposed grapes prior to vinification. Since it has now been established that smoke-derived volatile phenols are conjugated following smoke exposure (Hayasaka et al. 2010b), detection of marker compounds should involve acid hydrolysis of juice samples (as described by Kennison et al. 2008), small-scale fermentations or direct measurement of guaiacol glycoconjugates (Dungey et al. 2011). However, if smoke exposure occurs immediately prior to harvest then smoke taint could be minimised if fruit is harvested as soon as possible after the exposure. To avoid the carry-over yield reduction effect caused by high levels of smoke exposure, consideration may need to be given to retaining higher vine bud numbers in the season following smoke exposure.

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CHAPTER 5 UNPUBLISHED PAPER

5.1 UNPUBLISHED PAPER 1

Kennison, K.R., Williams, H.G. and Gibberd, M.R. (2011) The density and duration of smoke exposure to grapevines influences the development of smoke-like compounds, flavours and aromas in resultant wines. Paper submitted to the Australian Journal of Grape and Wine Research.

The density and duration of smoke exposure to grapevines influences the development of smoke-like compounds, flavours and aromas in resultant wines.

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Abstract

Background and aims:

The study aimed to determine the minimum amount of smoke exposure required to create smoke taint in wine as detected by wine sensory and chemical analysis.

Methods and results:

Smoke was applied to field-grown Merlot grapevines at high densities (5, 10 and 20% obs/m) for short durations (5, 10 and 20 min) and a low smoke density (2.5% obs/m) for long durations (10, 20, 40 and 80 min). The minimum smoke exposure that created a difference in wine characteristics was 5% obs/m for 5 min. Wines produced from 2.5% obs/m smoke for 80 min and 20% obs/m smoke for 20 min contained accentuated levels of guaiacol, 4-methylguaiacol and smoke related wine sensory characters.

Conclusions:

This study demonstrates the duration and density of smoke exposure required to determine the development of smoke related aromas, flavours and compounds in wine. The minimum smoke application of 5% obs/m for 5 min was found to alter wine characteristics. Higher smoke densities resulted in increased intensity of smoke related sensory characters in wine.

Significance of the study:

This is the first known paper investigating smoke density and duration effects on the development of smoke taint in wine providing information to the wine industry to determine the risk of potential smoke taint development subsequent to grapevine smoke exposure.

Keywords: *gas chromatography-mass spectrometry, grapevines, guaiacol, smoke taint,* Vitis vinifera, *volatile phenols, wine*

Introduction

Smoke derived taint in grapes and wine is an issue of increasing significance for wine production internationally. Viticulture regions in both the northern and southern hemisphere have been negatively affected by smoke derived taint with costs flowing on to wine markets and adversely impacting wine brands (Høj et al. 2003, Krstic et al 2007, Mira de Orduña 2010, Zybach et al. 2009). Due to climate change, an increase in fire events is occurring resulting in more frequent exposure of viticultural areas to smoke (Mira de Orduña 2010, Zybach et al. 2009).

The connection between the atmospheric smoke exposure to grapevines and the development of smoke-like aromas in resultant wine has been well documented (Høj et al. 2003, Kennison et al. 2007, 2009, Sheppard et al. 2009). Wines made from the fruit of vines exposed to smoke exhibit 'smoky', 'burnt', 'salami', 'smoked meat', 'burnt rubber', 'leather' and 'ash' aroma and flavour characteristics (Høj et al. 2003, Kennison et al. 2009). When smoke taint characteristics are of a noticeable or high intensity to the wine consumer, the wine product can be unpalatable and is unfit for purpose. These wines have been shown to contain elevated concentrations of key indicator compounds such as guaiacol and 4-methylguaiacol (Kennison et al. 2009, 2011). In controlled studies, smoke application to field-grown grapevines, results in an elevation of smoke related aromas and compounds in final wines (Kennison et al. 2011). In addition, smoke exposure to grapevines is cumulative with repeat smoke application from the post veraison period to harvest resulting in proportional increases in the concentration of smoke-like compounds and aromas in wine (Kennison et al. 2009).

The extent of development of smoke taint in wine following smoke exposure varies during the annual grapevine growth cycle (Kennison et al. 2011). Smoke exposure to grapevines (cv. Merlot) early in the grapevine growing season, from shoots 10 cm to full-bloom, imparted a negligible effect. However, smoke exposure to grapevines mid-season, from berries pea size to the onset of veraison, results in variable (low to medium) in smoke uptake and there is a high susceptibility to smoke uptake and taint

development late in the growing season, i.e. from 7 days post veraison to harvest (Kennison et al. 2011).

Scientific information on the effect of smoke density and duration on the chemical and sensory properties of food is limited (Codex Alimentarius Commission 2009). The smoking of food is a centuries old tradition utilized to impart smoke flavouring, colour and aroma and to improve food storage and preservation (Tóth and Potthast 1984, Fellows 2000). The magnitude of smoke influence on food is a function of smoke density, velocity, duration, temperature and humidity indicating that the rate of absorption by food is limited by the rate of deposition (Tóth and Potthast 1984, Boyle and Schmidt 1999, Ogbadu 2000, Fellows 2009). Rather than investigating the sensory implications of smoke exposure on food, studies have concentrated on the effect of smoke density, duration and generation conditions (such as temperature and humidity) on the food safety impacts in order to avoid bacterial contamination (Fellows 2009). There are no published studies that relate to fruit, grapes or wine.

Given that smoke events within the landscape are frequent and highly variable, the wine industry needs to be able to ascertain the critical level of exposure which may create an industrial concern. Research to date has investigated the role of bushfire smoke exposure to field-grown grapevines on the chemical and sensory effects of resultant wine. The research has been conducted subsequent to fire events with no capacity to measure the smoke density or the exposure duration (Hoj et al. 2003, Whiting and Krstic 2007). Controlled applications of smoke to field-grown grapevines have been conducted however attempts to quantify the smoke intensity in these experiments are limited. Smoke applications have either been prepared from a known fuel quantity (Sheppard et al. 2009), have had a single duration (30 min) (Kennison et al. 2008) or single and repeated smoke applications (n = 8) to the same grapevines for only one smoke density and duration (200 $\mu g/m^3$ for 30 min) (Kennison et al. 2009). All treatments have proven that the elevation of smoke related properties in grapes and wine, including compounds, aromas and flavours, is possible subsequent to smoke exposure however the minimal level of smoke exposure that leads to the development of smoke taint in wine has not

been determined. As such, the risks associated with smoke exposure are unable to be managed by the wine industry.

Optical properties of smoke derived from fire events are highly variable depending on the fire location and combustion conditions (Garland et al. 2008). The measurement of smoke can be complex requiring the employment of numerous equipment and analysis methodologies (Adam et al. 2004, Lee et al. 2005) such as the highly sensitive and reliable nephelometry equipment for the detection of smoke (Adam et al. 2004).

This study builds on previous research to investigate the effect of smoke density and duration on the development of smoke taint related sensory and chemical attributes in wine. The aim of the study was to determine the minimal amount of smoke required to create a commercially significant level of smoke taint in wine.

Methodology

Smoke application apparatus

Smoke apparatus and application was reproduced from previously proven methodology of smoke application to field-grown grapevines (Kennison et al. 2009). In brief, the smoke application apparatus was comprised of a steel framed tent covered in greenhouse fabric (Solaweave, Gale Pacific, Braeside, Vic., Australia) constructed to surround field-grown grapevines for the application of smoke at ambient temperature. A smoke generator, constructed from a steel drum (50 L) as a vessel to contain fuel and fire, was used to produce smoke to be applied to grapevines. A fuel of dry barley straw was ignited within the drum and the smoke from the fire was forced, by a 12 volt air pump, from the drum through outlet hosing into the tent. Smoke production was controlled by an outlet valve that could be physically manipulated to regulate the density and duration of smoke production.

Smoke treatments to field-grown grapevines

Smoke was applied to field-grown grapevines (cv. Merlot) within the grapevine growth period of 7 days post veraison to harvest as this is the period defined as possessing high sensitivity for smoke uptake by the grapevine and subsequent development of smoke taint in wine (Kennison et al. 2011). Smoke treatments were developed in line with the project aim of ascertaining the minimum amount of smoke density and duration required to create smoke taint in wine. Smoke was applied to field-grown grapevines at a range of high densities (5, 10 and 20% obs/m) and short durations (5, 10, 20 min). This smoke experiment will be referred to as 'high smoke density, short duration'. Subsequent to the initial smoke application, it was established that further investigation was required to determine the effects of a lower smoke density for a longer duration. Therefore, smoke was applied to field grown grapevines at one low density (2.5% obs/m) for long durations of 10, 20, 40 and 80 min. This smoke experiment will be referred to as 'low smoke density, long duration'. All smoke applications applied to field-grown grapevines were replicated in triplicate. A control treatment, consisting of fruit from unsmoked vines grown in identical conditions, was also included in each of the experiments and replicated in triplicate.

The reproducibility of smoke application was monitored by use of nephelometry equipment (VESDA Laser FOCUS^{TM} VLF-250, Victoria, Australia) that recorded the smoke density and duration over the course of the smoke treatment. Nephelometry equipment is routinely used for the detection of smoke, has been successfully used in past experimentation of smoke application to grapevines (Kennison et al. 2011) and found to be accurate and precise in measurement of the concentration of smoke (Williamson and Bowman 2008). Smoke density is recorded as the percentage of visual obscuration over a distance of one meter and recorded in measurements of % obs/m. The duration of smoke exposure was measured in minutes (min).

Wine production

Wines were produced from grapes of grapevines exposed to each smoke density and duration concentration and from fruit of control (unsmoked) vines. Fruit (approx. 11 kg

per treatment replicate, n = 3) was harvested from grapevines when the total soluble solids (TSS) reached 22.2 ± 1.5 °Brix measured by refractometry (Iland et al. 2004). Samples of smoked and control grape juice were analysed for free amino nitrogen (FAN) as per methodology described by Dukes and Butzke (1989). Fruit was destemmed and crushed, inoculated with EC1118 *Saccharomyces cerevisiae* yeast (Lallemand Inc., Montreal, Canada) at a rate of 200 mg/L. Musts were fermented on-skins in 15 L stainless steel fermentation vessels, the skin cap was plunged once daily until TSS approached 0 °Brix. All wines were pressed from skins at the same time, transferred to 5 L enclosed glass fermentation vessels and inoculated with *Oenococcus oeni* (Viniflora Oenos. Chr. Hansen, Denmark) for malolactic fermentation. On completion of malolactic fermentation (< 0.1 g/L malic acid) as determined by enzymatic analysis, wine was racked from malolactic lees, wine sulphur dioxide (SO_2) concentrations were adjusted to 30 ppm and wines were cold stabilized at 2 ^{o}C for 28 days. Subsequent to cold stabilization SO_2 concentrations were adjusted to a total of 60 ppm and the wine was then filtered (5 µm) and bottled.

Gas chromatography-mass spectrometry analysis

Guaiacol and 4-methylguaiacol are known to have 'smoky', 'toasted' and 'ash' aromas (Boidron et al. 1988) and were measured in all grape and wine samples. Guaiacol and 4-methylguaiacol have been detected in elevated concentrations in grapes and wines made from fruit of grapevines exposed to smoke (Kennison et al. 2007). As such, guaiacol and 4-methylguaiacol have been used as effective markers for the detection of smoke taint in grapes and wine following smoke exposure to vines. In this study, guaiacol and 4-methylguaiacol were analysed by gas chromatography-mass spectrometry (GC-MS) as per methods detailed by Pollnitz et al. (2000, 2004).

Wine sensory analysis

Experimental wines were analysed for the detection of difference by a panel of 130 regular wine consumers. Untrained wine consumers were used to determine if the regular wine consumer could detect a difference between the smoked and unsmoked wines and at what level of smoke density and duration a difference was readily

perceptible. Panellists were pre-screened and selected on the basis of being non-smokers, regular wine consumers, of good health, available, interested in wine tasting and over the age of 21 years. The analysis method employed was the triangle test (Meilgaard et al. 2007) as per the Australian Standard 2542.2.2 (2005). Wines were evaluated by panellists in a dedicated sensory facility containing 6 separate sensory booths with lighting used to mask colour differences in wines. Wine samples (20 ml) were presented to panellists in 3 digit coded ISO wine glasses that were lidded to avoid aroma release and contamination. An incomplete balanced block design was used such that each panelist assessed 9 wines but did not assess all wines however each wine produced in this research was tasted a total of 30 times during the wine sensory analysis. Samples of wine produced from fruit exposed to smoke (A) and the control (B) were presented so that all permutations of presentation order (i.e. AAB, ABA, BAA, BAB, ABB, BBA) were randomized across the panellists in order to control bias due to presentation order. Each panellist evaluated 3 triangle tests (a total of 9 wine samples per panellist).

Further wine sensory analysis, Quantitative Descriptive Analysis (QDA) (Meilgaard et al. 2007), was employed to quantitatively characterize the perceived differences in the aroma and flavour attributes of all wines. A panel of 11 people (six males and five females) was selected for QDA based on their interest in wine tasting, availability, being regular wine consumers, in good health, non-smokers and having experienced wine sensory education at a tertiary level. Panellists' ages ranged between 21 and 50 years. Prior to formal wine sensory assessment, all panellists underwent training sessions where they identified and agreed on a total of nine aroma and flavour descriptive terms and learnt to measure the intensity of these descriptors on an unstructured 100-point line scale. Prior to formal wine evaluation, all wines were informally tasted by a panel of five experienced wine tasters for the presence of off-flavours and to detect any wine faults. Formal wine evaluation was conducted in a dedicated sensory facility with each panellist assigned to an isolated tasting station. Wines (30 mL) were presented to panellists in three-digit coded standard ISO wine tasting glasses that were lidded to avoid contamination of the testing area and other samples. All wines were coded and

presented in a randomized order so that no two panellists received the same code or the same wine at any one time. In order to avoid sensory fatigue, panellists were required to wait at least 2 min between testing samples and were required to leave the tasting area to an external outdoor environment regularly.

Statistical analysis

All data were analysed using Genstat 11th Edition (VSN International Limited, Hemel Hempstead, UK). The significance of the main effects of treatments and, where appropriate, their interaction were analysed using analysis of variance (ANOVA) with mean comparisons performed by least significant difference (LSD) multiple comparison tests at $P \leq 0.05$. Correlation analysis was performed on some variables. Wine sensory data from triangle tests was analysed by use of statistical tables detailed by Meilgaard et al. (2007) and data from Quantitative Descriptive Analysis was analysed by ANOVA and Principal Component Analysis (PCA).

Results

Smoke effects on grapes and wine

1. High smoke density, short duration experiment

In this experiment smoke was applied to field-grown grapevines at a range of high smoke densities (5, 10 and 20% obs/m) for short durations (5, 10, 20 min). These treatments did not produce a difference in grapevine fruit yield or alter the yield components of bunch weight, berry weight or bunch number per vine at harvest (data not shown). Analysis of grape must at harvest also did not indicate a significant effect of treatments on total soluble solids (TSS) however a difference was detected in the free amino nitrogen (FAN) concentration. FAN concentration ranged from 88 to 179 mg/L and was highest (179 mg/L) in grape must from grapevines exposed to 20% obs/m smoke for 20 min (Table 1). The lowest FAN concentration (88 mg/L) was detected in the must from unsmoked (control) grapes.

The duration of grape must fermentation was influenced by the application of high smoke densities for short durations. The fermentation duration for unsmoked (control)

musts was 8.7 days with musts of grapes exposed to smoke completing primary fermentation in 5.7 to 8 days (Table 1). Musts produced from grapes exposed to 20% obs/m smoke for 20 min completed fermentation in the shortest amount of time (5.7 days).

Guaiacol and 4-methylguaiacol were measured as analytes of interest in the detection of the presence of smoke taint in grapes and wine. They were not detected in any grape samples from the high smoke density – short duration experiment. However, elevated levels of these compounds were detected in wines produced from fruit in the high smoke density for short durations experiment. For example, guaiacol and 4-methylguaiacol were elevated in wines produced from grapes exposed to 20% obs/m smoke for 20 min (6.3 and 2.7 µg/L, respectively) and 10% obs/m for 20 min (6.3 and 2.3 µg/L, respectively) in comparison to unsmoked (control) wines (2.3 and 1.3 µg/L, respectively) (Table 2). All smoke treatments were equal or higher in the concentration of guaiacol and 4-methylguaiacol than the control ranging from 2.3 to 6.3 µg/L guaiacol and 1.3 to 2.7 µg/L 4-methylguaiacol (Table 2).

For the concentration of guaiacol in wine, analysis of variance shows smoke density was not a source of variation on its own ($P < 0.07$). However, smoke duration ($P < 0.003$) and the interaction of density x duration ($P < 0.003$) were significant sources of variation. Graphical representation of this data (Figure 1) shows a positive interaction between smoke duration and the concentration of guaiacol for wines made from grapes exposed to 10 and 20% obs/m smoke for 5, 10 and 20 min. The 5% obs/m smoke exposure treatment does not follow the same trend with no significant ($P \leq 0.05$) effect of duration on the guaiacol concentration in wines.

2. Low smoke density, long duration

In experiment 2 smoke was applied to field grown grapevines at one (low) smoke density (2.5% obs/m) for long durations (10, 20, 40 and 80 min). The low smoke density –long duration treatments did not influence grapevine yield, bunch weight or berry weight (data not shown). However the TSS of grape must at harvest was reduced

by the application of 2.5% obs/m smoke for 80 min (to 22.3 ^{o}Brix) in comparison to all other grape musts including the control (23.2 ^{o}Brix) which contained higher TSS contents (Table 3). The ethanol content of wine was lower in all wines made from grapes exposed to smoke (ranging from 12.4 to 13.9% v/v) in comparison to the control (14.3% v/v) (Table 3). The lowest ethanol content occurred in wines made from grapes exposed to 2.5% obs/m smoke for 80 min (12.4% v/v).

FAN concentration in grape must at harvest was significantly higher in those musts produced from grapes of vines exposed to smoke ($P < 0.05$, Table 3). FAN concentration varied widely between treatments with the lowest levels (37 mg/L) detected in unsmoked (control) musts and the highest levels detected in musts produced from fruit of vines exposed to 2.5% obs/m smoke (60 to 71 mg/L) (Table 3). The fermentation duration was faster in all musts of grapes exposed to smoke than musts of control (unsmoked) fruit which completed fermentation in 16.7 days (Table 3). Grapes produced from smoked vines completed fermentation at 10.7 days for the 80 min smoke treatment and 12.7 days for the 10 min smoke treatment.

GC-MS analysis did not detect guaiacol or 4-methylguaiacol in berry samples from vines exposed to low smoke densities for long durations. Conversely, guaiacol and 4-methylguaiacol were detected in elevated concentrations in wines made from the same grapes (Table 4). Concentrations of guaiacol and 4-methylguaiacol were the lowest in wines made from unsmoked (control) fruit (1.7 µg/L and n.d. respectively) and highest in wines made from fruit of grapevines exposed to 2.5% obs/m of smoke for 80 min (10 and 2 µg/L respectively). A positive correlation existed between the duration of smoke application and guaiacol ($r = 0.76$) and 4-methylguaiacol ($r = 0.78$) concentration in wine.

Sensory analysis of experimental wines

Triangle tests of wines made from fruit of grapevines exposed to smoke showed that regular wine consumers could detect a difference in the majority of smoked wines when compared to unsmoked (control) wines. Wine consumers could detect a difference ($P \leq$

0.05 to 0.001) in all wines made from fruit of grapevines exposed to a low smoke density (2.5% obs/m) for long durations (results not shown). For wines made from fruit of vines exposed to high smoke densities for various durations, a significant difference ($P \leq 0.05$ to 0.001) was detected in all wines produced from grapes smoked for 20 min regardless of the smoke density (5, 10 or 20% obs/m) (Table 5). Furthermore, a difference ($P \leq 0.01$) was detectable in wines made from fruit exposed to 5% obs/m smoke for 5 min, 20% obs/m smoke for 5 min and 20% obs/m smoke for 10 min.

Smoke and wine related sensory attributes of all wines in this study were found to vary depending on the density and duration of smoke application applied to the field-grown grapevines (Figure 2). During QDA panellists identified and agreed on the aroma descriptors of 'red berry', 'eucalypt', 'smoke' and 'hospital' and palate attributes / flavours of 'red berry', 'eucalypt', 'smoke', 'dried meat' and 'ashy palate' to characterize both the smoke and wine related attributes of all wines. The detection of these key smoke related and wine related aromas and flavours varied and was dependent on both smoke density and duration of smoke exposure to grapevines in the experiment of 5, 10 or 20% obs/m smoke for 5, 10 or 20 min. Smoke related wine characteristics of 'dried meat flavour' (5.2), 'ashy palate' (5.1), 'smoke aroma' (4.5) and 'smoke flavour' (4.1) were intensified in wines made of fruit of grapevines exposed to 20% obs/m smoke for 20 min in comparison to all other wines (Figure 3). In comparison, these smoke-like descriptors were detected in low levels in unsmoked (control) wines that instead contained elevated detection of 'red berry aroma' (7.1) and 'red berry flavour' (7.4). All other wines showed variable detection of aromas and flavours however, in general, smoke related wine characters were detected at lower levels than the 20% obs/m smoke exposure for 20 min and wine-like characters were detected in concentrations lower than the unsmoked (control) wines (Figure 3).

Wines made from fruit of grapevines exposed to 2.5% obs/m smoke varied in their intensity of key descriptors that was dependent on the duration of smoke exposure (Figure 4). Wines made from unsmoked (control) grapes had elevated values for wine related descriptors of 'red berry flavour' (7.4) and 'red berry aroma' (7.1) and low

detection of the smoke related descriptors of ‘smoke flavour’ (0.5), ‘smoke aroma’ (0.9), ‘dried meat flavour’ (0.7) and ‘ashy palate’ (0.3) (Figure 4). This is in stark contrast to wines made from fruit of vines exposed to 2.5% obs/m smoke for 80 min that displayed heightened detection of ‘smoke aroma’ (5.1), ‘smoke flavour’ (3.6), ‘ashy palate’ (4.1), ‘dried meat flavour’ (3.5), ‘eucalypt aroma’ (4.7), ‘eucalypt flavour’ (4.9) with a low detection of ‘red berry flavour’ (4.8) and ‘red berry aroma’ (4.7). The intensity of key aromas and flavours of wines made from fruit of vines exposed to 2.5% obs/m smoke for 10, 20 and 40 min were found to be detected at levels in-between those reported for the unsmoked (control) and 80 min wines except for wines made from fruit of vines exposed to smoke for 40 min that had heightened ‘hospital aroma’ (3.8).

PCA of mean aroma and flavour descriptors accounted for 84.33% of the overall data variation comprised predominately of principal component 1 (PC1) (72.82%) and principal component 2 (PC2) (11.51%) (Figure 2). PC1 is characterized by positive loadings on the smoke related wine descriptors of ‘ashy palate’ (0.54), ‘dried meat flavour’ (0.47), ‘hospital aroma’ (0.29), ‘smoke aroma’ (0.19) and ‘smoke flavour’ (0.45) in contrast to the negative loadings on the wine related descriptors of ‘eucalypt aroma’ (-0.12), ‘eucalypt flavour (-0.11)’, ‘red berry aroma’ (-0.22) and ‘red berry flavour’ (-0.3). PC2 is converse to PC1 with negative loadings on ‘ashy palate’ (-0.03), ‘dried meat flavour’ (-0.06), ‘smoke aroma’ (-0.38) and ‘smoke flavour’ (-0.02) and positive loadings on ‘red berry aroma’ (0.21) and ‘red berry flavour’ (0.01) however negative loadings were retained on ‘eucalypt aroma’ (-0.61) and ‘eucalypt flavour’ (-0.65) and positive loading retained on ‘hospital aroma’ (0.11).

Clear relationships were evident in the wine sensory data between the descriptors characterizing smoke related attributes and those characterizing the wine related attributes. Correlation analysis (not shown) revealed a strong positive correlation between the descriptors of ‘smoke flavour’ and ‘ashy palate’ ($r = 0.91$), ‘smoke flavour’ and ‘dried meat flavour’ ($r = 0.95$) and ‘ashy palate’ and ‘dried meat flavour’ ($r = 0.92$) ($n = 8$, $P < 0.05$). Similarly, a positive correlation was evident between ‘red berry aroma’ and ‘red berry flavour’ ($r = 0.76$). Conversely strong negative correlations were

established between the wine-like descriptor of 'red berry flavour' and 'ashy palate' (r = -0.85), 'dried meat flavour' (-0.74) and 'smoke flavour' (r = -0.85) with a visual representation of these relationships presented in the PCA biplot (Figure 2).

Interestingly, a relationship was also evident between the concentration of guaiacol and 4-methylguaiacol in wine and the sensory descriptor of 'dried meat flavour' and 'ashy palate'. Correlation analysis revealed 'dried meat flavour' to be positively correlated with both wine guaiacol (r = 0.64) and wine 4-methylguaiacol (r = 0.76) whilst 'ashy palate' showed positive correlation with wine 4-methylguaiacol only (r = 0.65). Correlation between guaiacol and other wine and smoke related aromas and flavours was weak therefore showing guaiacol to be an inadequate indicator of the intensity of key aromas in smoke tainted wines.

Discussion

This study demonstrates that the density and duration of smoke application to field-grown grapevines determines the concentration of smoke related compounds and the development of smoke-taint characteristics in wine. Previous research has shown that smoke exposure to field-grown grapevines effects the chemical composition and sensory properties of resultant wine and, depending on the timing of smoke exposure, to create smoke taint in wine (Kennison et al. 2009, 2011). This study builds on previous research and, as such, is the first paper to investigate the effect of high smoke densities (5, 10 and 20% obs/m) for short durations (5, 10 and 20 min) and a low smoke density (2.5% obs/m) for long durations (10, 20, 40 and 80 min) on the development of smoke taint compounds and characteristics in wine.

The length of smoke exposure had a cumulative effect on the concentration of smoke compounds in wine. Demonstrating that longer periods of smoke exposure to grapevines have a higher potential to taint resultant wines. The concentration of guaiacol and 4-methylguaiacol varied among wines depending on the density and duration of smoke application to field-grown grapevines. Smoke density x duration and smoke duration on its own were significant sources of variation ($P < 0.003$) for the concentration of

guaiacol in wine. The influence of smoke duration was evident in those wines produced from grapes exposed to 10 and 20 % obs/m however the influence of duration was not evident for the 5% obs/m smoke. Guaiacol and 4-methygluaiacol were elevated in wines produced from fruit of grapevines exposed to 20% smoke for 20 min (6.3 and 2.7 µg/L, respectively) and 10% for 20 min (6.3 and 2.3 µg/L, respectively) yet concentrations were higher in wines produced from a lower smoke density (2.5%) for a longer duration (40 min 7.7 and 1.7 µg/L, respectively) (80 min 10 and 2 µg/L, respectively). A 3 year duration study on Merlot by Kennison et al. (2011) showed a single smoke exposure of a high smoke density (PM_{10} level of 200 $\mu g/m^3$) for 30 minutes duration, resulted in wine guaiacol concentrations of < 1 to 60.7 µg/L and 4-methylguaiacol concentrations of < 1 to 14.1 µg/L depending on the timing of smoke exposure. Furthermore, 8 repeated smoke applications to the same field-grown grapevines imparted a cumulative effect on the concentrations of guaiacol (388 µg/L) and 4-methylguaiacol (93 µg/L) in resultant wine (Kennison et al. 2009). Similarly, in this current study, the duration of smoke exposure was positively correlated with the concentration of guaiacol in wines produced from smoke exposed grapes.

This study demonstrates that the density and duration of smoke application to field-grown grapevines influences the sensory properties of wines. The intensity of smoke related descriptors of 'smoke flavour', 'ashy palate' and 'dried meat flavour' were accentuated in wine made from a low smoke density (2.5%) for a long duration (80 min) and high smoke densities (10 and 20%) for lesser durations (10 and 20 min). However, smoke related sensory descriptors were even evident in those wines made from grapevines exposed to low smoke densities for short durations (5% for 5 min). There was conjecture over the relative importance of density and duration depending on the analysis method. Instrument analysis (GC-MS) indicated the phenol concentration of smoke affected wines to be influenced predominantly by the duration of smoke exposure whereas the key contributing factor to the development of the smoke related aromas of 'dried meat flavour' ($P < 0.002$), 'smoke aroma' ($P < 0.016$) and 'smoke flavour' ($P < 0.002$), as determined by wine sensory analysis, was found to be smoke density. The density of smoke exposure has been of key importance in the smoking of food products

and is well documented to affect the absorption of smoke aromas, flavours and volatile phenols with the higher the smoke density the greater absorption of smoke characteristics by food (Boyle and Schmidt 1999, Ogbadu 2000, Fellows 2009). In grapes, the effect of smoke duration, from repeated smoke exposures (n = 8), to grapevines has been shown to result in the accentuation of wine aromas of 'smoked meat', 'leather', 'burnt rubber' and 'disinfectant' in comparison to wines made from fruit of grapevines exposed to one smoke exposure (Kennison et al. 2009). This study therefore shows the potential negative influence of smoke events of a high smoke density and/or a prolonged duration in vineyards on the creation of smoke related characters in resultant wines.

The key analytes of interest, the phenols guaiacol and 4-methylguaiacol, have been measured in smoked foods to indicate the extent of smoke exposure and deposition (Bratzler et al. 1969, Chan et al. 1975, Ogbadu 2000). Interestingly, they were undetectable in any berry samples produced from grapevines exposed to smoke during this study. Although undetectable in berries, the measurable guaiacol and 4-methylguaiacol concentrations increased during the fermentation process and were found in detectable concentrations in final wines. Previously, Merlot grapes exposed to smoke have shown similar results with low detection of guaiacol (1 µg/L) and 4-methylguaiacol (<1 µg/L) in berries and the subsequent release of these compounds by strong acid (pH 1.0) and enzyme (*ß*-glucosidase) hydrolysis (Kennison et al. 2008). Recent advances in the analysis of smoke taint has resulted in the discovery of a glucoside (ß-$_D$-glucopyranoside) of guaiacol in grapes and leaves following grapevine exposure to smoke (Hayasaka et al. 2010b, Dungey et al. 2011) and additional (n = 7) glycosylated metabolites in smoke exposed grapes and wine (Hayasaka et al. 2010c). The extraction rate of the additional glycosylated metabolites, from grapes to wine, is reported at 78% for Chardonnay and 67% for Cabernet sauvignon (Hayasaka et al. 2010c). In this study, guaiacol and 4-methylguaiacol concentration in wine have been effective indicators of the presence of smoke exposure to grapevines.

Smoke exposure was shown to adversely affect the fruit ripening ability of those grapevines exposed to smoke for long durations. Smoke application of 2.5% obs/m for 80 min decreased the TSS (^{o}Brix) of grapes at harvest by over 1 ^{o}Brix. This resulted in the ethanol content of final wines also being decreased by an average of 1.9% v/v. This observation is supported by previous studies where a single heavy smoke exposure for 30 min decreased the TSS content of fruit at harvest by an average of 1.6 ^{o}Brix and a repeated exposure treatment (8 successive smoke exposures for 30 min each) resulted in an average TSS decrease by 3 ^{o}Brix (Kennison et al. 2009). The decrease in the grapevines ability to ripen fruit may be a consequence of the smoke and its effect on the photosynthetic capacity of the grapevine. Smoke exposure to plants is known to reduce plant photosynthetic capacity with inhibition of plant gas exchange (Davies and Unam 1999, Gilbert and Ripley 2002). A 1 min smoke exposure to *Chrysanthemoides monilifera* reduced CO_2 assimilation rate, stomatal conductance and internal CO_2 concentration which did not recover, to the control levels, for up to 24 hours (Gilbert and Ripley 2002). Our own data (unpublished) supports these findings for grapevines and shows that exposures of greater than 16 min resulted in incomplete recovery after 5 days.

Smoke application to grapevines increased the concentration of free amino acids (FAN) in musts. Previous studies have shown the fermentation rate of must to be influenced by nitrogen content (Ough 1964, Monteiro and Bisson 1992) which was also apparent in our current study with fermentation accelerated of all musts derived from smoked wines in comparison to unsmoked (control) musts. Previous experiments of field-based smoke applications to grapevines, and smoke applications to grape bunches have also shown an increase the fermentation rate of Chardonnay (Kennison et al. 2007) and Merlot (Kennison et al. 2009). As previously considered (Kennison et al. 2009) FAN increase may be a result of direct nitrogenous compound deposition from smoke onto the grape surface (Nussbaum et al. 1993, Stulen et al. 1998) or due to tissue damage caused by elevated smoke conditions (Heath 1980). However, further investigation is required to indentify the cause of both the increase of FAN and the acceleration of fermentation of smoke-exposed grapes.

In summary, this study has investigated the effect on the development of smoke related compounds, aromas and flavours in resultant wines after the application of a range of smoke densities and durations to field-grown grapevines. Sensory tests with regular wine consumers showed the minimum amount of smoke application to create a detectable difference in wine was found to be 5% obs/m for 5 min. Accentuated levels of guaiacol (10 µg/L), 4-methylguaiacol (2 µg/L) and smoke related aromas and flavours were detected in wines made from fruit of grapevines exposed to 2.5% obs/m smoke for 80 min. Hence the intensity of smoke taint in wine was influenced by both smoke duration and smoke density. As such, this research provides information to the wine industry to determine the risk of the potential development of smoke taint in wines following smoke exposure to grapevines.

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TABLES

Table 1. Concentration of free amino nitrogen (FAN mg/L) and fermentation rate (days) of grape must produced from fruit of grapevines exposed to smoke at high smoke densities (5, 10 and 20%) for short durations (5, 10 and 20 min).

Treatment	FAN (mg/L)	Fermentation (days)
Control	88.3^{d}	8.7^{a}
5% 5 min	93.0^{d}	8.0^{ab}
5% 10 min	109.0^{cd}	8.0^{ab}
5% 20 min	171.3^{ab}	6.0^{e}
10% 5 min	103.3^{d}	7.3^{bcd}
10% 10 min	114.3^{cd}	6.3^{de}
10% 20 min	100.3^{d}	7.7^{abc}
20% 5 min	133.0^{bcd}	6.7^{cde}
20% 10 min	153.0^{abc}	6.7^{cde}
20% 20 min	179.0^{a}	5.7^{e}

Means followed by the same letter within columns are not significantly different at $P \leq 0.05$, $n = 3$.

Table 2. Concentration (µg/L) of guaiacol and 4-methylguaiacol detected in wines made from fruit of vines exposed to high smoke densities (5, 10 and 20%) for short durations (5, 10 and 20 min).

Treatment[†]	Concentration (µg/L) of[‡]	
	guaiacol	4-methylguaiacol
Control	2.3^{c}	1.3^{c}
5% 5 min	4.0^{bc}	2.0^{abc}
5% 10 min	4.3^{b}	2.0^{abc}
5% 20 min	3.3^{bc}	2.0^{abc}
10% 5 min	2.3^{c}	1.3^{c}
10% 10 min	3.7^{bc}	2.0^{abc}
10% 20 min	6.3^{a}	2.3^{ab}
20% 5 min	3.0^{bc}	1.7^{bc}
20% 10 min	3.7^{bc}	2.0^{abc}
20% 20 min	6.3^{a}	2.7^{a}

[†]Grapevines were exposed to smoke at high smoke densities (% obscuration / m) for short durations (min)

[‡]For each analyte, means followed by the same letter are not significantly different at $P \leq 0.05$, $n = 3$.

Table 3. Total soluble solids (TSS ^{o}Brix), free amino nitrogen (FAN mg/L) and fermentation rate (days) of grape must from fruit harvested from smoked and unsmoked vines and alcohol content (% v/v) in resultant wines. Smoked grapevines were exposed to smoke at one low density (2.5%) for long durations of 10, 20, 40 and 80 min.

Smoke duration (min)	Must[†]			Wine[†]
	TSS (°Brix)	FAN (mg/L)	Fermentation (days)	Alcohol (% v/v)
Control	23.2^{a}	36.7^{c}	16.7^{a}	14.3^{a}
10	23.7^{a}	70.7^{ab}	12.7^{b}	13.9^{ab}
20	23.5^{a}	76^{a}	11.3^{bc}	13.8^{ab}
40	23.2^{a}	68.7^{ab}	11.3^{bc}	13.3^{bc}
80	22.3^{b}	59.7^{b}	10.7^{c}	12.4^{c}

[†]Means followed by the same letter within columns are not significantly different at $P \leq 0.05$, $n = 3$.

Table 4. Concentration (µg/L) of guaiacol and 4-methylguaiacol detected in wines made from fruit of vines exposed to a low smoke density (2.5% obs/m) for long durations (0, 10, 20, 40 and 80 min).

Smoke duration (min)	Concentration (µg/L) of[†]	
	Guaiacol	4-methylguaiacol
Control	1.7[d]	n.d.
10	2.6[c]	n.d.
20	3.3[c]	0.3[b]
40	7.7[b]	1.7[a]
80	10.0[a]	2[a]

[†]For each analyte, means followed by the same letter within columns are not significantly different at $P \leq 0.05$, $n = 3$; n.d. = not detected.

Table 5. Correct number of responses in a triangle test of wines made from fruit of vines exposed to high smoke densities (5, 10 and 20% obs/m) for long durations (5, 10 and 20 min).

Treatment	No. of correct responses[†]
5% 5 min	18**
5% 10 min	13
5% 20 min	19***
10% 5 min	13
10% 10 min	13
10% 20 min	15*
20% 5 min	17**
20% 10 min	17**
20% 20 min	20***

[†]Significance indicated by $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$ as defined by Meilgaard et al. (2007). Each wine tasted a total of 30 times by regular wine consumers.

FIGURES

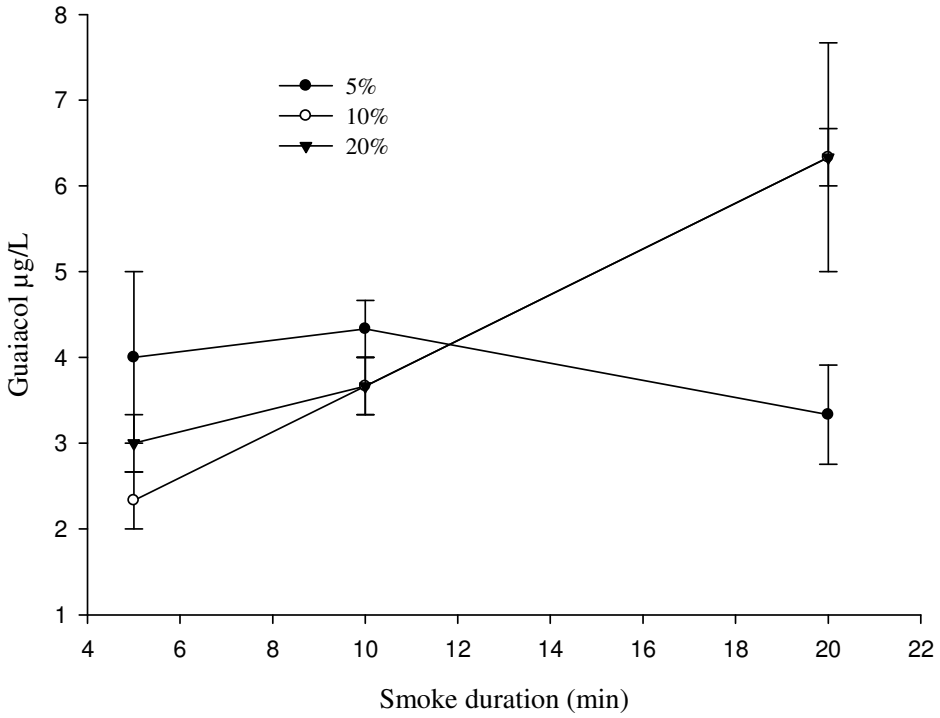

Figure 1. Concentration of guaicol in wines made from fruit of grapevines exposed to 5, 10 and 20% obs/m smoke for 5, 10 and 20 min. Error bars indicate two standard errors of the mean.

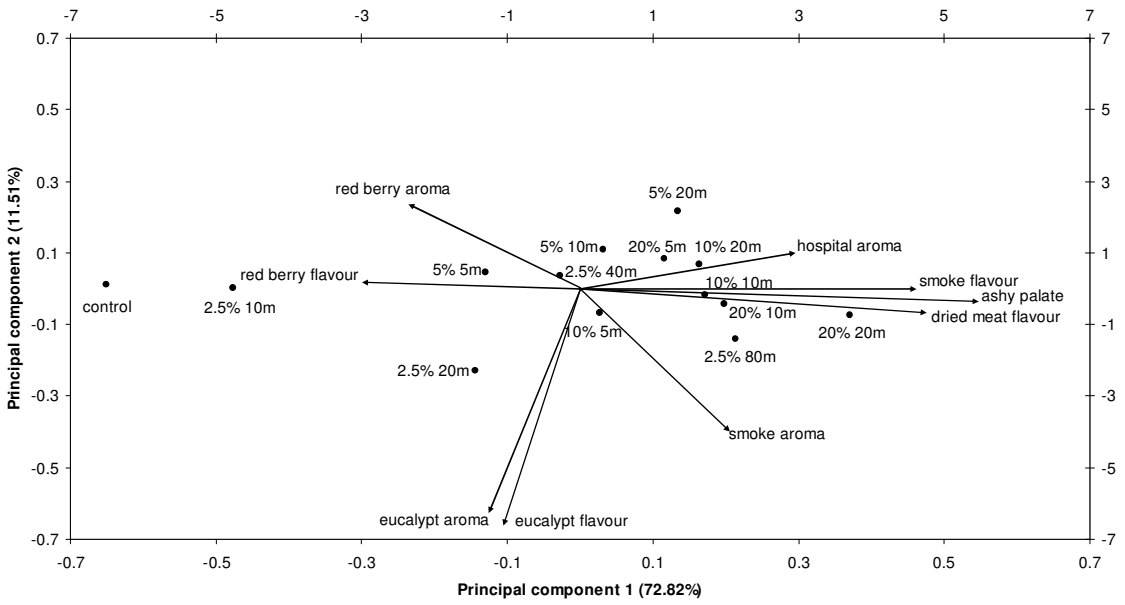

Figure 2. PCA biplot of mean wine sensory scores (•) of wine made from fruit of unsmoked grapevines (control) or wines made from fruit of grapevines exposed to 2.5% obs/m smoke for 10, 20, 40 or 80 min and 5, 10 or 20% obs/m smoke for either 5, 10 or 20 min.

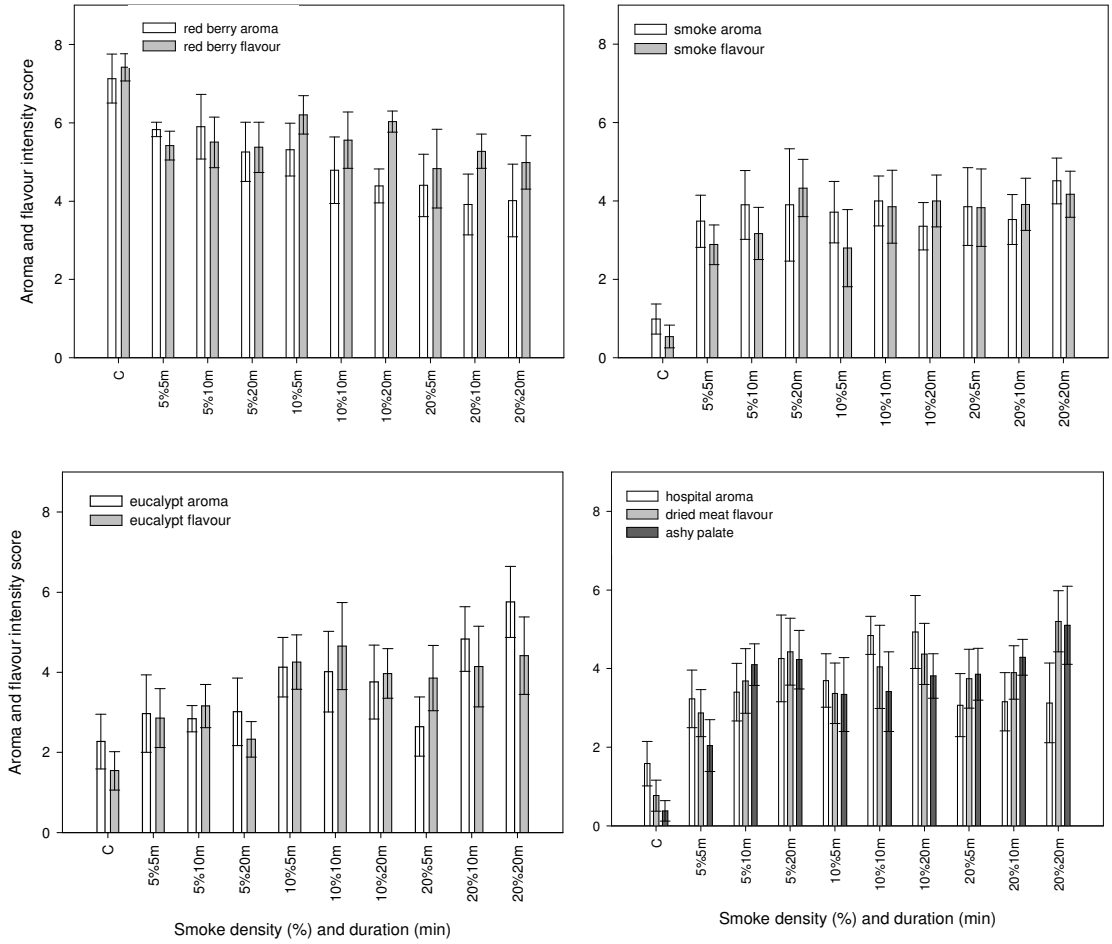

Figure 3. Mean intensity scores for 'red berry aroma', 'red berry flavour', 'smoke aroma', 'smoke flavour', 'eucalypt aroma', 'eucalypt flavour', 'hospital aroma', 'dried meat flavour' and 'ashy palate' for wines made from grapes of vines exposed to either 5, 10 or 20% obs/m smoke for either 5, 10 or 20 min and wines made from unsmoked grapes (control). Scale represents 0 = non-detectable to 8 = highly detectable aroma or flavour. Error bars indicate two standard errors of the mean.

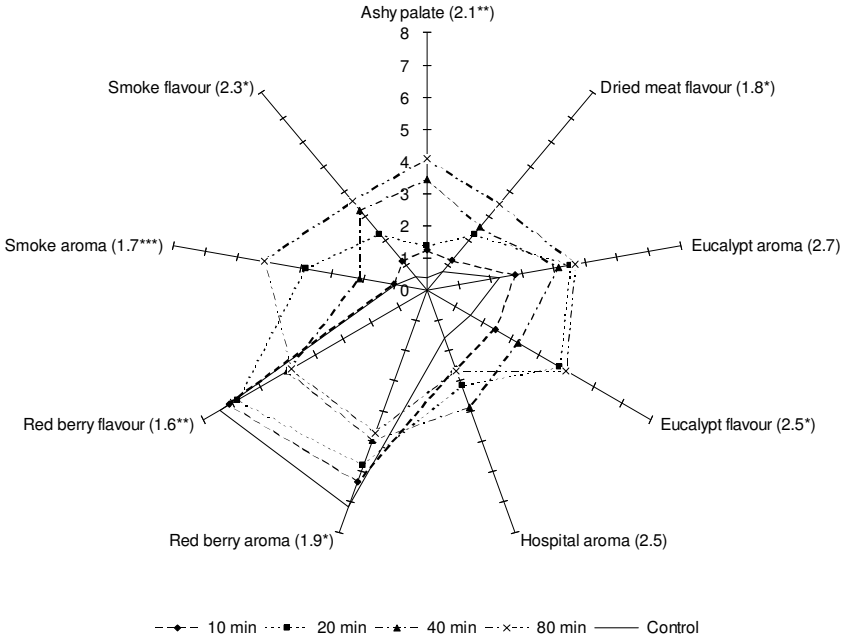

Figure 4. Mean intensity ratings of smoke related characteristics of 'smoke aroma', 'smoke flavour', 'ashy palate', 'dried meat flavour', 'hospital aroma' and wine related characteristics of 'red berry flavour', 'red berry aroma', 'eucalypt aroma' and eucalypt flavour' in wines made from fruit of grapevines exposed to 2.5% obs/m smoke for 10, 20, 40 or 80 min and wines made from unsmoked grapes (control). Scale represents 0 = non-detectable to 8 = highly detectable aroma or flavour. LSD is indicated in parenthesis with significance at $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.

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CHAPTER 6 REVIEW AND DISCUSSION

This thesis has been instrumental in enhancing the knowledge of the effects of smoke on grapevines, grapes and wine leading to the reduction of negative smoke taint characteristics in wine. Key findings of this study include:

- Establishing and proving the link between smoke exposure to grapevines and the identification of marker volatile phenols and smoke-like sensory characteristics in wine;
- The seasonal timing of smoke exposure to grapevines to influence the chemical composition and sensory properties of wine;
- Identifying that smoke exposure to grape bunches and field-grown grapevines affects the sensory and chemical properties of resultant wines;
- The density and duration of smoke application to field-grown grapevines influences the intensity of smoke taint in resultant wines.

As such, this research produced the first known refereed paper detailing the effects of a purposeful application of smoke to grapevines being the development of smoke taint in wine. The research established the direct link between smoke exposure and the development of smoke taint in wine. This proved the cause and effect response of smoke on grape production and led to the development of methodology and direction for further research. Since then, the incidence and severity of smoke exposure to grapevines has greatly increased to become a developing challenge for the global wine industry. The research developed in this thesis has provided valuable information to understand the impacts of smoke taint in wine.

An outcome of consequence from this research has been the identification of the key periods of grapevine sensitivity to smoke uptake and development of smoke taint in wine (Kennison et al. 2011). Key periods of grapevine sensitivity to smoke uptake have been identified based on the grapevine phenological stage of growth. Initial research identified the peak period of grapevine sensitivity to smoke uptake to be at 7 days post veraison. Further to this research, an investigation of smoke effects throughout the

entire grapevine growing season identified 3 key periods of grapevine sensitivity to smoke uptake. These periods have been defined as (1) from shoots 10 cm in length to full-bloom resulting in low levels of smoke taint in wine; (2) from berries pea size to the onset of veraison resulting in variable levels of smoke taint in wine; and (3) from 7 days post veraison to harvest resulting in high levels of smoke taint in wine. These findings provide applications and benefits for wine production industry. For instance, knowledge of the effects of smoke exposure to grapevines at particular seasonal growth stages provides an indication of the risk of smoke taint development in wine at any one time. Consequently if smoke exposure occurs, vignerons are able to determine appropriate management including if grape testing for the presence of smoke compounds or management techniques to minimise or avoid smoke taint in wine are required.

Further to the understanding of the timing of grapevine sensitivity to smoke uptake, this research has advanced the understanding of the influence of smoke duration and density on the development of smoke taint in wine. Smoke emissions have been proven to be highly variable in the environment (Garland et al. 2008) with the concentration of smoke exposure, and its associated effects, on grapevines unknown. This thesis has explored the effects of a range of smoke durations and densities on the development of smoke taint in wine. Results have proven that smoke exposure to grapevines alters the chemical composition and sensory properties of wine even at low smoke densities and durations of exposure. Wine sensory analysis has demonstrated the alteration of wine attributes from smoke exposure to result in taint-like characters with the minimum amount of smoke required to create smoke taint aroma and flavour characteristics in wine determined to be 5 % obs/m for 20 min. As such, this research has proven that the duration and density of smoke exposure is of prominent effect on the chemical and sensory properties of wine.

Smoke exposure to grapevines is cumulative in its effect on the development of smoke taint in wine. During this study, repeated smoke exposures to the same field-based grapevines resulted in an accumulation of smoke related marker compounds and aromas in resultant wines (Kennison et al. 2009). The repeated smoke applications created a

greater duration in smoke exposure to the grapevine which reduced the vines physiological ability to ripen berries as evident in the decreased total soluble solids content (TSS) of fruit, at harvest, from smoke exposed vines. Also other effects of the repeated smoke applications were observed in the form of physical damage, necrotic leaf lesions, on those grapevines exposed to repeated smoke application. The physiological phenomenon of smoke on grapevines was not investigated in this study and provides an additional opportunity for further research in this area.

A greater understanding of the development of smoke taint during the fermentation process of winemaking has been gained from this study (Kennison et al. 2008). Samples taken during the fermentation of smoke affected fruit demonstrated the progressive release of key smoke marker compounds to occur. That is, in initial juice samples at harvest, the level of smoke related marker compounds were low and during the fermentation of juice these compounds were released to be detected at elevated levels in the final wine. This presented an implication for the under assessment of smoke taint in grapes prior to fermentation. Further investigations revealed the smoke related marker compounds were released by acid and enzyme catalysed conditions and, as such, this methodology was employed for the determination of smoke taint in grape juice.

Information gained from this study has been directly adopted by the wine industry to reduce the negative influence of smoke taint in grape and wine production. Results from this research are currently being applied by the Department of Environment and Conservation (DEC), the Department of Agriculture and Food WA (DAFWA) and the University of Western Australia for the development of a smoke taint risk reduction tool. This smoke risk reduction tool is being developed for the wine industry nationally to predict the seasonal timing of grapevine sensitivity to smoke uptake and the impact of smoke exposure on taint development. Key risk factors developed for this tool have been derived from published research contained within this thesis. Furthermore, information from this research is currently being utilised by forest management agencies (DEC) to assist with the timing of prescribed burns in order to minimise the risk of smoke exposure to grapevines.

Throughout this experimentation, the development of novel research methodology for the application of smoke to field grown grapevines was successfully pioneered. In this study, the controlled application of smoke to grapevines was an essential research element required for the research hypotheses to be tested. A basic smoke application methodology was previously developed by Dixon et al. (1995) for the application of smoke to native seeds. Our study built on this previous research to successfully develop, refine and employ a smoke generator and tent apparatus for smoke application to grapevines in the field. Our research was unique in the sense that it was the first known controlled application of smoke to perennial plants on a trellis structure. Due to the successful operation of the smoke application apparatus, similar methodology has subsequently been employed by other authors (Ristic et al. 2011).

Opportunities for further research have been noted from this study. In this study, a limited number of grapevine varieties were employed in field research and, as such, an opportunity exists to research the effect of smoke on alternative grapevine varieties. Field research detailed within this thesis predominantly concentrates on *Vitis vinifera* cv. Merlot and cv. Verdelho. These varieties were selected based on their availability over the duration of the study and their current use in industry. The selection of a limited number of varieties in this research has also been due to the limited time available to conduct this study, limited funding and physical resources. Since the conclusion of this study, additional research has been undertaken and is currently focusing on the opportunities in this area to investigate the effects of smoke application to a wide range of grapevine varieties.

Additional research opportunities have been identified from this study and concentrate on the area of chemical analysis of smoke related compounds. During this study, the smoke-derived compounds guaiacol and 4-methylguaiacol have been used as indicators for the presence of smoke taint in grapes and wines. As such, they have been effective indicators of the presence of smoke exposure and the development of smoke taint in wine however are limited in quantifying the true taint intensity. To address this issue,

both wine chemical and sensory analysis have been employed in this study. Recently, additional compounds that are produced in smoke are being investigated as to their contribute to the sensory smoke taint. The determination of additional smoke compounds responsible for smoke taint in wine is outside the scope of this PhD however researchers are currently actively seeking to identify additional compounds that contribute to the sensory smoke taint.

APPENDICES

APPENDIX I CO-AUTHOR STATEMENTS

APPENDIX II COPYRIGHT PERMISSION LETTERS

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Title: Smoke-derived Taint in Wine: Effect of Postharvest Smoke Exposure of Grapes on the Chemical Composition and Sensory Characteristics of Wine

Author: Kristen R. Kennison et al.

Publication: Journal of Agricultural and Food Chemistry

Publisher: American Chemical Society

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