School of Science
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A Study into the Effects of Pyrolysis Fuels, Pyrolysis Conditions and the Identification of Chemical Markers in Grapes and Wine as Smoke Taint

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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.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature:

Date:

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Abstract

The accumulation of smoke taint compounds in wine grapes from an exposure to bushfire smoke poses great risk to the long term viability of Australian wine companies. Vineyards that have been exposed to smoke generated by wildfires can produce wines that have burnt, metallic, smoky aromas and flavour attributes with low consumer acceptance and as such, investigations into the formation and amelioration of smoke taint in wine are of great importance to the Australian wine industry.

In this study, taxonomically distinct groups of vegetation fuels with different lignin composition were used to generate smoke, under conditions that reproduce wildfire pyrolysis temperature profiles, for fumigating grapevines at the start of grape ripening. The primary aim was to examine the influence of the lignin makeup of vegetation on lignin pyrolysis products (potential smoke taint compounds) that accrue in wine as a result of the exposure of grapes to smoke. Incidental to this was the quantification of hollocellulose, lignin and monolignol components of vegetation fuels subjected to frequent fire events in the Margaret River region and an analysis of phenols generated at wildfire temperatures for each vegetation type. Despite large differences in fuel composition, a predominance of phenol and cresols (~10% of total emissions) were generated as pyrolysis emissions in trials. These findings were corroborated in emission samples taken at prescribed burning where sampling was made at the fire front. Twenty two phenols found in smoke were quantified as potential taint in wines. This has more than doubled the number of compounds identified as putative taint in wines made from smoke exposed vines.

The results showed that, regardless of fuel type, the commonly reported smoke taint compounds, guaiacol and 4-methylguaiacol, represented about 20% of total phenols in wines. Quantitatively, syringol derivatives dominated the total phenol pools both in volatile and glycoconjugated forms while the contributions of phenol and its derivatives were generally similar to the guaiacols. Fuel lignin makeup was not found to be a good indicator of the types of lignin pyrolysis products that become elevated in fruit and

wines. Gymnosperm exposed fruit was found to contain elevated levels of syringols compared to controls despite an absence of syringol derived pyrolysates in gymnosperm smoke. The mechanism for syringol uptake as glycoconjugates in smoke exposed fruit is therefore not a simple absorption and glycosylation of the syringols present in smoke as hypothesised by other researchers and may also be the case for smoke borne phenols in general. Subsequent vineyard trials to investigate syringol derivative uptake included vine exposure to cellulose pyrolysis and pure phenol vapour exposures, of which neither successfully replicated the accumulation of syringol glycoconjugates in grapes from gymnosperm exposure.

The concentrations of putative taint phenols in wines made from smoke exposed vines were found to be negatively correlated to vine canopy leaf area as well as the leaf area per bunch, suggesting direct uptake by berries may be a significant contributor to accumulation of smoke-derived phenols. Earlier work speculated foliar uptake and subsequent translocation and sequestration in berries as a possible pathway, however in this study, controlled, replicated exposures of vines to smoke in field trials discounted this theory.

Previous studies have claimed differences in cultivar sensitivity to smoke taint where they have examined wildfire exposed commercial fruit and wines, however it has never been clear if the fuel source, pyrolysis conditions, smoke age, smoke density, smoke duration or phenological differences have caused the variations in taint. In this thesis, cultivar differences in putative smoke taint accumulation in grapes were examined by exposing three cultivars to smoke at the same phenological stage with replicated fuel pyrolysis. When smoke exposure events occurred at a comparable stage of berry development there was no difference among the cultivars in the accumulation of total putative taint. This has significance for wine companies seeking to minimise the impact of smoke taint.

Significant differences in the extraction of taint from smoke exposed grapes were found to occur due to traditional winemaking methods. White varieties fermented without skin

contact retained approximately 30% of the phenols present in fruit compared to over 75% in red wines made with skin contact and within the white winemaking methods, minimisation of skin contact by whole bunch pressing as opposed to crushing and pressing extracted 50% less putative taint. To assess whether the glycosidase activity of lactic acid bacteria contributes significantly to hydrolysis of glycoconjugated phenols to volatile phenols, a paired comparison of malolactic fermentation (MLF) and non-MLF Merlot wines was made. The extraction of putative taint from fruit into wine occurred during alcoholic fermentation and no changes in the quantity or distribution of taint were found from MLF.

A sensory assessment of the wines found clear differences in aroma and taste due to smoking application. Differences in taste were also apparent between some of the smoking treatments driven by both phenol composition and concentrations. Vanillin, acetovanillone, syringaldehyde and acetosyringone were closely aligned to fruit aroma and taste and the presence of these compounds may mask some of the negative sensory descriptors of smoke taint flavour. A large number of glycoconjugated phenols were associated with negative taste descriptors and may deconjugate in the mouth when tasting smoke affected wine. Glycoconjugates of phenol, *m*-cresol and *p*-cresol were found to be the most likely drivers for the negative flavour descriptors in smoke affected wine. Previous studies have used volatile guaiacol concentrations as an indicator of smoke taint and reported the smoky aroma and flavour of tainted wines is due to the presence of elevated volatile guaiacol. This study found volatile and glycoconjugated 4-methylguaiacol concentration was highly correlated to smoke aroma and taste and these sensory descriptors were not dependent on guaiacol concentrations.

Publications

Kelly, D., Zerihun, A., Singh, D. P., Vitzthum von Eckstaedt, C., Gibberd, M., Grice, K. and Downey, M. (2012). Exposure of grapes to smoke of vegetation with varying lignin composition and accretion of lignin derived putative smoke taint compounds in wine. Food Chemistry, 135, 787-798.

Singh, D., Zerihun, A., Kelly, D., Cain, N., Nankervis, P., and Downey, M. (2012). A GC-MS based analytical method for detection of smoke taint associated phenols in smoke affected wines. Current Bioactive Compounds, 8, 190-199

Kelly, D., Zerihun, A., Hayasaka, Y. and Gibberd, M. (2014). Wine making practice affects the extraction of smoke-borne phenols from grapes into wines. Australian Journal of Grape and Wine Research. 20(3), 386-393.

Presentations

David Kelly, Ayalsew Zerihun, Davinder P. Singh, Christiane Vitzthum Von Eckstaedt, Mark Gibberd, Kliti Grice and Mark Downey.
A study into the effects of pyrolysis fuels, pyrolysis conditions and the identification of chemical markers in grapes and wine as smoke taint. Presentation at the Department of Environment and Conservation Bushfire Smoke Meeting, Pemberton, February 9th, 2011.

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A study into the effects of pyrolysis fuels, pyrolysis conditions and the identification of chemical markers in grapes and wine as smoke taint. Presentation at the National Smoke Taint meeting, February 11th, 2011.

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David Kelly (2012). Smoke taint in wine unchanged by differences in vegetation. Grapegrower and Winemaker pp 59-60 582.

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CHAPTER 1

INTRODUCTION AND OVERVIEW

1.1 Introduction

Much of the global wine grape crop is produced in Mediterranean-type environments (Jones et al. 2005) where fire is a frequent occurrence (Keeley et al. 2011). While fire and the resultant smoke play an influential role in shaping natural ecosystems in these environments (Keeley et al. 2011), smoke from such fires can also potentially taint grapes and consequently wines if smoke drifts through vineyards in the landscape while vines are bearing fruit (Whiting & Krstic 2007). Wine grapes exposed to smoke from wildfires and prescribed burns produce wines with elevated levels of glycoconjugated and volatile phenols that impart burnt, smoky and dirty aromas and flavour attributes (Whiting & Krstic 2007, Singh et al. 2012, Hayasaka et al. 2010, Parker et al. 2012). Over the past decade, viticultural areas in Australia have reported large financial losses from wines being unsellable due to the formation of 'smoke taint' where in some part of the growing season their vines have been exposed to smoke (Whiting & Krstic 2007).

Smoke-borne compounds that are considered responsible for smoke taint in grapes or wines are thought to originate primarily from pyrolysis of the lignin component of vegetation fuels (Hayasaka et al. 2010a, Singh et al. 2012) analogous to that which occurs in smoke used for curing/flavouring of food (Gilbert & Knowles 1975, Tóth & Potthast 1984, Wittkowski et al. 1992). Lignin is derived mainly from polymerisation of three monolignol precursors: *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (Pettersen 1984, Fahmi et al. 2007, Weng & Chapple 2010), which respectively constitutes the *p*-hydroxyphenyl, guaiacyl and syringyl units of lignin. When pyrolysed, these lignin units release respectively phenol, guaiacol and syringol along with their substituted forms such as methyl, ethyl, propyl, vinyl, allyl and propenyl derivatives (Gilbert & Knowles 1975). The lignin makeup broadly varies with vegetation type. The lignin of grasses contains all three precursors (Ralph & Hatfield 1991, Fahmi et al. 2007,

Buranov & Mazza 2008) and in angiosperm hardwood lignin, syringyl units dominate while most of the balance is from guaiacyl units with a minor contribution of *p*-hydroxyphenyl units (Pettersen 1984, Rencoret et al. 2011). In the lignin of gymnosperms, such as pines, there is an absence of syringyl units and guaiacyl units predominate with the balance *p*-hydroxyphenyls (Lebo et al. 2001, Pettersen 1984, Weng & Chapple 2010).

Several variables can affect the composition of lignin pyrolysis products in smoke and subsequently the types of lignin-derived putative smoke taint compounds that accrue in wines made from smoke exposed grapes. These include, firstly, the monolignol composition of pyrolysed vegetation. Even for a given vegetation, its lignin makeup may vary as a function of its age (Rencoret et al. 2011), the proportion of various plant components (Yokoi et al. 1999) and state of decay of the fuel which can alter the monolignol composition due to preferential demethoxylation of the dimethoxy- or methoxy-substituted phenylpropanoid units, i.e., degradation of syringyl and/or guaiacyl units, by rot-fungi (Vane et al. 2005, Faix et al. 1991, Schmidt 2006, Higuchi 1990 and references therein). Secondly, pyrolysis conditions, particularly temperature and oxygen availability (Simon et al. 2005, Guillén & Ibargoitia 1996, Tóth & Potthast 1984) can affect lignin pyrolysis products. While lignin may start to pyrolyse at temperatures as low as 280-290^{o}C (Browne 1958, Butt 2006), the composition of lignin pyrolysis products varies as temperature increases, with yields of the organoleptically important phenolic compounds in smoke reportedly peaking in the region of 559°C (Guillén & Ibargoitia 1996) and 650°C (Tóth & Potthast 1984). The above variables underscore the need to define vegetation type, fuel and pyrolysis conditions and smoke composition in order to compare results across studies as well as to explore the links between these factors and the types of putative taint compounds that accrue in grapes and wines. However, such definitions are rare in most of the smoke taint studies in grapes and wines to date.

Early work on smoke taint in grapes and wines focussed on two guaiacyl lignin-derived phenols: guaiacol and 4-methylguaiacol (Kennison et al. 2007, 2008, Sheppard et al.

2009). However, it is apparent that smoke affected wines have sensory properties additional to those expected from these two phenols (Kennison et al. 2007). This suggests other phenols in smoke are contributing to the undesirable sensory effects. Recently, Hayasaka et al. (2010a) and Singh et al. (2012) have shown that there are indeed several lignin pyrolysis products in bushfire smoke affected wines at elevated levels. Although these findings broaden our understanding of the range of putative taint compounds that can accrue in wine as a result of smoke exposure, they do not allow for exploring the link, if any, between vegetation type of the smoke source and the types of taint compounds that accrue in grapes or wines.

The fermentation of smoke exposed fruit increases the levels of guaiacol and 4-methylguaiacol throughout the fermentation, resulting in elevated levels of these volatile taint phenols in wine (Kennison et al. 2008). Kennison et al. (2008) also demonstrated the levels of these volatile phenols increased from enzymatic hydrolysis of smoke affected juice suggesting they were present in glycoconjugated form. Hayasaka et al. (2009) confirmed the presence of glycoconjugated phenols in smoke exposed grapes and in a broader study (Hayasaka et al. 2010a) found taint phenols to be predominantly glycoconjugated and not as free volatiles.

Although a direct absorption of phenol and *p*-cresol in maize has been reported by Beattie and Seibel (2007) the mechanism of accretion of smoke borne lignin pyrolysates in wine grapes is not well understood. Some of the possibilities include direct uptake of lignin pyrolysis products in smoke emissions by berries and/or foliar uptake and subsequent translocation and sequestration in berries. Tracer studies have shown grape vine leaves and berries take up phenols, although only trace quantities are subsequently translocated from the leaves to the berries (Hayasaka et al. 2010b). If the mechanism of uptake in berries was primarily through the leaves, then a positive relationship between vine canopy size (absorptive surface area) and taint concentration from smoke exposure would be expected. This relationship is easily investigated through controlled, replicated smoke exposures of fruit bearing vines and an analysis of taint levels.

When a vineyard is impacted by smoke, grape growers and winemakers endeavour to know the likely levels of smoke taint in grapes and the expected levels of taint extracted into wines. However, several factors are likely to influence the level of accretion of smoke-borne putative taint compounds in grapes and wines. Firstly, from the work reported to date, it is unclear whether different wine grape cultivars accumulate similar levels of smoke-borne phenols under comparable smoke exposure conditions. Putative cultivar differences in levels of phenols from wildfire smoke exposed fruit have been reported (Singh et al. 2012, Dungey et al 2011, Hayasaka et al. 2010) but determination of cultivar differences from such data is difficult due to variations in smoke exposure (intensity and duration) as well as in the occurrence of a smoke exposure event vis-à-vis vine phenology (Kennison et al. 2009).

Secondly, our current understanding of transformations and estimates of the expected levels of phenols in finished wines due to differences in traditional winemaking is incomplete. Recently, there have been advances in the analysis of phenols in smoke affected fruit and resultant wines (Hayasaka et al. 2010, Ristic et al. 2011). Estimates of the expected proportions of volatile and glycoconjugated phenols extractable from grapes into wines, however, may not be fully inferred from these reports either due in part to the use of non-standard winemaking (for example white wines fermented on skins as in Hayasaka et al. 2010a) or to the limited range of phenol glycoconjugates reported (Ristic et al. 2011). In smoke affected grapes, a high proportion of glycoconjugated phenols are sequestered in the skin (Dungey et al. 2011) and skin maceration and contact during winemaking may affect the extraction of glycoconjugated phenols into wine. It is thus imperative to understand the likely extraction levels of a comprehensive range of phenols from smoke impacted grapes under the commonly used white and red winemaking practices.

Thirdly, the organoleptic impact of smoke taint in wine is influenced by the distribution of volatile and glycoconjugated phenols (Parker et al. 2012). Red wine ferments are often subjected to malolactic fermentation (MLF) by inoculation with lactic acid bacteria. The metabolic activity of lactic acid bacteria can influence wine aroma complexity by hydrolysing wine aroma glycoconjugates (Ugliano et al. 2003, D'Incecco

et al. 2004), however the effect of MLF on the distribution of glycoconjugated phenols in smoke tainted wine is yet to be reported.

Wines with unpalatable levels of smoke related attributes (Høj et al. 2003) contain a large number of volatile and glycoconjugated phenols (Kelly et al. 2012, 2014, Hayasaka et al. 2010a, 2013). Although the sensory profile of smoke taint is related to the volatile phenol composition, glycoconjugated phenols may be deconjugated to the volatile forms in tasting smoke affected wine (Parker et al. 2012). A detailed exploration of the volatile and glycoconjugated phenols (Kelly et al. 2012) as drivers for the harsh smoke taint descriptors reported by Parker et al. (2012) has not been reported.

1.2 Thesis rationale

Understanding the mechanism for the sequestration of putative smoke taint phenols in wine grapes is essential to developing a strategy to mitigate the impact of wildfire smoke on the viticultural industry. As the composition of lignin pyrolysis products in smoke are altered by several factors the need to define vegetation type, fuel and pyrolysis conditions and smoke composition is the first step in investigating vine response to smoke exposure. While deliberate exposures of grapes to smoke are known to produce wines with elevated levels of volatile and glycoconjugated phenols (Kennison et al. 2007, Hayasaka et al. 2010c), an examination of fuel composition and smoke has not been made to determine if the lignin composition of vegetation fuels quantitatively and qualitatively influences the putative smoke taint compounds that accrue in wines under standardised exposure conditions.

In accumulating taint, it is unclear whether different wine grape cultivars accumulate similar levels of smoke-borne phenols under comparable smoke exposure conditions. Putative cultivar differences in levels of phenols from wildfire smoke exposed fruit have been reported (Singh et al. 2012, Dungey et al. 2011, Hayasaka et al. 2010), but determination of cultivar differences from such data is difficult due to variations in smoke exposure.

An analysis of smoke affected fruit and subsequent wines made with varying degrees of skin contact and maceration will provide estimations of volatile and glycoconjugated phenol extraction from traditional winemaking. While other researchers have reported extraction ratios of a very limited number of phenols and glycoconjugated phenols (Ristic et al. 2011), it is unknown if all glycoconjugated phenols extract or deconjugate to the same degree. Where a more comprehensive analysis of smoke affected fruit and wines has been made, non-standard winemaking has been used that does not allow a comparison of extraction from traditional winemaking methods. A detailed analysis of volatile and glycoconjugated phenols in fruit and wines made with different winemaking methods will provide industry with estimations of putative taint concentrations in finished wines from a detailed analysis of smoke affected fruit.

A sensory assessment of wines with the volatile and glycoconjugated phenol concentrations determined will allow a detailed analysis of the drivers for the sensory descriptors of smoke tainted wine reported by Parker et al. (2012). Previous researchers have focussed on volatile guaiacol concentrations as indicators of smoke taint (Kennison et al. 2007, 2008, Sheppard et al. 2009), however an exploration of the phenols reported by Singh et al. (2012) and Kelly et al. (2012) will reveal the organoleptic impact these compounds have in wine.

1.3 General Aim of Research

1. To examine the influence of vegetation lignin composition on the types of putative smoke taint compounds that accrue in grapes and wines.

2. Determine whether there are cultivar differences in uptake of smoke-borne phenols and accumulation in grapes.

3. Determine the likely proportions of glycoconjugated phenols that are extracted from grapes into wines under traditional red and white winemaking techniques, including when fruit is crushed and de-stemmed and when fruit is whole bunch pressed.

4. Assess whether the glycosidase activity of lactic acid bacteria contributes significantly to hydrolysis of glycoconjugated phenols to volatile phenols, by a paired comparison of MLF and non-MLF Merlot wines.

5. Determine the phenol compounds responsible for the aroma and taste descriptors of smoke affected wines.

CHAPTER 2

LITERATURE REVIEW

2.1 A review of smoke taint in wine

Smoke taint in wine is attributable to the exposure of grapevines to smoke from the pyrolysis of biomass, from a period just prior to veraison to harvest (Kennison et al. 2009). Wines made from smoke exposed grapes exhibit 'smoky', 'burnt', 'ash' characters with an 'acrid', 'cigarette', 'ashtray' and hard metallic, unpleasant finish (Simos 2005). Wines with smoke taint attributes have been described over many years and they have occurred in a number of regions in Australia, Canada, the United States of America and South Africa (Whiting & Krstic 2007).

Initial investigations into smoke taint (Kennison et al. 2007, Kennison et al. 2008) relied on existing wine analysis methods of volatile phenols (Pollnitz et al. 2000, Pollnitz et al. 2004) and concentrated on two compounds: guaiacol and 4-methylguaiacol. Kennison et al. (2007) claimed smoke taint descriptors were apparent in wines made from smoke exposed fruit at levels below the reported threshold values of guaiacol and 4-methylguaiacol and as such, it was not conclusive these two phenols were solely responsible for smoke taint in grapes and wine. Hayasaka et al. (2010a) reported six additional phenols, found in smoke at prescribed burning in South Australia, were also present at elevated levels in smoke exposed fruit and the organoleptic influence of these compounds to contribute to the perception of taint in smoke affected wine was confirmed by Parker et al. (2012). Many additional phenols present in vegetation smoke (Nolte et al. 2001) may also accumulate in smoke exposed grapes and Singh et al. (2012) confirmed twenty one of these were present at elevated levels in wines made from bushfire smoke exposed fruit. The organoleptic effect of most of these phenols as taint in smoke affected grapes and wine has not been established.

Recent work has shown that putative smoke taint compounds are predominantly sequestered as glycoconjugates in grapes (Hayasaka et al. 2010a, Dungey et al. 2011). The presence of glycoconjugated phenols had previously been reported in non-smoke exposed grapes (Sefton 1998, Wirth et al. 2001, Lopez et al. 2004), however their levels were significantly elevated in grapes exposed to vegetation smoke (Dungey et al. 2011). Singh et al. (2011) and Singh et al. (2012) have also found glycoconjugated phenols in wines made from non-smoke affected fruit, possibly from the degradation of lignin in grapes during winemaking (Macheix & Fleuriet 1998). These putative taint glycoconjugated phenols in smoke exposed fruit remain predominantly glycosidically bound in wines (Hayasaka et al. 2010a, Singh et al. 2011, Singh et al. 2012, Kennison et al. 2008, Wilkinson et al. 2011), highlighting the importance of quantifying both volatile phenols and glycoconjugated phenols in smoke taint studies.

Research investigating the formation of smoke taint in grapes and wine has either used commercial samples where the fuel source and emissions were unknown as they were made from wildfire smoke exposed vines (Hayasaka et al. 2010a, Singh et al. 2011, Singh et al. 2012, Dungey et al. 2011) or have applied smoke to vines experimentally using a model fuel source without controlling the pyrolysis or emissions (Kennison et al. 2008, Kennison et al. 2009, Kennison et al. 2011, Fudge et al. 2012). Without control of pyrolysis or the mass of fuel pyrolysed, past research has been limited in exploring relationships between vine phenology and fuel emissions, and as a result, investigations into vine response to smoke have not uncovered the mechanisms leading to putative taint accumulation. Research by Kennison et al. (2009) investigating phenological timing, density and duration, used a model fuel source (dry barley straw) to expose vines where the mass of fuel and pyrolysis conditions were not controlled or recorded. Previous research, where smoke was generated and entrapped over vines in a tent structure, pyrolysed a model fuel source and held smoke particulate obscuration (PM 2.5) above a predetermined percentage by adding more fuel to an incinerator during the trial. Replicated trials have not standardised the mass of fuel pyrolysed or the pyrolysis temperature and most studies have used this methodology of holding particulate obscuration above a nominal 30% despite measuring obscuration with equipment that

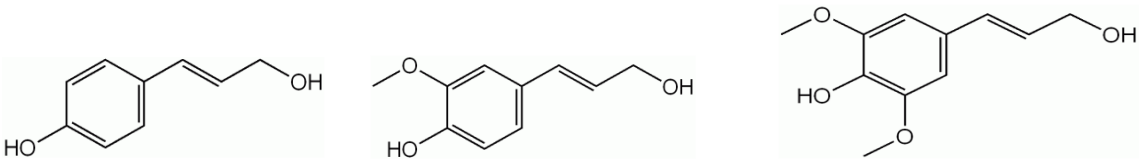
peaks at 32% (Kennison et al. 2008, Kennison et al. 2009, Sheppard et al. 2009, Kennison et al. 2011, Fudge et al. 2012, Ristic et al. 2013). Although measurements of obscuration are useful in determining the particulate matter with aerodynamic diameters less than 2.5µm suspended in air as smoke, the link between particle obscuration and gaseous phenol concentration has not been established. In order to adequately study the accumulation of smoke taint in fruit and wines a comprehensive review of pyrolysis emissions must be made that includes the fuel source and pyrolysis conditions.

2.2 Chemical composition of wood

The pyrolysis of biomass in a wildfire includes a combination of species components, from elevated canopy layer fine fuels to surface layer large fuels and accumulated dead material (Gould et al. 2007). The chemical composition of wood cannot be defined precisely for a given tree species or even within different components of a tree. The composition varies with each functional part of a tree (leaf, stem, branch, trunk and root), the type of wood (compression or tension), climate, soil conditions and geographic location (Pettersen 1984). There are two major chemical components: lignin (18-35%) and carbohydrates (65-75%) with the balance extraneous materials (4-10%) in the form of organic extractives (fatty acids, polyhydric alcohols and terpenes) and inorganic minerals (Guillén & Manzanos 1999).

The carbohydrate component comprises cellulose (40-50%) and hemicelluloses (25-35%). Cellulose is a glucan polymer of linear chains of 1-4-β-bonded anhydro-glucose units. The number of glucose units that make up a chain is termed the degree of polymerization and can vary even within the same site of a single tree. Hemicelluloses, which contribute as structural components in plants, are chains of polysaccharides synthesized from glucose, mannose, galactose, xylose, arabinose, 4-O-methyl glucuronic acid and galacturonic acid. They are macromolecules of much lower weight than cellulose and their carbohydrate makeup varies within a plant (Pettersen 1984).

Lignin is a phenolic substance consisting of an irregular array of hydroxyl and methoxy substituted phenylpropane units. It is formed from the enzymatic dehydrogenative polymerization of three phenylpropanoid monomers; *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (Figure 2.1, Lebo et al. 2001).



p-Coumaryl Alcohol Coniferyl Alcohol Sinapyl Alcohol

Figure 2.1 Monolignol Structures

The makeup of lignin monomers varies between grasses, softwoods and hardwoods. Softwood lignins contain coniferyl alcohol monomer units (~90%) with the remainder mostly consisting of *p*-coumaryl alcohol units (Lebo et al. 2001). Hardwood lignins contain predominantly sinapyl and coniferyl alcohol units with the ratio of monomers varying between species (Pettersen 1984). Grasses contain small amounts of *p*-coumaryl alcohol in addition to coniferyl and sinapyl alcohol monomers with residues of *p*-coumaric, hydroxycinnamic and ferulic acids attached to the lignins through ester and ether linkages (Lewis & Paice 1989).

Essential oils are volatile organic, condensable compounds obtained from plants. In most *Eucalyptus* species, 1,8-cineole and α-pinene predominate in the essential oil present in the leaves (Butt 2006). *Pinus radiata* is poor in essential oil content (0.01% of fresh foliage weight) which mostly consists of α and β- pinene (Cool & Zavarin 1992).

2.3 Pyrolysis of wood and biomass

In order to study the pyrolytic process of biomass that leads to smoke taint contamination, the thermal degradation of wood must first be considered. Wood will not

combust from the direct application of heat, but undergoes degradation to produce flammable gases and vapours. These will combust under the right conditions and if enough heat is retained by the biomass it will continue to burn leaving only inorganic residues as ash. The heating of biomass in a fire causes active pyrolysis and the pyrolysis products combust only by mixing with enough oxygen in the presence of a flame (Browne 1958). In a wildfire the process of pyrolysis and combustion may occur almost simultaneously, where the fuel surface is irradiated so intensely, spontaneous ignition occurs. In wood of sufficient thickness, combustion occurs in the exposed upper layer of alpha cellulose leaving a thin surface layer of burnt fuel backed by unaltered cellulose (Martin 1956).

Hawley (1952) defines ignition temperature as the point at which flammable gases are first produced, and introduced the concept of pyrolysis temperature degradation zones. Browne (1958) adopted these zones which occur in succession or simultaneously in wood of sufficient thickness.

Zone A — Below 200^{o}C
Production of water, carbon dioxide, formic acid, acetic acid and glyoxal occurs.

Zone B — 200^{o}C-280^{o}C
Carbon monoxide is produced and the emission of water vapours cease. The reaction proceeds exothermically.

Zone C — 280^{o}C-500^{o}C
Combustible volatile gasses and flammable tars as smoke particles are produced. Secondary reactions occur in the volatile products and the reaction proceeds exothermically.

Zone D — Above 500^{o}C
Charcoal residue remains which provide an active site for secondary reactions.

Figure 2.2 Pyrolysis temperature zones (Brown 1958)

2.4 Pyrolysis of cellulose

Cellulose pyrolysis produces the highest proportion of flammable gases of the three wood components (Browne 1958). The initial heating to 100^{o}C causes the loss of bound water followed by the splitting of cellulose macromolecules and the formation of free radicals between 260 and 300^{o}C (Beall & Eickner 1970). As the cellulose is heated above 300^{o}C, the polymer rapidly fragments to yield 1,6-anhydro-β-D-glucopyranose (levoglucosan), 1,6-anhydro-β-D-glucanfuranose, water, carbon monoxide, carbon dioxide and tar degradation products containing carboxyl, carbonyl and hydro peroxide groups (Shafizadeh & Chin 1977). Open chained polyhydroxy aldehydes, ketones or acids readily pyrolyse to simpler hydroxyl aldehydes, ketones and acids which undergo secondary reactions to form formaldehyde, acetone, glyoxal, glycolic aldehyde, glycolic acid, lactic acid, formic acid and acetic acid (Browne 1958).

Cellulose pyrolysis can also produce phenol and *o*-cresol where these compounds occur in minor amounts from carbohydrate rearrangement reactions (Almendros et al. 1997, Pastorova et al. 1994, Pouwels et al. 1989, Tóth & Potthast 1984). These phenol pyrolysates are usually associated with the pyrolysis of lignin.

2.5 Pyrolysis of hemicelluloses

Hemicelluloses can be one of several polysaccharide heteropolymers that include glucose, xylose, mannose, galactose, rhamnose and arabinose. Composition varies significantly between species and as such the decomposition products significantly vary (Browne 1958). Hemicelluloses are cross linked glycans (less than 3000 units) with side chains and an open structure. They are the least pyrolytically stable of the three wood components and evolve more gases and less tar than cellulose pyrolysis (Browne 1958).

The pyrolysis conditions of hemicelluloses determine the relative yields of volatile gases, char and tar. As in cellulose decomposition, water is given off at 100^{o}C, followed

by exothermic decomposition from 170^{o}C to 325^{o}C (Browne 1958). During pyrolysis, hemicellulose undergoes random dehydration reactions and glycosidic bonds are cleaved to yield monomeric glycosyl type compounds (Shafizadeh & Chin 1977), including furfural which decomposes to furan (Browne 1958).

2.6 Pyrolysis of lignin

Lignin is an amorphous, three dimensional phenylpropane polymer consisting of *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol monolignol monomers (Coscia et al. 1961). The distribution of lignin closely follows the direction of cellulose microfibrils in the secondary cell walls (Hepler et al. 1970, Fromm et al. 2003). The pyrolysis products of lignin are the most important components of smoke flavouring and the most significant compounds in the determination of different fuel types in smoke taint (Gilbert & Knowles 1975, Simon et al 2005, Hayasaka et al. 2010a). The primary products of lignin pyrolysis are closely related structurally to the monolignol makeup of the biomass fuel source (Simoneit et al. 1993). The pyrolysis products are phenols and phenolic ethers, typically the homologs and derivatives of phenol, guaiacol (2-methoxyphenol) and syringol (2, 6-dimethoxyphenol). The substituent groups are largely methyl, ethyl, propyl, propenyl, allyl and vinyl. The side chains occur almost exclusively in the para position to the phenolic hydroxyl group and generally do not exceed three carbons in length (Goos 1952). As hardwoods contain roughly equal coniferyl and sinapyl alcohol monomers (Pettersen 1984) and softwoods contain 90% coniferyl alcohol monomers with negligible sinapyl monomers (Lebo et al. 2001), the combustion of softwoods and hardwoods produce different ratios of coniferyl alcohol and sinapyl alcohol derived products. While the pyrolysis of sinapyl alcohol produces syringol and its derivatives, a demethoxylation of the sinapyl alcohol structure also produces guaiacol and its derivatives (Faix et al. 1987, Wittkowski et al. 1992, Asmadi et al. 2011). Therefore, the pyrolysis of hardwoods produces both syringol and guaiacol derivatives while softwoods will produce only guaiacol derivatives (Schauer et al. 2001).

Schauer et al. (2001), in studying fireplace emissions, found guaiacol and guaiacol derivatives as products in the smoke of both hard and softwoods but with different emission rates. They found guaiacol, its derivatives, and resin acids are emitted in significant quantities from pine wood combustion. In an analysis of *Eucalyptus camaldulensis* Dehn., Yokoi et al. (2001) found the pyrolysates from lignin were of the order 60:40 syringol to guaiacol units. Ferulic acid, together with dehydro-ferulic acid, is a component of ligno-cellulose, giving rigidity to cell walls by cross-linking polysaccharides to lignin. Its decomposition yields guaiacol, vanillin, acetovanillone and vanillic acid. In the pyrolysis of lignin, even though a large number of products occurs, a relatively small number of compounds accounts for a large proportion of phenolic yield. The pyrolysis of *Eucalyptus regnans* F. Muell, for example, produces guaiacol, vanillin, eugenol, isoeugenol, syringol and syringaldehyde. Furfural and methylacetyl furfural account for a large proportion of the carbohydrate yield (Butt 2006).

2.7 Pyrolysis conditions

The combustion products of biomass pyrolysis are greatly influenced by the pyrolysis conditions, which include temperature, flame residence time, oxygen availability and secondary reactions in smoke (Simon et al. 2005). Temperature is the dominant factor in determining the pyrolysis products of wood. While the polycyclic aromatic hydrocarbon content increases with increasing temperature from 400 - 1000^{o}C (Toth & Blaas 1972), the organoleptically important compounds only increase from 400 - 650^{o}C with a maximum somewhere in this region (Butt 2006). Daun et al. (1972) found smoke generated at 400^{o}C is more concentrated in flavour compounds than that produced at 500^{o}C or 600^{o}C and Toth and Potthast (1984) have reported that increasing temperature from 450^{o}C to 600^{o}C increased the syringol four fold in hard wood smoke. The formation of lignin derived phenols in smoke also leads to the secondary formation of pyrocatechols above 400^{o}C as strong exothermic reactions take place. Connors et al. (1980) refer these reactions to the reactive nature of the methoxy groups of guaiacol and syringol. For example, 4-ethylguaiacol pyrolyses to 4-ethylphenol and 4-ethylpyrocatechol

(4-ethylbenzene, 1,2 diol) and Toth and Potthast (1984) note a relative decrease in guaiacol occurs with an increase in pyrocatechols as the temperature increases from 600^{o}C to 1000^{o}C.

2.8 Wildfires and prescribed burning in Australia

Australia's topography, climate and vegetation combine to produce one of the most severe fire environments in the world (Australasian Fire Authorities Council, 2006). The distribution of flora that exists today has evolved from an adaptation to a severe fire regime that has been a part of the Australian ecosystem for millions of years (Australasian Fire Authorities Council, 2006). In the southwest of Western Australia, forests are burnt in both wildfires and by prescribed burning. Although each fire type will have many parameters in common, the former is an uncontrolled fire that fire agencies will endeavour to control and extinguish in the shortest possible time and the latter is the deliberate use of fire to conserve biodiversity and create areas of low fuel density. A wildfire is most likely to occur in times of minimum fuel moisture (December-March) and minimum air relative humidity (usually between 11am and 4pm), while a prescribed burn is usually carried out in conditions favourable for low intensity burning. Fuel load reduction from prescribed burning of forested land reduces the rate of spread, flame height and intensity of a wildfire which is a product of wind speed, fine fuel moisture, fuel hazard score (spatial arrangement, load and size of fuel) and topography (Gould et al. 2007).

2.9 The composition of fuels in wildfires and prescribed burning

Investigations of fuel structure, fuel dynamics and fire behaviour of dry *Eucalyptus* forests have separated the fuels of a forest fire into five distinct zones:

1. Over story tree and canopy layer
2. Intermediate tree and canopy layer
3. Elevated fuel layer
4. Near surface fuel layer
5. Surface fuel layer

(Gould et al. 2007)

The levels of fuel burnt are not consistent throughout an entire fire and are influenced by topography, wind strength and wind direction. As a general rule, fire burns with greatest intensity travelling uphill, least intensity downhill, increases in intensity with increasing winds and won't burn the over story or intermediate tree and canopy layer without sufficient surface fire intensity (Cheney 1994). Therefore, while the surface and near surface fuels always burn in a wildfire and the elevated fuels usually burn, intermediate and over story fuels only burn during intense fire behaviour when the fire is said to be 'crowning'. Optimally, prescribed burning only burns the surface and near surface fuels with limited elevated fuel combustion. While this does not reduce the intermediate and over story fuels available to burn in a wildfire, a reduction in available surface and near surface fuels reduces surface fire behaviour to reduce the likelihood of a fire crowning (Scott & Reinhardt 2001).

Gould et al. (2007) found that southern jarrah forests (*Eucalyptus marginata* Donn ex Sm.) reach a steady state load of surface fuel of around 14 tonnes per hectare (t/ha) with a surface fuel depth of 25-30 mm, but may continue to build up the elevated fuels for over thirty years. Contributing to this is the bark fuel consumed on standing trees as available fire fuel load in the elevated fuel layer. The steady state loads of unburnt

forests are species specific with the forest floor mass of Karri forests (*Eucalyptus diversicolor* F. Muell.) peaking at around 30 t/ha in areas unburnt for 16 years (O'Connell 1987).

2.10 Wildfire and prescribed burning pyrolysis temperature

The combustion of vegetation in a fire occurs in consecutive stages: distillation, endothermic pyrolysis, exothermic pyrolysis, ignition, flaming combustion and smouldering. The pyrolysis products of each stage will vary significantly and analysis of smoke plumes cannot distinguish the emissions from each stage as the smoke generated is a product of every stage (Greenberg et al. 2005). In a review of biomass burning emissions, Reid et al. (2005) described smouldering as a surface process that occurs when most of the volatiles have been expelled from the cellulose fuel.

As pyrolysis temperature is the single most influential factor in determining the chemical makeup of smoke, it is important to understand the temperature profile of wildfires and prescribed burns (Martin et al. 1969). In dry *Eucalypt* forests, bushfire flame temperatures peak at 1000^{o}C to 1100^{o}C and closely follow the combustion stages detailed by Greenberg et al. (2005): a short period of sub 100^{o}C is followed by a rapid spike to a maximum temperature of over 1000^{o}C as the flaming period begins, decreasing rapidly to a longer period of 200 - 400^{o}C as flaming ends with extended smouldering combustion temperatures between 100^{o}C and 200^{o}C (Figure 2.3, Gould et al. 2007).

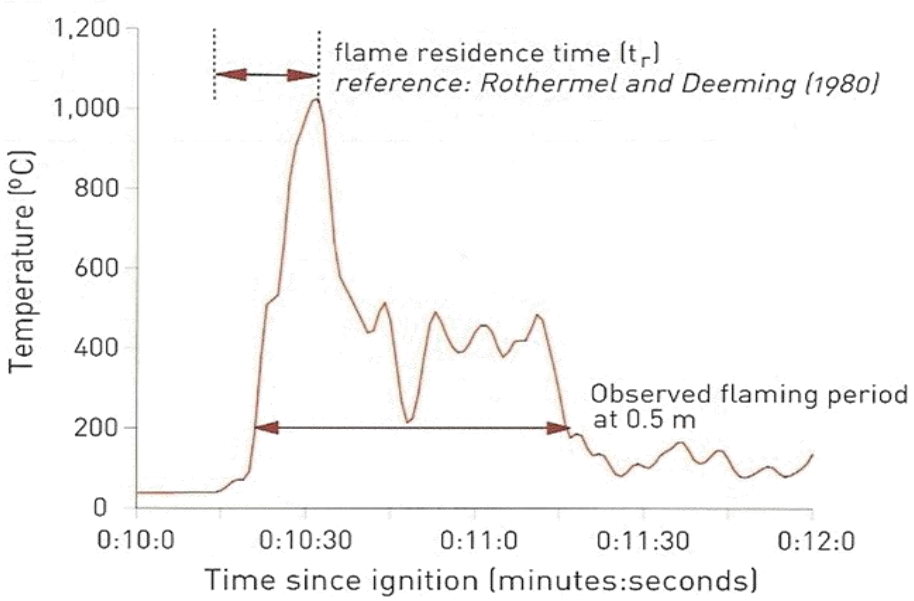


Figure 2.3 Observed flaming period at 0.5 m height in an experimental fire. Source: Gould et al. (2007).

Gould et al. (2007) have also found flame temperatures are independent of fuel type and fuel accumulation (t/ha) (Figure 2.3, Gould et al. 2007).

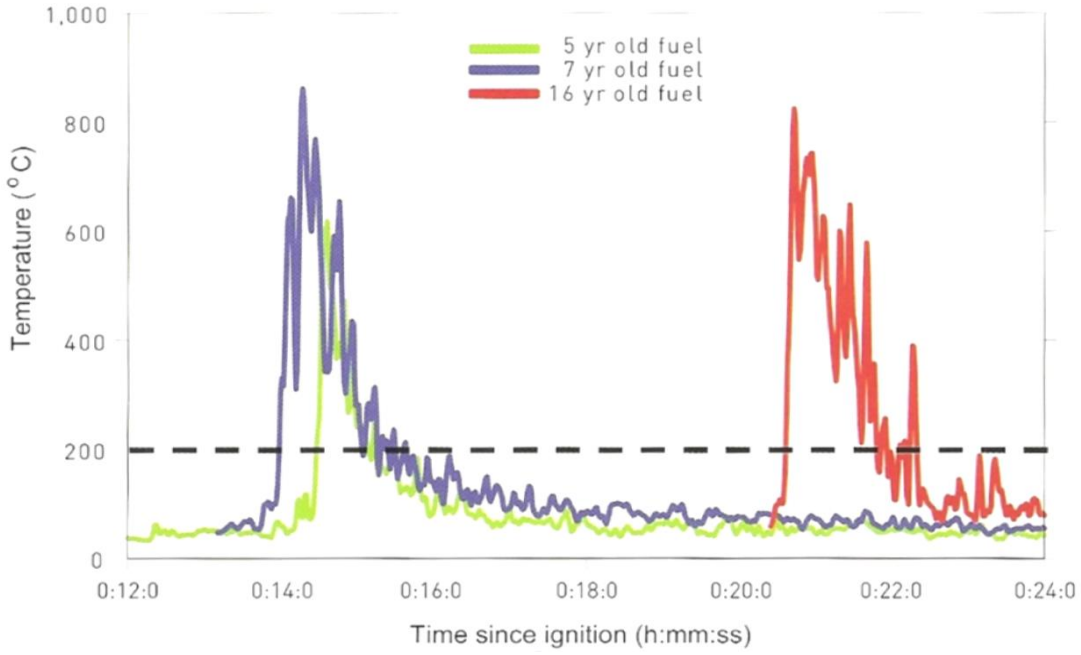


Figure 2.4 Comparison of temperature profiles of fuels of different ages with five year old fuel at 5.1 t/ha, seven year old fuel at 7.4 t/ha and sixteen year old fuel at 9.1 t/ha. Source:Gould et al. (2007).

Prescribed burning is the use of fire to achieve defined forest management objectives, including fuel reduction, conservation of biodiversity and the re-establishment of forests (Australasian Fire Authorities Council 2006). The conditions of prescribed burning vary as much as wildfires from fuel type, topography, fuel moisture content, fuel spatial arrangement and climatic conditions. The difference is in the design of the fire prescription by land management agencies to achieve a result where the fuel is reduced in a defined area. The burns are usually carried out immediately before and after the summer fire season as they utilise the same resources required to fight wildfires in summer. While the fuel moisture content is typically as low as 7% in open *Eucalypt* forests (Gould et al. 2007) during peak fire season, the fuel moisture content during prescribed burning is usually much higher. Karri forests carry much higher fuel moisture content through summer and are usually only burnt after the fire season ends when the moisture levels are at their lowest. While prescribed burning is designed to be of low intensity, typically taking out only the surface and low under-story fuel, variability in topography, wind strength, fuel moisture and fuel arrangements cause some areas to be burnt with wildfire like intensity and others to be relatively untouched. While the bulk of emissions may therefore be from lower intensity pyrolysis, a broad spectrum of fire intensity will still exist and the products of a prescribed burn may only be different from that of a wildfire in the proportions of pyrolysis products.

2.11 Fuel components consumed in a wildfire

Fire events in Australia occur in native forests, pastures and plantations where the available fuels are composed of fresh leaf, twigs, branches, bark, root structure, fruit, trunk and deposited material of each fuel component in various stages of decomposition (O'Connell & Menage 1982, Burrows 1994). The ratio of these components available as fuel varies with fire 'age', defined as the time (years) since the fuel has last been subjected to a fire event (O'Connell & Menage 1982), including both wildfires and prescribed burning. With the composition of cellulose, hemicelluloses, lignin and monolignols changing for components of a species, the ratios of deposited components in various stages of decomposition will likely also have compositional differences as the

proportions of leaf, twigs, branches, bark etc. changes with the age of the fuel accumulation in a forest (O'Connell & Menage 1982, Burrows 1994). Vane et al. (2005) found in the decay of the cultivated angiosperm apricot wood, lignin decomposition occurred at different rates for syringyl and guaiacyl units. This may also hold true for the surface forest components available as fuel in a fire event. While the forest floor fuels may reach a maximum level where decomposition matches new deposits, the ratio of syringol units to guaiacol units may be less in lower layers of fuel compared to new deposits. The forest fuels used in this study have been assembled from the components that typically burn in a 10 year old fuel accumulation.

The forest fuels used in this study also contained essential oils expected to volatilise in a wildfire (Mazurek & Simoneit 1997). The essential oil eucalyptol (1,8-cineole), which imparts a distinctive aroma described as 'eucalyptus' in wine, was first reported by Herve et al. (2003). The accumulation of 1,8-cineole in red wines from fruit grown in close proximity to *Eucalypt* forests has been reported by Capone et al. (2012), who determined the uptake to be predominantly in the grape skin from absorption of 1,8-cineole. The consumer acceptance of 1,8-cineole in red wine is 27.5 µg/l (Saliba et al. 2009). Singh et al. (2012) have considered 1,8-cineole to be a taint compound in examining smoke taint in wines, however there is yet to be an examination of the composition of vegetative fuels, essential oils in their pyrolysis emissions and the formation of smoke-derived 1,8-cineole as taint in wine.

2.12 Sensory assessment of smoke tainted wines

Early assessments of smoke affected wines noted significant taint occurred in wines where volatile guaiacol and 4-methylguaiacol were below sensory thresholds (Kennison et al. 2009), suggesting taint may also be attributable to other smoke-derived phenols. A large proportion of phenols present in smoke affected wines are glycoconjugated (Kelly et al. 2012 and 2014) and Parker et al. (2012) demonstrated they may play an important role in imparting smoke taint flavours in smoke affected wines. Singh et al. (2012) have reported a large number of phenols are present in smoke affected wines however the

contribution each of these volatile phenols and their glycoconjugates plays in imparting off aromas and flavours is unknown.

2.13 Research methodology of this thesis

To date there has not been any reported research where grapevines have been exposed to replicated, controlled pyrolysis of fuels. In this thesis, fuels typically burnt in wildfires and prescribed burning in the Margaret River wine region have been examined to investigate relationships between putative smoke taint phenol accumulation in the fruit of smoke exposed vines and the monolignol composition of the fuels. Differences in cultivar susceptibility to smoke exposure have also been studied where the mass of fuel and pyrolysis temperature have been replicated and differences in taint extraction examined from traditional winemaking practices. A sensory evaluation has been used to examine wine phenol composition impact on smoke tainted wine.

2.14 General workflow of the research

Step 1. Identify fuel types typically burnt in wildfires and in yearly prescribed burning programs by the Department of Parks and Wildlife and the Department of Fire and Emergency Services. The fuels chosen for this study were done in consultation with Department of Parks and Wildlife research scientists and also included fuels with large lignin and monolignol differences.

Step 2. Compile fuels with a standardisation of fuel age using existing research and an examination of burnt and unburnt fuel stands after fires.

Step 3. Develop a pyrolysis chamber capable of pyrolysing fuels at wildfire temperatures as described by Gould et al. (2007). The chamber needed to reliably replicate the same temperature time profile over a large number of trials and operate in dry vineyard paddocks without risk of starting a fire. The outlet temperature needed to be cooled considerably to avoid increasing the tent air temperature in vineyard exposures.

Step 4. Quantify carbohydrate, ash, fuel moisture, lignin and monolignol content of the fuels. Characterise fuel emissions produced during vineyard trials.

Step 5. In collaboration with other researchers, develop new analysis techniques for a large number of phenols suspected of accumulating in fruit and wines as putative taint.

Step 6. Expose vines to replicated emissions from each fuel and examine the differences in accumulation of putative taint phenols in fruit and wines.

Step 7. Examine cultivar differences to the accumulation of putative taint.

Step 8. Develop industry guidelines for the extraction of taint using different traditional winemaking techniques.

Step 9. Conduct sensory trials on wines to examine drivers of smoke taint related descriptors from paired comparisons of phenol composition.

CHAPTER 3

CHARACTERISATION OF FUELS AND FUEL PYROLYSIS EMISSIONS

3.1 Introduction

The work reported here was designed to investigate the differences in smoke-derived taint from fuel sources in the Margaret River wine region (33º57′S, 115º01′E), in the south west of Western Australia. The viticultural region is surrounded by farmland, forested crown land and privately owned forested allotments. This research has looked at the fuels in the region to further the understanding of smoke taint in wine for two distinct outcomes. The first was to investigate if significant differences occur in smoke-derived taint from the bushland fuel types in the region. Every year, the Western Australian Department of Parks and Wildlife (DPAW) conducts a large prescribed burning program which poses a high risk to viticulturists by enveloping their vineyards in smoke for prolonged periods. While the smoke from a prescribed burn is usually highly visible, its influence on a vineyard may be dependent on the variety and phenological stage of development (Kennison et al. 2011). Concurrent with DPAW's burning program, local government bushfire brigades and landowners also conduct burns which may also impact vineyards with smoke. Attributing the accumulation of taint to a specific fire event, when typically several small and large scale fire events occur during a season's berry growth, requires distinct chemical differences in taint formation from those fire events. Exposures of vines to smoke of varying lignin composition will examine the influence lignin composition has on the types of putative smoke taint compounds that accrue in fruit and wines. The second consideration in the selection of fuels for this study was to investigate if sequestration of putative taint phenols in smoke exposed fruit is a simple absorption and conjugation of the phenols present in smoke. As lignin is the source of gaseous phenols in smoke (Schauer et al. 2001), pyrolysis trials of fuels with large variations in proportionate lignin and monolignol composition were used to investigate relationships between fuel composition and the derived putative taint in smoke exposed grapes and wine. The research described

here has therefore included an angiosperm fuel low in lignin content and a gymnosperm fuel that is relatively high in lignin but lacking sinapyl alcohol monolignols.

In examining the effects monolignol composition has on putative taint accumulation in fruit and wine from vines exposed to the pyrolysis products of fuels present in the Margaret River region, the following sequence of determinations was required:

1. Identification of fuels and fuel component compositions from existing literature and an analysis of fire affected forests.
2. Quantification of fuel cellulose, hemicellulose, lignin, ash, essential oil and fuel moisture content.
3. Quantification of fuel monolignol content.
4. Development of a pyrolysis chamber to replicate wildfire temperature profiles.
5. Quantification of phenols generated in pyrolysis trials.
6. Quantification of phenols generated in prescribed burning.

In the Margaret River viticultural region, the vegetation of the surrounding landscape mainly contains the hardwood species: jarrah (*Eucalyptus marginata* Donn ex Sm), karri (*E. diversicolor* F. Muell) and marri (*Corymbia calophylla* Lindl.); plantations of the softwood species radiata pine (*Pinus radiata* D. Don) and pasture grasses such as wild oats (*Avena fatua* L). For this study, five biomass fuels representing each of these main vegetation types were used as fuel sources. With the component percentages of cellulose, hemicelluloses, lignin and monolignols changing between different parts of a fuel's components (Pettersen 1984), and through biodegradation of those components on the forest floor (Vane et al 2005), a standardisation of the fuels to a defined fire event was required. The 'age' of a block of fuel in fire terms is the time in years since the block was burnt by a wildfire or prescribed burn and the passage of a fire through a forest or plantation of a defined fuel age will consume set proportions of fuel components (Gould et al. 2007). The components consumed changes as the age of the fuel changes (O'Connell & Menage 1982, Burrows 1994). The component proportions for a ten year fire event were chosen for this study for the tree species and for the grass

fuel, an annual species, a local Margaret River site was chosen where cured, intact plants could be collected. The tree fuel components were assembled as percentages by mass of air-dried leaf, bark, twig (branch or stem less than 6 m.m. in diameter), stick (branch or stem greater than 6 m.m. in diameter) and duff. The component compositions for karri fuel were assembled using the proportions reported by O'Connell and Menage (1982) and the jarrah compositions as reported by Burrows (1994). The marri compositions were matched to the jarrah compositions as the two usually coexist in areas around Margaret River. The pine plantation composition was made from observations of burnt and unburnt pine plantation sections after a fire in Bridgetown, Western Australia (33° 57' S, 116° 08'E). For wild oats, all of its above ground biomass was considered a single component (100% fuel source) since all of it combusts during a fire event. The proportions for each fuel are listed in Table 3.1.

Table 3.1 Percentage of 'biomass' component used for reconstituting fuels for smoke generation as well as lignocellulose and lignin composition analysis.

Fuel	Biomass fuel component					Total
	Leaves	Duff	Bark	Twigs ∅ < 6 mm	Wood ∅ > 6 mm	
Karri	50.1	26.7	3.8	14.3	5.1	100
Jarrah	16.9	53.4	3.9	25.8	0.0	100
Marri	16.9	53.4	3.9	25.8	0.0	100
	Needles	Twigs ∅ < 5 mm	Wood ∅ 5-20 mm	Wood ∅ > 20 mm		
Pine	90.0	4.0	5.0	1.0	-	100
	Straw, blade and panicle					
Wild oats	100					100

The inclusion of large amounts of duff in the hardwood fuels was expected to decrease the lignin percentage of the compiled fuel as well as change the sinapyl alcohol/coniferyl alcohol ratio from a demethoxylation of sinapyl alcohol to coniferyl alcohol (Vane et al. 2005).

The fuels were sourced from forests and plantations in the Margaret River region known to have had no fire impact for ten years. Fresh fuels were continually collected to minimise potential degradation from rot that may change the lignin and monolignol ratio after the fuels were collected. The fuels were stored in thin layers for several weeks to equilibrate moisture contents. After drying, each of the tree fuels for smoke generation was compiled from foliage, duff, bark, twigs (Ø < 6 mm) and round wood (Ø ≥ 6 mm) (Table 3.1).

3.2. Materials and methods

3.2.1 Fuel pyrolysis for vineyard trials

A pyrolysis chamber was constructed to imitate the temperature profile of a wildfire as described by Gould et al. (2007). As pyrolysis temperature is the single most influential factor in determining the chemical makeup of smoke (Martin et al. 1969), the chamber needed to reliably replicate the same temperature profile for fuels with different physical structure. The mass of fuel burnt in each trial was standardised at one kilogram to allow comparisons between the lignin and monolignol content pyrolysed in each fuel. A steel 30 litre open topped drum with a raised steel mesh fuel screen and 37 mm air inlet pipe, to force air from underneath the fuel load, was placed into a sealed 218 litre steel drum with a 76 mm diameter, 6m long, flexible steel outlet to deliver smoke for vineyard trials.

The fuel combustion rate and temperature were controlled by forcing air into the inlet pipe with a petrol operated Stihl BG 85 leaf blower (Stihl International GmbH, Waiblingen, Germany). The time and temperature profiles were recorded with a Center

309 data logger (Center Technology Corporation, Taipei, Taiwan) and K type exposed junction thermocouples (KD Instruments, Perth, Western Australia), positioned at the fuel screen and 12, 24 and 36 cm above the fuel screen.

Figure 3.1 Inner fuel drum with fuel mesh screen and air inlet.

Figure 3.2 Inner fuel drum loaded with wild oats fuel and positioned in outer steel drum.

Figure 3.3 Assembled pyrolysis chamber with Stihl leaf blower, K type thermocouples and flexible steel smoke outlet.

For each trial, one kilogram of fuel was loosely arranged on the fuel screen and ignited at the bottom before the steel lid was sealed on the outer drum. After ignition the burning fuel would draw air into the drum through the fuel screen before the leaf blower was accelerated to full power (air flow at 81.8ms^{-1}) and maintained until the temperature recorded by the thermocouple positioned 12 cm above the fuel screen reached 1050 ^{o}C. At this point the leaf blower would be removed with the rapidly burning fuel continuing to draw air as the pyrolysis rate started to slow down. The fuels were allowed to smoulder until completely consumed. This produced a short period of sub 100^{o}C pyrolysis followed by a rapid spike to 1050^{o}C decreasing rapidly to a longer period of 200 to 400^{o}C as the flaming ended with final smouldering combustion temperatures of 100^{o}C to 200^{o}C.

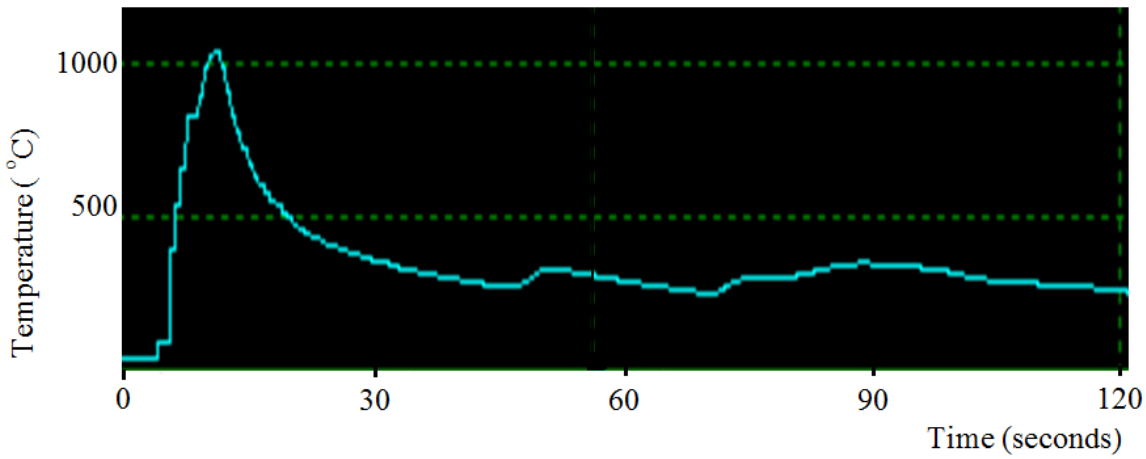


Figure 3.4 Typical thermocouple response during pyrolysis of fuel.

3.2.2 Fuel analysis

One kilogram lots of each fuel were combined from the components listed in Table 3.1 and ground to 0.5 mm using an IKA MF10 mill (IKA Works, Staufen, Germany).

3.2.3 Fuel moisture, ash and essential oil content

The fuel moisture content was determined gravimetrically after heating to 105 ^{o}C for 48 hours (van Wagner 1967) and the ash content by gravimetric determination after combustion at 600 ^{o}C for eight hours (ASTM D1102–84.). The essential oil content was determined by steam distillation (Hughes 1970, Li & Madden 1995). Each analysis was determined in triplicate.

3.2.4 Determination of cellulose, hemicelluloses and Klason lignin

The fuels were analysed for cellulose, hemicellulose and lignin at the Western Australian Chemistry Centre (Perth, Western Australia). Each analysis was performed in triplicate using the following methods:

Enzymatic Neutral detergent fibre **(ENDF*)*:** The plant material was pre-treated with the enzyme α-amylase overnight to remove the starch, (McQueen & Nicholson 1979) and then boiled with buffered neutral detergent solution. The residue was collected on a course sintered glass filtering crucible (van Soest 1963, van Soest & Wine 1967) and the weight of the dry residue measured to give the ENDF content, after allowing for ash content.

Acid detergent fibre **(ADF):** The plant material was simmered in acidic detergent solution for 1 hour and then filtered on a coarse sintered glass crucible (AOAC method 973.18). The ADF was equal to the weight of the dry residue, after allowing for ash content.

Klason Lignin: The percentage lignin content was determined by reacting the fibre residue from the ADF determination with 72% sulphuric acid. (AOAC method 973.18c)

Cellulose and Hemicellulose content: The percentage cellulose content was determined by the subtraction of the lignin values from the total ADF value and the percentage hemicellulose content was determined by the subtraction of the ADF from the ENDF values. Each analysis was performed in triplicate.

3.2.5 Monolignol analysis by Pyrolysis GC-MS (*Py* GC-MS)

Approximately 0.1 mg of the ground samples were weighed into quartz tubes and flash pyrolysed for 20 seconds at 550 °C using a CDS Analytical 5250 Automated Pyroprobe (Oxford, Pennsylvania, USA). The transfer line from the pyroprobe to the GC-MS system was operated at 300 °C and the GC-MS analyses were performed on an HP

6890A Gas Chromatograph (Hewlett Packard, Santa Clara, California, USA) interfaced to an HP 5973A Mass Selective Detector. A 60 m x 0.25 mm x 0.25 µm DB-5MS capillary column (Agilent J&W, Santa Clara, USA) was used for the analyses with a helium carrier in constant flow mode at 1.2 ml/min with a 40:1 inlet split. The GC oven was cooled to -20 ºC, held for one minute and then heated at 8 ºC/min to 40 ºC. The oven temperature was then ramped to 320 ºC at 4 ºC/min and held at 320 ºC for 25 minutes. The mass selective detector was scanned between *m/z* 20 and 620, at 2.48 scans per second with an electron energy of 70 eV. All the analyses were carried out in triplicate. The lignin-derived compounds were identified by comparing their mass spectra with the NIST and/or Wiley spectral libraries or by comparison to reported spectra in literature (Ralph & Hatfield 1991). Individual lignin pyrolysates were quantified by their relative percentage area of the total ion chromatograms of all lignin pyrolysates.

3.2.6 Smoke analysis by Thermal Desorption GC-MS (TD GC-MS)

Samples of smoke generated during the vineyard smoke exposure experiments and prescribed burning were collected using a Markes Unity 2 thermal desorption (TD) unit (Markes International Ltd, Llantrisant, UK). The thermal desorption sampling tubes were packed manually with approximately 280 mg Tenax-TA adsorbent (60-80 mesh) with minimal compression. All tubes were thermally conditioned for 4 hours at 330 °C prior to their first use and for 30 min at 310 °C prior to every sampling event. In the vineyard trials, smoke was drawn through a series of three Tenax-TA TD tubes where the first tube, the primary sample, drew air samples from the tent, the second tube captured any compounds that broke through the primary tube and a third tube was used to entrap compounds breaking through the second tube. Samples were drawn through the chain of three TD tubes at a rate of 200 ml/min with a miniport diaphragm pump (KNF Neuberger GmbH, Breisgau, Germany) for 30 minutes. The tubes were stored at 4 °C until analysed. All smoke sampling was performed in triplicate.

Prescribed burning of marri fuel was sampled at the Department of Parks and Wildlife's Bodega block near Margaret River, in May 2009. The block had a predominance of

marri with heavy, mixed fuel understory and a total fuel loading of 14 tonnes per hectare. The fire carried through with an approximate burn height of five meters and only large logs and standing trees remained. Ahead of the fire front (Figure 3.5), dense thick smoke was generated which partially cleared in the smouldering phase. The fine fuels and duff were completely combusted in the flaming fire front and only large branches and logs continued to smoulder.

Figure 3.5 Smoke sampling at the DPW's Bodega block near Margaret River.

The smoke from a prescribed burn of wild oats was sampled in April, 2010 in the paddock used for the collection of the wild oats (Figure 3.6). The site contained a high predominance of wild oats with some unidentified weed species interspersed. The paddock was ignited in the most upwind point and the fire was allowed to carry for thirty meters before sampling began.

Figure 3.6 Smoke sampling of wild oats paddock.

The passage of the fire front completely consumed the wild oats and left very little other unburnt matter. Visually, the smoke generated ahead of the fire front was much cleaner compared to the marri and karri prescribed burns, suggesting the grassland fire generated lower amounts of partially pyrolysed fuel. Low density smoke was generated during the smouldering phase after the fire front had passed with very little residual combustion.

Prescribed burning of karri fuel was sampled at the Department of Parks and Wildlife's Leeuwin Block on the Leeuwin ridge near Augusta (34º 19'S, 115º 07'E) in May 2011. The block contained a high fuel loading in excess of 20 tonnes per hectare and a large variety of understory species with thick accumulations of karri leaf, bark and twigs on the forest floor. The smoke density ahead of the fire front was visually dense (Figure 3.7) and remained dense during the smouldering phase. Large sections of partially combusted duff, twigs and logs remained unburnt and continued to smoulder for a long period of time. Despite there being large amounts of partially combusted fuels

remaining, several sections of the forest crowned (the fire carried through the tree canopy) around the sampling point, a sign usually associated with intense fire activity.

Figure 3.7 Smoke sampling of karri forest.

In the prescribed burning sampling events, the samples were drawn through a series of three Tenax-TA TD tubes as described above at a rate of 200ml/min with an SKC 222-3 personal air sampler (SKC, Eighty Four, Pennsylvania, USA). The sample pump was held in front of the flaming fire front for approximately one minute before the fire front passed over the sampler holding the sample pump and Tenax-TA tubes at a height of one meter. The total sample time for each prescribed burn was thirty minutes.

The smoke sampling Tenax-TA tubes were analysed by thermal desorption gas chromatography mass spectrometry (TD GC-MS) on an HP 6890A Gas Chromatograph interfaced to an HP 5973A Mass Selective Detector and Unity 2 single tube, two stage desorption unit as described in Vitzthum von Eckstaedt et al. (2011) and Bates et al. (2008). The Tenax-TA tubes were thermally desorbed at 300 ºC for five minutes (first

stage desorption) and the desorbed samples were transferred in a helium gas stream (>25 ml/min) to a cold trap (10 ºC) to refocus the samples. The refocusing cold trap was subsequently heated at 100 ºC/sec to 300 ºC and held isothermally for 1 min (second stage desorption). The samples desorbed from the refocusing trap were transferred, in a helium stream at 1ml/min, to the GC via a deactivated fused silica capillary transfer line that was maintained at 120 ºC. Samples were run on a 60 m x 0.25 mm x 0.25 μm Agilent DB5-MS column with a helium carrier gas in constant flow mode at 1.1 ml/min. The GC was set at constant pressure and run in splitless mode. All sample splits were carried out at the desorption stage. The DB-5MS column was run with an initial temperature of 40 ºC ramped at 4 ºC/min to 300 ºC and held isothermally for 10 min. The lignin-derived phenols were identified by comparing their mass spectra with the NIST and/or Wiley spectral libraries or by comparison to reported spectra in literature (Ralph & Hatfield, 1991). Individual lignin phenols were quantified by their relative percentage area of the total ion chromatograms.

3.2.7 Statistical analysis

Data were analysed using SPSS 20 (IBM® SPSS® Statistics). Reported treatment effects are significant at $p < 0.05$.

3.3 Results

3.3.1 Lignocellulosic composition, essential oil and fuel moisture content

The cellulose contents of all fuels varied over a relatively narrow range (24 to 29%) except in oat for which cellulose made up about half of the fuel mass (Table 3.2). The hemicellulose contents of the tree fuels (hardwood or softwood) were also similar averaging at 8.6%, which was substantially lower than the 28% for oats fuels. The lignin content of the five vegetation fuel types fell into three distinct groupings. The oats fuel at 7.8% had the lowest lignin content, the three hardwoods with an average of 26% were intermediate and the softwood pine fuel, with a lignin concentration nearly six times that of oats, had the highest level of 44.5%. (Table 3.2). The essential oil content of the *Eucalypt* karri was more than double that of the *Eucalypt* jarrah fuel (9.3 mL/kg versus

4.6mL/kg respectively) and comparable to the marri fuel (9.1 mL/kg) (Table 3.3). The oats and pine fuels contained less than 0.5 mL/kg essential oil. The moisture content of the tree fuels was between 7.9 and 8.7% and the cured oats fuel less than half at 3.8%.

Table 3.2 Lignocellulosic compositions, moisture and ash content (percentage) of the fuels used for smoke generation. Data are mean ± 1 standard error, (n = 3).

Fuel	Cellulose	Hemicellulose	Lignin	Moisture Content	Ash
Jarrah	24.2 ±0.4	6.1 ±0.2	23.5 ±0.1	8.4 ±0.1	1.7 ±0.1
Karri	28.4 ±0.1	8.1 ±0.2	29.3 ±0.3	8.7 ±0.1	4.0 ±0.1
Marri	29.4 ±0.7	9.0 ±0.2	24.9 ±0.2	7.9 ±0.1	8.3 ±0.4
Oats	48.9 ±0.1	28.0 ±0.3	7.8 ±0.2	3.8 ±0.1	2.5 ±0.2
Pine	23.7 ±0.4	9.4 ±0.2	44.5 ±0.4	8.5 ±0.1	1.8 ±0.7

Table 3.3 Essential oil content (mL/kg) of fuels used for smoke generation. Data are mean ± 1 standard error, (n = 3).

Fuel	Essential Oil
Jarrah	4.6 ±0.1
Karri	9.3 ±0.1
Marri	9.1 ±0.1
Oats	< 0.5
Pine	< 0.5

3.3.2 Fuel lignin composition

The lignin pyrolysis products of fuels from the *Py* GC-MS analysis are shown in Table 3.4. All the angiosperm fuels (the three hardwoods and oats) contained lignin pyrolysates from all three lignin units (Table 3.4). As expected, the pine fuel contained no syringyl products. For the pine and oat fuels, 70-80% of the total lignin products pyrogram peak area was contributed by guaiacyl derivatives. For the hardwood fuels, ≥ 80% of the lignin pyrolysis products were of syringyl and guaiacyl phenols.

Table 3.4 Lignin composition of fuels based on *Py* GC-MS analysis.
Fuel samples were analysed in triplicates; nq, not quantified, nd not detected.
Data are mean ± 1 standard error, (n = 3).

Lignin unit	Lignin pyrolysates	Relative abundance (% of total lignin-derived pyrolysates) by fuel type				
		Jarrah	Karri	Marri	Oats	Pine
p-Hydroxyphenyls	Phenol	5.9	8.7	3.4	4.6	6.8
	o-Cresol	2.4	1.7	0.8	1.1	3.2
	m- and *p*-Cresol	8.1	3.1	2.4	1.8	6.9
	2,4-Dimethylphenol	1.2	0.8	0.5	0.3	1.8
	4-Ethylphenol	2.4	1.2	1.0	1.1	2.3
	4-Hydroxybenzaldehyde	nq	nq	nq	0.8	nq
	4-Allylphenol	nq	nq	nq	0.5	0.8
	Subtotal	20.0±0.2	15.5±0.6	8.1±0.5	10.2±0.2	21.8±0.1
Guaiacyls	Guaiacol	3.0	3.7	9.4	9.4	6.4
	4-Methylguaiacol	3.6	4.9	4.2	3.1	11.9
	4-Ethylguaiacol	4.1	1.4	0.9	3.1	3.1
	4-Vinylguaiacol	14.0	10.2	12.7	41.3	26.9
	Eugenol	1.0	0.8	1.0	1.3	2.6
	4-Propylguaiacol	nq	nq	nq	nq	0.8
	Vanillin	2.4	2.0	2.8	3.9	4.6
	trans Isoeugenol	1.2	1.3	1.2	0.4	1.9
	cis Isoeugenol	5.5	2.4	7.8	5.5	12.7
	Homovanillyl Alcohol	nq	nq	nq	0.8	nq
	Acetovanillone	2.2	3.0	1.7	1.2	1.5
	Homovanillic Acid	2.0	3.1	nq	nq	5.8
	Subtotal	39.0±0.7	32.8±0.6	41.7±2.0	70.0±0.6	78.2±0.1
Syringyls	Syringol	8.0	6.6	5.0	6.7	nd
	4-Vinylsyringol	11.6	15.2	18.6	4.7	nd
	4-(2-Propenyl)-syringol	1.8	2.4	3.0	0.8	nd
	Z-4-(1-Propenyl)-syringol	1.8	1.7	1.7	0.5	nd
	Syringaldehyde	4.0	5.2	4.1	1.2	nd
	E-4-(1-Propenyl)-syringol	10.4	14.3	15.1	3.1	nd
	Acetosyringone	3.4	6.3	2.7	2.4	nd
	3,5-Dimethoxy-4-hydroxycinnamaldehyde	nq	nq	nq	0.4	nd
	Subtotal	41.0±0.9	51.7±0.9	50.2±0.9	19.8±0.6	nd
	Total	100.0	100.0	100.0	100.0	100.0

Of the *p*-hydroxyphenyl derivatives, phenol and the three isomers of cresol were the dominant pyrolysis products (accounting for 75-87% of peak areas), irrespective of fuel type (Table 3.4). The balance was mainly made up by dimethylphenols and 4-ethylphenol. With respect to the guaiacyl lignin products, 4-vinylguaiacol was the single most abundant pyrolysate in all the five fuel types, and particularly so in wild oats and pine fuels. Other pyrolysates with high relative abundance included guaiacol, 4-methylguaiacol and 4-ethylguaiacol, vanillin, *cis*-isoeugenol and acetovanillone (Table 3.4). For the angiosperm fuels, the relative abundances of the syringyl-derived pyrolysates were broadly comparable among the hardwood trees. However, in the wild oats, the relative abundances of the syringyl-derived products, except syringol, were consistently less than those of the hardwoods.

A canonical variate analysis (Figure 3.8) and a cluster analysis (Figure 3.9) (Chatfield & Collins 1980) of the *Py* GC-MS data show distinct groupings of the fuels with respect to their lignin composition. The fuels are grouped into three broadly different groups representing grass, pine and the three hardwood trees based on similarity of their lignin makeup.

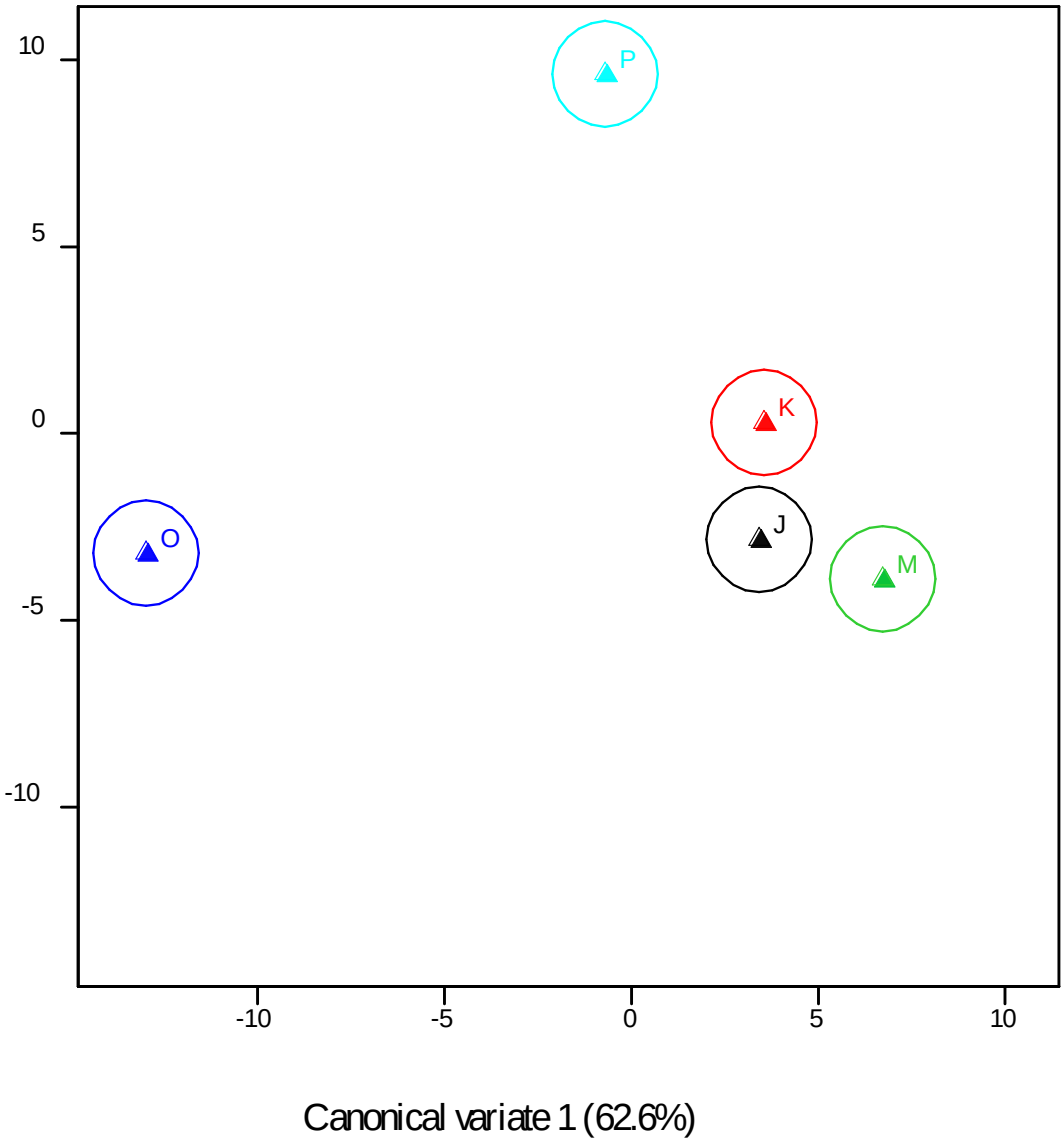


Figure 3.8 Canonical variate analysis of *Py* GC-MS data showing distinct grouping of fuels with respect to their lignin composition. Fuel types are: O, wild oats; P, pine; K, karri; J, jarrah; and M, marri. The circles around each fuel are the 95% confidence regions.

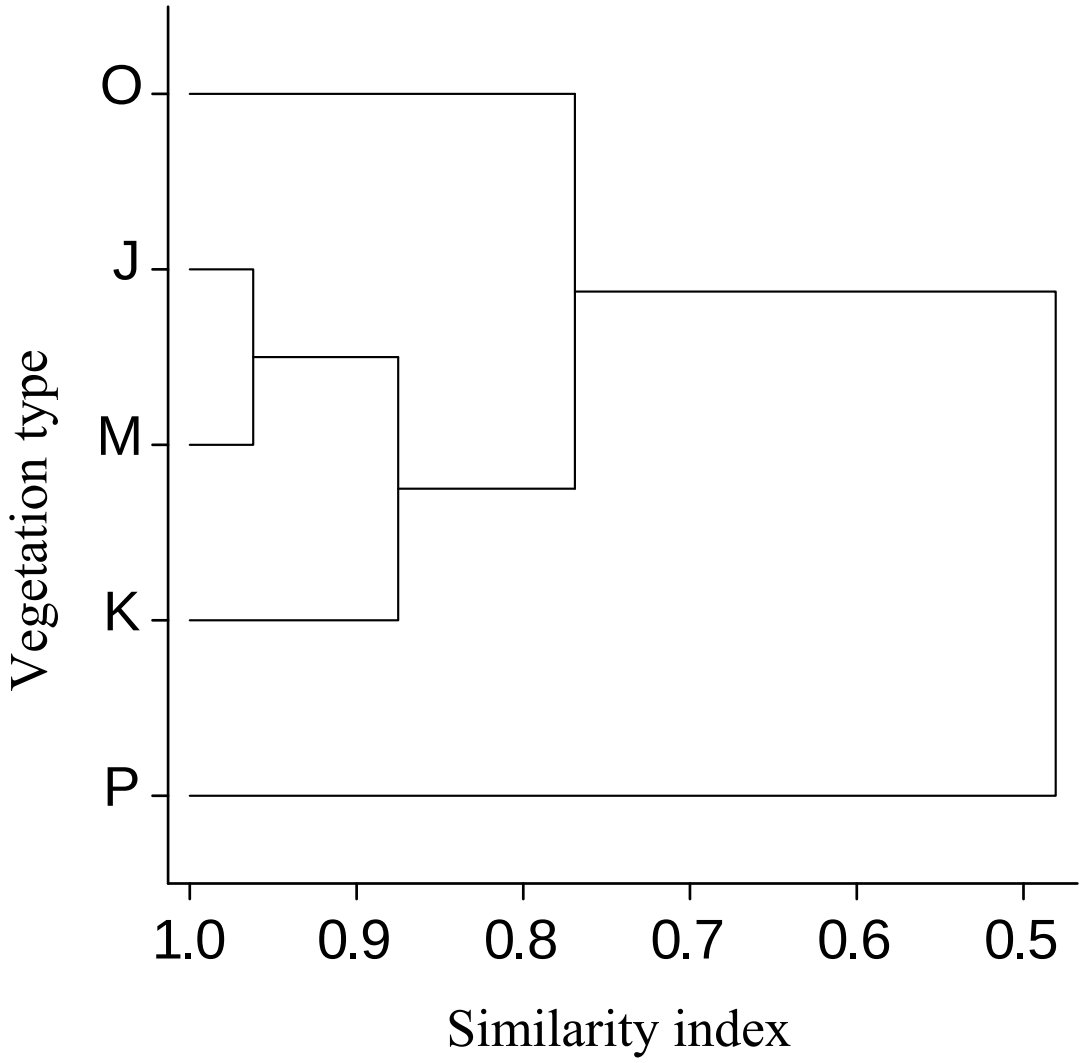


Figure 3.9 Cluster analysis of fuel lignin composition showing the fuels as falling into three distinct groups based on similarity of their lignin makeup.

3.3.3 Volatile phenols in smoke emissions

The sampling of smoke emissions used a train of three Tenax TA absorption tubes in series to entrap compounds desorbed from the primary tube into the two breakthrough tubes during the sampling procedure. Thermal desorptions of the second and third tubes from all of the vineyard and prescribed samples showed there were no phenols breaking through from the primary tube. Smoke emissions sampled from the pyrolysis of five fuels during the vine smoke exposure experiment as well as from prescribed burns of three of these vegetation types (marri, karri and wild oats) showed a range of lignin pyrolysates (Table 3.5).

Table 3.5 Volatile phenols in smoke emissions from various fuel types.

Data are based on triplicate sample analysis; nd, not detected, nq, not quantified.

		Relative abundance of lignin pyrolysates in smoke emissions from							
		vineyard smoke exposure experiments					prescribed burns		
Lignin units	Compounds in smoke	Jarrah	Karri	Marri	Oats	Pine	Karri	Marri	Oats
	Phenol	54.53	48.69	45.82	56.73	33.44	35.14	14.80	34.23
	o-Cresol	9.96	8.99	9.00	11.79	7.51	6.73	3.06	5.37
	m and *p*-Cresol	17.44	23.45	9.16	13.90	14.38	11.93	13.27	7.38
	2,4-Dimethylphenol	1.59	1.42	1.33	1.56	2.89	4.39	1.02	1.34
	3,5-Dimethylphenol	nq	nq	1.23	0.44	1.28	nd	nq	0.67
p-Hydroxyphenyls	2-Ethylphenol	nq	0.52	0.75	nq	1.47	nd	nq	nq
	4-Ethylphenol	3.68	1.80	2.40	2.67	4.76	2.93	3.06	4.70
	4-Vinylphenol	nd	nd	nd	nd	8.66	nd	nd	9.40
	4-Allylphenol	nd	nd	nd	nd	0.78	nd	nd	nd
	Subtotal	87.19	84.87	69.69	87.10	75.17	61.13	35.20	63.09
	Guaiacol	6.40	7.34	12.31	6.23	8.43	22.55	11.73	8.72
	4-Methylguaiacol	2.54	3.15	5.81	3.11	4.81	7.98	9.69	4.70
	4-Ethylguaiacol	1.78	2.55	4.90	1.45	2.43	5.42	6.63	2.01
Guaiacyls	4-Vinylguaiacol	0.25	0.45	2.40	0.67	3.99	1.54	13.27	8.72
	Eugenol	0.19	0.22	1.23	0.11	1.60	nd	1.02	0.67
	Vanillin	0.63	0.37	0.21	0.33	0.69	nd	nd	4.03
	4-Propylguaiacol	0.19	0.15	0.75	nd	1.19	nd	nd	nd
	cis- and trans- Isoeugenol	nq	nd	nd	0.22	1.47	nd	nd	1.34
	Acetovanillone	nd	nd	nd	nd	0.23	nd	nd	nd
	Subtotal	11.98	14.23	27.60	12.12	24.83	37.48	42.35	30.20
Syringyls	Syringol	0.82	0.90	2.72	0.78	nd	1.39	22.45	6.04
	4-Methylsyringol	nd	nd	nd	nd	nd	nd	nd	0.67
	Subtotal	0.82	0.90	2.72	0.78	nd	1.39	22.45	6.71

The smoke generated from each of the five fuel types during the vineyard smoke exposure experiment contained relatively high levels of phenol, averaging approximately 8% of the total absorbed fuel emissions (data not shown). Overall, phenol, *o*-cresol, *m*-cresol and *p*-cresol accounted for approximately 82% of the total phenols in the jarrah, karri and oats fuels emissions, 64% in marri fuel emissions and 55% in the pine fuel emissions. The *p*-hydroxyphenyl derivatives were the dominant phenols in the vineyard trials, accounting for at least 69% in marri fuel emissions and up to 87% in jarrah fuel emissions (Table 3.5). The gymnosperm pine emissions differed markedly from the angiosperm emissions in having a high 4-vinylphenol content (8% in pine but not detected in the angiosperm emissions). The guaiacyl derivatives were less dominant, ranging from 12% in jarrah fuel emissions, to 27% in marri fuel emissions. Of the guaiacyl derivatives, the dominant compounds were guaiacol, 4-methylguaiacol, 4-ethylguaiacol, eugenol and vanillin. Syringol was the only significant pyrolysate from the syringyl group in angiosperm smoke samples. No syringol or substituted syringols were detected in the emissions from the pine fuel which contained 75% *p*-hydroxyphenyl derivatives and 25% guaiacyl derivatives (Table 3.5).

The smoke emissions from prescribed burns of wild oats pastures and marri and karri dominated forests all produced similar lignin pyrolysate profiles (Table 3.5) as those obtained in smoke emissions of the respective fuels during the vineyard experiment. The largest difference occurred in there being a lower proportion of *p*-hydroxyphenyl derivatives, particularly in the marri prescribed burning emissions, where syringol was the highest pyrolysate (22%) and the guaiacyl derivatives accounted for 42% (Table 3.5).

The smoke samples were also examined for the essential oil eucalyptol (1,8-cineole, Table 3.6). The karri fuel used in vineyard trials contained over 50% non-degraded leaf matter and the emissions contained a high 1,8-cineole content (8% of total emissions). This was much lower in karri prescribed burning emissions (1.3% of total emissions). By comparison, the vineyard trials of the non *Eucalypt* marri contained only 0.2% 1,8-cineole and there was no 1,8-cineole detected in emissions of oats and pine fuels and marri prescribed burning trials (Table 3.6).

Table 3.6 Essential oil 1,8-cineole in smoke emissions from various fuel types.

Data are based on triplicate sample analyses; nd, not detected.

	Relative abundance (%) of 1,8-cineole in smoke emissions							
	vineyard smoke exposure experiment					prescribed burns		
	Jarrah	Karri	Marri	Oats	Pine	Karri	Marri	Oats
1,8-Cineole	1.2	8.0	0.2	nd	nd	1.3	nd	nd

3.4 Discussion

3.4.1 Lignocellulosic compositions of the fuels used for smoke generation

In this study, taxonomically distinct groups of vegetation fuels with different lignin composition were used to generate smoke, under conditions that reproduce bushfire pyrolysis temperature profiles, for fumigating grapevines at the start of grape ripening. The primary aim was to examine the influence of the lignin makeup of vegetation on lignin pyrolysis products (potential smoke taint compounds) that accrue in wine as a result of exposure of grapes to smoke. The behaviour of a bushfire event is largely determined by a range of factors, including fuel structure and fuel properties (Gould et al. 2007) and all components of vegetation, including partially decomposed litter from the soil surface, contribute to the resulting smoke. Each of the fuels used here was compiled in proportion to components of the respective vegetation fuel types that are pyrolysed during a decadal bushfire event - the typical forest fire management burn cycle in the Margaret River wine region. In fuels comprised variously of dried fresh matter and decomposed components, it is pertinent that the fuels retain at least compositional variation characteristic of the vegetation types used. In this respect, the holocellulose components of fuels showed the expected dichotomy. The tree fuels, whether hardwood or softwood, had low concentrations of cellulose and hemicelluloses,

whereas wild oats had high levels of carbohydrate fraction. In terms of the total lignin concentrations - the prime source of putative smoke taint compounds - the fuel sources fell into three distinct levels: low (oat), medium (hardwoods) and high (softwood), consistent with the broad ranking of these vegetation types (Lebo et al. 2001). The radiata pine fuel had a substantially higher lignin concentration than expected due to the high (90%) contribution of needles, which have lignin concentrations in the range of 37% (in freshly senesced needles) to greater than 50% (in old, partially decomposed needles) (Girisha et al. 2003).

Previous studies (Kennison et al. 2008, Sheppard et al. 2009) have made comparisons of putative lignin-derived smoke taint phenols in wines made from smoke exposed fruit in vineyard trials without controlling or measuring the mass of fuel pyrolysed. In standardising the fuel mass pyrolysed in the vineyard trials, this study was able to investigate the effect lignin composition has on the emission of lignin-derived phenols. One kilogram of fuel was pyrolysed in each vineyard exposure, with the mass of lignin pyrolysed ranging from a low of 78 g in the wild oats replicates to a high of 445 g in the pine replicates.

3.4.2 Fuel lignin composition

A detailed analysis of the lignin composition of the five fuel types, by Pyrolysis Gas Chromatography-Mass Spectrometry (*Py* GC-MS), indicated that while the angiosperms (hardwood trees and the wild oats fuels) contained lignin pyrolysis products from all three lignin units, the gymnosperm softwood contained only guaiacyl and *p*-hydroxyphenyl units (Table 3.4). The hardwood angiosperms were dominant in syringyl and guaiacyl derivatives whereas the angiosperm grass had a high predominance of guaiacyl units. Guaiacol and substituted guaiacols were the principal components of the gymnosperm pine fuel lignin. As expected the gymnosperm contained no syringyl derivatives (Kjällstrand et al. 2000, Nolte et al. 2001, Greenwood et al. 2002, Pettersen 1984), although trace levels are sometimes reported (*e.g.*, Kristensen et al. 2009) in spite of the general absence of the requisite syringyl lignin

biosynthetic enzymes (Weng &Chapple 2010). Despite the inclusion of partially degraded components in the fuel samples, the relative proportions of the lignin derivatives were still distinct enough to characterise the fuels into the three (grass, hardwood and softwood) lignin types (Higuchi 1990, Pettersen 1984, Buranov & Mazza 2008) (Table 3.4, Figure 3.8 and Figure 3.9).

3.4.3 Volatile phenols in smoke emissions

Smoke emissions from the vineyard smoke exposure experiment and the prescribed burns of three of the vegetation types contained phenol, guaiacol and several of their substituted forms as well as syringol and trace levels of 4-methylsyringol. Emissions from the radiata pine fuel contained no syringyl derivatives consistent with the *Py* GC-MS results presented here and with earlier reports that members of the gymnosperm taxa lack syringyl lignin (Kjällstrand et al. 2000, Nolte et al. 2001, Schauer et al. 2001, Bari et al. 2009), although some exceptions have been noted (*e.g.*, Fine et al. 2001). Volatile phenol emission profiles from the prescribed burns and from smoke generated during vineyard experiments were broadly similar, indicating that (1) the pyrolysis conditions during the vineyard smoke exposure successfully simulated bushfire/prescribed burn conditions, and (2), as a result, the vines and grapes described subsequently in the following chapters were exposed to the types of volatile phenols that would occur in bushfire and/or prescribed burn smoke. The differences between the emissions generated in the vineyard trials and prescribed burns is likely attributable to the presence of other species as understory. Although the emissions profiles of volatile phenols from the prescribed burns and vineyard experiment were similar in this study (*c.f.*, Table 3.5), the relative abundances, especially of syringyl and guaiacyl derivatives, were lower than those reported from prescribed burn emissions in the Adelaide Hills, South Australia (Hayasaka et al. 2010a). However, in the latter case, sampling was carried out in winter when conditions are likely to favour smouldering, which increases the yield of these methoxyphenols (Kjällstrand et al. 2000). A key difference in sampling to the work reported by Hayasaka (2010a) is the sampling proximity and, therefore, age of the emissions. Both the vineyard and prescribed burning emissions in this study were

sampled at the source of combustion. As the composition of smoke in the atmosphere changes over time due to a number of factors including temperature (Kamens et al. 1988), sunlight, ozone and nitrous oxide concentration (Kamens, et al. 1985), the high relative concentrations of phenol and cresols found in this study may be due to the emissions being sampled before changes could occur.

Lignin pyrolysate compositions of fuels from the *Py* GC-MS analyses and those from TD GC-MS analyses of smoke emissions indicated broadly comparable results for the quantitatively significant lignin derivatives (*cf*. Tables 3.4 and 3.5). For example, in fuels and their smoke emissions, the same five compounds dominated the total pool of *p*-hydroxyphenyl derivatives (*cf*. Tables 3.4 and 3.5). The guaiacyl lignin derivatives were also similar between the *Py* GC-MS of fuels and TD GC-MS of the smoke samples. However, while high levels of *cis*-, *trans*-isoeugenol and acetovanillone were observed from *Py* GC-MS analysis, only trace levels were found in smoke emissions. The most noticeable difference between the *Py* GC-MS fuels and TD GC-MS of smoke samples occurred in the syringyl products (*cf*. Tables 3.4 and 3.5). From the syringyl group, only syringol was present at quantifiable levels from the vineyard as well as prescribed burn smoke emissions whereas the *Py* GC-MS of fuel samples showed six additional syringyl derivatives at substantial relative abundance levels.

CHAPTER 4

THE EFFECT OF SMOKE EXPOSURE ON THE ACCUMULATION OF LIGNIN-DERIVED PUTATIVE SMOKE TAINT COMPOUNDS IN WINE

4.1 Introduction

The pyrolysis products of lignin are closely related structurally to the monolignol makeup of the biomass fuel source (Simoneit et al. 1993). The pyrolysis products are phenols and phenolic ethers, typically the homologs and derivatives of phenol, guaiacol and syringol with substituent groups almost exclusively in the para position to the phenolic hydroxyl group (Goos 1952). Nolte et al. (2001) have identified 34 phenols present in smoke from the pyrolysis of angiosperm fuels and Hayasaka et al. (2010a) have identified eight lignin-derived volatile and glycoconjugated phenols as putative taint compounds in fruit exposed to bushfire smoke. The 34 lignin-derived phenols identified in smoke by Nolte et al. (2001) were considered possible putative smoke taint compounds in wine for this study. Singh et al. (2011) quantified volatile and glycoconjugated guaiacol and 4-methylguaiacol in wines where the bound phenols were released after acid hydrolysis. This method was expanded to include quantification of 19 volatile phenols and their glycoconjugates (Singh et al. 2012, Appendix 1).

In the work reported here, a controlled and replicated smoke generation experiment was carried out using vegetation fuel sources that differed in their lignin makeup. The aim was to expose fruit-bearing mature Merlot vines to the resultant smoke and to examine the accretion of putative smoke taint compounds in wines in relation to the lignin makeup of the fuels as well as the smoke emissions. For this purpose, five distinct vegetation types with varying lignin composition were used. Each fuel type was (1) reconstituted in proportion to biomass components that burn in a decadal fire event and (2) pyrolysed under conditions that reproduce wildfire temperature profiles (Gould et al. 2007) as described in Chapter 3.

The putative smoke taint analysis of merlot wines made from control and smoke exposed vines and the correlations of phenol concentrations in wines to lignin composition, harvest and vine size assessments are described here.

4.2 Materials and methods

4.2.1 Vineyard trials

The smoke exposure experiment was set up as a completely randomised block design in a commercial vineyard containing 10 years old *Vitis vinifera* L. cv. Merlot vines. To minimise variability in experimental units within a block, each block was carefully selected for vines of uniform canopy size and crop load. The treatments, for smoke generation and exposure, consisted of the five vegetation fuel types described above plus a control (i.e., vines not exposed to smoke). Within each block, the treatments plus the control were randomly allocated to experimental units. Each experimental unit consisted of a panel of five vines. Experimental units were separated by at least two panels of vines to avoid smoke cross contamination. Each block containing the full treatment structure was replicated five times, thus there were a total of 30 experimental units. Smoke exposure of the experimental vines was carried out 14 days post-veraison (EL 36), between the hours of 9am and 3pm, in a purpose built tent as described by Kennison et al. (2008). The 63 m^3 tent (6m long, 3.5m wide by 3m high) was made of galvanised steel sections to hold an outer shell of Solarweave (Gale Pacific, Braeside, Victoria, Australia) greenhouse plastic with the end sections split and sealed around the vertical shoot positioned trellis wire. For smoke generation, 1 kg of fuel sample was combusted inside the pyrolysis chamber (Figure 3.3) and the resulting smoke delivered via the 6m flexible steel tube. Two electric fans were used to evenly distribute smoke during the exposure. Each smoke exposure event lasted 30 min. The smoke density, defined as an obscuration by particulate matter 2.5 μm or less ($PM_{2.5}$), was recorded for the entire duration of each smoke exposure event using a VESDA Laser Focus VLF-250 nephelometer (Xtralis, Mawson Lakes, South Australia). In each case, obscuration exceeded the instrument's maximum reading of 32%. Control vines were similarly enclosed for the same duration to minimise differences in environmental conditions between smoke treated and control vines.

Figure 4.1 Vineyard trial with Solarweave tent and pyrolysis chamber.

Figure 4.2 Vineyard trial with Solarweave tent and pyrolysis chamber.

Figure 4.3 Vineyard trial with smoke inlet during a non-smoked control replicate.

4.2.2 Harvest and vine size assessments

The fruit from each replicate panel was harvested separately at commercial maturity, total soluble solids ~ 23°Brix (Reichert AR 200 digital refractometer, Reichert Inc., New York, U.S.A.), six weeks after smoking treatments. The mass of fruit from each replicate (A&D FG-30KB , A&D Ltd.,Toshima ku, Japan), the number of bunches and the mass of 200 randomly selected berries were determined (Mettler Toledo AB 204-S, Mettler Toledo GmbH, Greifensee, Switzerland). Leaf area per panel was estimated from the product of average leaf area per cane and the total number of canes per panel, where the total number of canes was recorded for each replicate and the leaves from two replicate canes from each vine were removed and counted. The mass of each cane's leaves were immediately measured and the total leaf area for each cane calculated from the average mass of fifty randomly selected 16 cm^2 leaf disk cuttings. The total replicate leaf area was calculated from a multiplication of the replicates average leaf area per cane and the total number of replicate canes.

4.2.3 Winemaking

The fruit from each replicate was kept separate and the wine made individually for each of the 30 replicates. Each lot was crushed and de-stemmed with a 100 ppm potassium metabisulphite (Chem Supply AR grade, Gillman S.A. Australia) addition and the total acids adjusted to 7.0 g/l with the addition of tartaric acid (Sigma- Aldrich, Sydney, Australia). The must was inoculated with 300 mg/l Saccharomyces cerevisiae EC1118 (Lallemand Inc., Montreal, Canada) and 100 mg/l diammonium phosphate (Sigma-Aldrich, Sydney, Australia) added as a ferment nitrogen supplement. Each replicate was fermented on skins in open neck 25 litre glass demijohns and hand plunged at 8 hour intervals. The specific gravity and temperature were recorded every twelve hours. At 3 °Brix (~8 days) the must was pressed off skins and each replicate fermented to dryness (<1g/l residual sugars) in closed 15 litre demijohns (16 days). Each replicate was racked from gross lees and inoculated with *Oenococcus oeni* (Viniflora CH 16, CHR Hansen, Denmark) at 10 mg/l to initiate malolactic conversion. The replicates were kept at 23°C until malic acid stability was reached (<0.1 g/l malic acid, 19-60 days), sulphured with the addition of 60 mg/l potassium metabisulphite, cold stabilised at - 4°C for 21 days, filtered to 0.2 μm (Sartorius Sartopure 2 Maxicap, Sartorius, Gottingen, Germany) and bottled under food grade nitrogen with stelvin closures. The final alcohol, volatile acidity (acetic acid) and malic acid concentration was measured by FTIR (Oeno Foss Type 4101, Foss, Hillerød, Denmark).

4.2.4 Volatile and glycoconjugated putative smoke taint compounds in wine

Lignin-derived putative smoke taint (volatile and glycoconjugated) compounds in wines were extracted and analysed using GC-MS as detailed in Singh et al. (2012, Appendix1). In this analysis, the glycoconjugated lignin pyrolysis products in wine refers to values determined after strong acid hydrolysis.

4.2.5 Wine metals analysis

Triplicate samples of each wine replicate were heated to 150 ^{o}C for one hour to remove the ethanol and adjusted to pH 2 with nitric acid (Ajax Chemicals, AR Grade). The

samples were analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) with a Perkin Elmer IC-PMS ELAN 9000 (Perkin Elmer, Shelton, Connecticut, U.S.A.) and ICP-OES Optima 5300DV (Perkin Elmer, Shelton, Connecticut, U.S.A.).

4.2.6 Statistical analysis

Data were analysed using SPSS 20 (IBM® SPSS® Statistics). Reported treatment effects are significant at $p < 0.05$.

4.3 Results

4.3.1 Putative smoke taint compounds in wines

4.3.1.1 Effect of smoke exposure on total (volatile plus glycoconjugated) lignin-derived smoke taint compounds in wines

Smoke exposure of vines 14 days after the onset of grape ripening caused significant increases in the total (volatile plus glycoconjugated) concentrations of lignin-derived compounds in wines. This increase in total concentrations of lignin-derived compounds was fuel type dependent and ranged from 74% (in the karri smoke treatment) to 146% (in the wild oats smoke treatment) (Table 4.1). Wines from unsmoked, control grapes contained a relatively high background level (311 μg/L) of total (volatile plus glycoconjugated) lignin degradation products (Table 4.1). Significant increases also occurred across all three lignin type derivatives, however the average magnitude of increases differed in the following order: guaiacyl derivatives (362%) > *p*-hydroxyphenyl derivatives (221%) > syringyl derivatives (72%) (Table 4.1). Irrespective of fuel type or smoke exposure, syringyl derivatives dominated the total pool of lignin-derived compounds, accounting for between 54 and 78% (Table 4.1). The remainder was approximately equally apportioned between the *p*-hydroxyphenyl and guaiacyl groups of compounds in oat and pine smoke-affected wines, while in the control and hardwood smoke affected wines, the *p*-hydroxyphenyl derived compounds accounted for a consistently higher proportion than the guaiacyl derivatives.

Table 4.1 Effects of smoke exposure and fuel type on levels (μg/l) of total phenols (sum of volatile and glycoconjugated phenols) in wines. Data shown are ± 1 standard error (n=5); nd, not detected. [1] Excludes Vanillin and Acetovanillone.

		Treatment					
Lignin unit	Putative taint	Control	Jarrah	Karri	Marri	Wild Oats	Pine
p-Hydroxyphenyls	Phenol	5.2±0.2	29.1±1.0	20.0±1.0	40.1±2.4	46.1±1.4	35.6±2.1
	o-Cresol	8.9±0.3	21.8±0.4	16.2±0.9	32.3±1.0	34.4±1.2	26.9±1.7
	m-Cresol	6.3±0.3	21.4±0.6	18.6±0.8	32.6±1.5	31.2±0.7	30.2±1.5
	p-Cresol	9.3±0.3	22.4±0.5	19.2±0.7	34.1±1.5	32.4±0.5	31.3±1.7
	4-Ethylphenol	12.4±0.6	20.7±1.1	17.7±0.7	30.5±1.3	29.4±0.8	22.8±0.7
	Subtotal	**42.1±1.8**	**115.4±1.3**	**91.7±2.7**	**169.6±7.3**	**173.5±3.4**	**146.8±7.4**
Guaiacyls	Guaiacol	19.2±0.5	54.7±2.9	44.5±1.4	69.6±2.9	125.1±1.4	84.4±6.8
	4-Methylguaiacol	8.1±0.9	29.4±2.4	18.4±1.0	35.2±2.6	33.9±1.9	58.3±7.4
	4-Ethylguaiacol	nq	1.9±0.1	2.0±0.1	5.9±0.2	6.2±0.2	4.9±0.5
	4-Propylguaiacol	nq	1.2±0.1	5.1±0.7	7.6±0.2	5.8±0.5	4.3±0.2
	4-Vinylguaiacol	nq	6.3±0.4	6.7±0.4	8.1±0.5	6.9±0.9	5.9±0.3
	Vanillin	252.2±19.4	221.8±33.6	270.6±18.8	209.0±14.2	177.6±13.4	166.2±9.6
	Acetovanillone	276.0±8.6	328.2±18.4	339.2±9.2	302.0±4.0	329.3±12.5	322.7±13.3
	Subtotal[1]	**27.3±0.8**	**93.5±4.2**	**76.7±1.3**	**126.4±4.5**	**177.9±2.0**	**157.8±14.3**
Syringyls	Syringol	82.2±1.3	168.0±18.1	133.7±9.8	153.1±15.2	154.4±8.6	129.9±5.9
	4-Methylsyringol	nq	8.4±0.6	8.8±0.5	13.1±0.8	7.9±0.4	6.8±0.2
	Syringaldehyde	48.8±2.0	67.3±8.5	86.2±2.3	54.5±2.9	47.9±2.4	52.3±2.7
	Acetosyringone	110.5±9.1	205.4±15.6	143.2±15.0	213.4±19.7	203.0±19.4	217.3±11.0
	Subtotal	**241.5±11.0**	**449.1±42.7**	**371.9±27.4**	**434.1±36.0**	**413.2±23.1**	**406.3±10.2**
	Total lignin-derivatives[1]	**311.2±11.1**	**658.0±44.2**	**540.1±29.6**	**731.3±45.0**	**764.6±23.7**	**711.0±27.5**

The smoke-derived compounds in wines showed no correspondence with the lignin composition of the pyrolysed fuels. Wines from grapes exposed to smoke of the pine fuel, the lignin of which contains no syringyl moieties or detectable syringyl derivatives in its smoke, unexpectedly contained high levels of syringyl products (Table 4.1). Indeed, smoke of gymnosperm and angiosperm fuels elicited equivalent levels of total syringyl derivatives in wines.

Of the *p*-hydroxyphenyl lignin derivatives, phenol, *o*-cresol, *m*-cresol, *p*-cresol and 4-ethylphenol were consistently present in both smoke affected and unaffected wines. However, in smoke affected wines all these compounds were significantly elevated (Table 4.1). While phenol was generally the single largest component of the *p*-hydroxyphenyl derivatives, the combined cresols (volatile plus glycoconjugated *o*-cresol, *m*-cresol and *p*-cresol) dominated (67-75%) the total *p*-hydroxyphenyl pool (Table 4.1).

Of the guaiacyl group, vanillin and acetovanillone were present at considerably higher total (volatile plus glycoconjugated) concentrations than any other guaiacyl derived phenol. Nonetheless, the levels of these two compounds were independent of smoke exposure (Table 4.1). The guaiacyl derivatives that increased due to smoke exposure were guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-propylguaiacol and 4-vinylguaiacol (Table 4.1). Of the total pool of five guaiacyl derivatives that responded to smoke exposure, guaiacol was by far the largest (54-70%) component with 4-methylguaiacol a distant second with 19-37% contribution (Table 4.1).

Control wines contained moderately high (241 μg/L) total (volatile plus glycoconjugated) concentrations of the syringyl-derived compounds syringol, syringaldehyde and acetosyringone (Table 4.1). In smoke-affected wines, total concentrations of all these syringyl compounds, including 4-methylsyringol, were significantly elevated with the exception of syringaldehyde in pine and wild oats smoke affected wines (Table 4.1). Unexpectedly, wines made from grapes exposed to pine fuel

smoke contained syringol and acetosyringone which respectively were 1.6 and 2 times the levels found in control wines (Table 4.1).

Some of the lignin pyrolysis products that were present in *Py* GC-MS (fuels) and/or TD GC-MS (smoke emissions) were either not detected or present below their detection limits in wines (~ 5 μg/L) (*cf*. Tables 3.4, 3.5 and 4.1).

4.3.1.2 Effect of smoke exposure on glycoconjugated lignin-derived smoke taint compounds in wines

Averaged across fuel types, the total pool of glycoconjugated lignin derivatives was more than 2.8 times the levels found in control wines (Table 4.2). Once again, the largest response to smoke exposure occurred in the total concentration of the guaiacyl derivatives (4.3 fold) followed by *p*-hydroxyphenyl (3 fold) with the least responsive being the total of syringyl products (1.8 fold).

Control wines contained measurable levels (3.6 - 12.4 μg/L) of glycoconjugates of phenol, *o*-cresol, *m*-cresol, *p*-cresol and 4-ethylphenol (Table 4.2). While no additional glycoconjugates of *p*-hydroxyphenyl origin were detected in smoke affected wines, smoke exposure increased the concentrations of these five compounds by up to eight-fold (Table 4.2). The effects of fuel type and/or the smoke exposure treatments on the levels of the glycoconjugated *p*-hydroxyphenyl derivatives closely tracked the effects observed on the total (volatile plus glycoconjugated) *p*-hydroxyphenyl compounds (*cf*. Table 4.1 and 4.2). Thus, karri smoke affected wines, while significantly different from the control wines, contained lower concentrations of each of the glycoconjugated *p*-hydroxyphenyl compounds than were found in wines from grapes exposed to smoke from any of the other four fuels (Table 4.2). Similarly, regardless of fuel type or smoke exposure, the cresol isomers as a group dominated (56-60%) the glycoconjugated pool of *p*-hydroxyphenyl derivatives (Table 4.2).

Table 4.2 Effects of fuel type and smoke exposure on glycoconjugated phenol levels (μg/l) in Merlot wines. Data shown are ± 1 standard error (n=5); nd, not detected.

		Treatments					
Lignin unit	Bound phenols	Control	Jarrah	Karri	Marri	Wild Oats	Pine
p-Hydroxyphenyls	Phenol	3.6±0.2	21.7±0.4	15.4±0.4	29.8±0.6	30.4±0.6	26.3±0.7
	o-Cresol	6.9±0.2	17.1±0.3	13.7±0.4	26.4±0.6	26.2±0.5	21.5±0.7
	m-Cresol	5.5±0.2	17.5±0.4	16.1±0.4	27.2±0.6	25.1±0.4	25.1 ±0.5
	p-Cresol	8.2 ±0.2	18.3±0.4	16.5±0.4	27.7±0.8	25.8±0.3	26.1±0.6
	4-Ethylphenol	12.4±0.6	17.7±0.7	16.0±0.5	26.7±0.6	24.1±0.5	19.2±0.3
	Subtotal	**36.6±2.0**	**92.3±1.8**	**77.7±1.4**	**137.8±4.6**	**131.6±2.2**	**118.2±4.0**
Guaiacyls	Guaiacol	15.1±0.4	38.9±1.2	37.4±0.7	54.3±0.7	87.8 ±0.8	64.3±2.8
	4-Methylguaiacol	7.6 ±0.5	22.7±1.0	14.9±0.4	26.0±0.9	24.9±1.0	46.6±2.9
	4-Ethylguaiacol	nd	1.9±0.1	2.0±0.1	3.1±0.1	3.5±0.1	3.3±0.2
	4-Propylguaiacol	nd	nd	4.0±0.4	5.1±0.2	4.3±0.3	4.3±0.2
	4-Vinylguaiacol	nd	6.3±0.4	6.7±0.4	8.1±0.5	6.9±0.9	5.9±0.3
	Subtotal	**22.7±0.7**	**69.8±3.2**	**65.0±1.4**	**96.6±1.8**	**127.4±1.6**	**124.4±10.5**
Syringyls	Syringol	9.8±0.6	64.1±8.1	34.7±2.6	52.0±4.9	52.7±2.6	26.5±2.5
	4-Methylsyringol	nd	nd	nd	nd	nd	nd
	Syringaldehyde	48.8±2.0	67.3±8.5	86.2±2.3	54.5±2.9	47.9±2.4	52.3±2.7
	Acetosyringone	13.7±0.7	30.6±4.5	24.0±1.6	25.0±1.5	10.9±0.5	9.6±0.8
	Subtotal	**72.3±5.9**	**162.0 ±38.3**	**144.9±10.2**	**131.5±13.1**	**111.5±4.2**	**88.4±9.4**
	Total	**131.6±5.8**	**324.1±25.9**	**287.6±10.6**	**365.9±15.0**	**370.5±10.9**	**331±15.1**

The total concentrations of glycoconjugated guaiacyl derivatives (guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-propylguaiacol and 4-vinylguaiacol) in smoke affected wines were 3 to 5.5 times the level in unsmoked, control wines (Table 4.2). Of the fuel types, the pine and oat fuels yielded significantly higher total concentrations of glycoconjugated guaiacyl derivatives in wines than any of the hardwood fuels.

Generally, smoke exposure also significantly elevated individual glycoconjugated compounds of guaiacyl lignin origin. Quantitatively, however, guaiacol and 4-methylguaiacol were the dominant components, respectively accounting for 56-69% and 20-38% of the total glycoconjugated guaiacyl derived compounds in smoke affected wines. Interestingly, the glycoconjugated 4-methylguaiacol levels of wines from pine smoke exposed grapes were 1.9 times those of the wines affected by the other fuel types. In control wines, glycoconjugated 4-ethylguaiacol, 4-propylguaiacol and 4-vinylguaiacol were below quantitation limits as was 4-propylguaiacol from the jarrah treatment.

Control wines contained substantial background levels (72 μg/L) of total glycoconjugates of syringyl compounds (Table 4.2). Smoke exposure further raised the already high background concentrations, depending on vegetation fuel type, by 22-124% (Table 4.2). However, syringol was the only compound significantly elevated across all fuel types including the pine fuel. Whilst exposure of grapes to smoke of the gymnosperm fuel significantly increased the syringol concentration in wines compared to the control (26.5 *vs* 9.8 μg/L), the effect of the gymnosperm fuel smoke was less than the effect of smoke from the angiosperm fuels, i.e. hardwoods and wild oats (Table 4.2). Interestingly, no glycoconjugated 4-methylsyringol was detected (< 1.5 μg/L) in any of the wines. Glycoconjugated syringaldehyde and acetosyringone were the other syringyl-derivatives affected by smoke. In particular, while wines from the control, pine or wild oats treatments contained comparable levels of these compounds, smoke from the hardwood fuels significantly elevated concentrations of glycoconjugated syringaldehyde and acetosyringone in wines (Table 4.2).

4.3.1.3 Effect of smoke exposure on volatile lignin-derived smoke taint compounds in wines

The total level of volatile lignin derivatives in smoke affected wines was, on average, nearly double the levels found in control wines (Table 4.3). Smoke exposure significantly increased the total concentrations of volatiles in each of the three lignin types, however the relative responses were different. Thus, relative to the respective levels in control wines, the volatile guaiacyl derivatives were the most responsive to smoke exposure (~ 6.5-times), followed by *p*-hydroxyphenyl products (~ 5-fold). The syringyl derivatives were the least responsive (1.7-times) reflecting the high background level of syringyl derivatives in wines. However, irrespective of smoke exposure, the syringyl derivatives were the dominant (77-94%) components of the total pool of volatile phenols in the wines (Table 4.3). The remainder of the total volatile phenols pool was approximately equally contributed by the *p*-hydroxyphenyl and guaiacyl derivatives.

Table 4.3 Effects of fuel type and smoke exposure on volatile phenol levels (μg/L) in Merlot wines. Data shown are ± 1 standard error (n=5); nd, not detected.

		Treatments					
Lignin unit	Volatile phenols	Control	Jarrah	Karri	Marri	Wild Oats	Pine
p-Hydroxyphenyls	Phenol	1.6 ±0.1	7.4 ±0.2	4.6 ±0.2	10.3 ±0.8	15.7 ±0.3	9.3 ±0.6
	o-Cresol	2.0 ±0.1	4.7 ±0.1	2.5 ±0.2	5.9 ±0.2	8.2 ±0.2	5.4 ±0.3
	m-Cresol	0.8 ±0.1	3.9 ±0.2	2.5 ±0.2	5.4 ±0.4	6.1 ±0.2	5.1 ±0.4
	p-Cresol	1.1 ±0.1	4.1 ±0.1	2.7 ±0.1	6.4 ±0.3	6.6 ±0.2	5.2 ±0.4
	4-Ethylphenol	nd	3.0 ±0.1	1.7 ±0.1	3.8 ±0.4	5.3 ±0.1	3.6 ±0.3
	Subtotal	**5.5 ±0.3**	**23.1 ±1.3**	**14.0 ±1.4**	**31.8 ±3.2**	**41.9 ±1.5**	**28.6 ±3.7**
Guaiacyls	Guaiacol	4.1 ±0.2	15.8 ±0.5	7.1 ±0.2	15.3 ±1.2	37.3 ±0.4	20.1 ±0.9
	4-Methylguaiacol	0.5 ±0.1	6.7 ±0.3	3.5 ±0.3	9.2 ±0.6	9.0 ±0.2	11.7 ±1.0
	4-Ethylguaiacol	nd	nd	nd	2.8 ±0.1	2.7 ±0.1	1.6 ±0.1
	4-Propylguaiacol	nd	1.2 ±0.1	1.1 ±0.1	2.5 ±0.1	1.5 ±0.1	nd
	4-Vinylguaiacol	nd	nd	nd	nd	nd	nd
	Subtotal	**4.6 ±0.3**	**23.7 ±1.2**	**11.7 ±0.5**	**29.8 ±2.9**	**50.5 ±0.7**	**33.4 ±3.8**
Syringyls	Syringol	72.4 ±1.0	103.9 ±2.3	99.0 ±3.2	101.1 ±3.7	101.7 ±2.5	103.4 ±1.5
	4-Methylsyringol	nd	8.4 ±0.6	8.8 ±0.5	13.1 ±0.8	7.9 ±0.4	6.8 ±0.2
	Syringaldehyde	nd	nd	nd	nd	nd	nd
	Acetosyringone	96.8 ±5.7	174.8 ±6.9	119.2 ±7.5	188.4 ±10.9	192.1 ±10.6	207.7 ±8.6
	Subtotal	**169.2 ±10.4**	**287.1 ±18.7**	**227.0 ±18.5**	**302.6 ±26.1**	**301.7 ±23.3**	**317.9 ±10.4**
	Total	**179.3±7.1**	**333.9±11.5**	**252.7±12.5**	**364.2±19.6**	**394.1±15.2**	**379.9±14.3**

Of the *p*-hydroxyphenyl derived volatiles, while phenol was the single largest (32-38%) component in wines from smoke exposed grapes, regardless of smoke exposure, the cresols as a group once again dominated (50-70%) (Table 4.3). Of the volatile guaiacyl derivatives, only guaiacol and 4-methylguaiacol were consistently found in quantifiable levels regardless of smoke exposure. As expected, however, the concentrations of these analytes were significantly higher in wines from smoke exposed grapes than from control grapes (Table 4.3). The other guaiacyl lignin derivatives (4-ethylguaiacol, 4-propylguaiacol and 4-vinylguaiacol) were present at trace levels. Therefore, volatile guaiacol and 4-methylguaiacol were the dominant volatile components jointly accounting for 82-100% of the total pool of guaiacyl derivatives, as was the case with the glycoconjugated forms (*cf.* Table 4.2). Control wines contained substantial levels of volatile syringol and acetosyringone (72 and 97 μg/L, respectively). Smoke exposure, regardless of fuel type, significantly increased the levels of these compounds (Table 4.3). 4-methylsyringol was not detected in control wines but was present at low levels in all wines made from grapes exposed to smoke. Although all wines, irrespective of smoke exposure, had moderate levels (≥ 48 μg/L) of glycoconjugated syringaldehyde, 19 months after bottling, no volatile syringaldehyde was detected (< 3 μg/L) in any of the wines.

Smoke exposure also significantly increased (37-88%, depending on fuel type) the total concentrations of volatile syringyl derivatives. Regardless of smoke exposure, >96% of the total volatile pool of syringyl derivatives in wines was contributed by acetosyringone (53-65%) and syringol (33-46%). Surprisingly, the wines from vines exposed to the smoke of pine fuel showed the highest concentration of total volatile syringyl derivatives (318 μg/L).

4.3.2 Distribution of the lignin-derived compounds between the volatile and glycoconjugated pools

In all wines, after 19 months of bottle storage, both the *p*-hydroxyphenyl and guaiacyl derivatives were predominantly present as glycoconjugates (Figure 4.4), with the volatile components of each group accounting for less than 30% of the total pools. Most of the syringyl derivatives (61-78%) were present in volatile form (Figure 4.4).

Nonetheless, even after 19 months, some syringyl derivatives such as syringaldehyde were detected only in the glycoconjugated form.

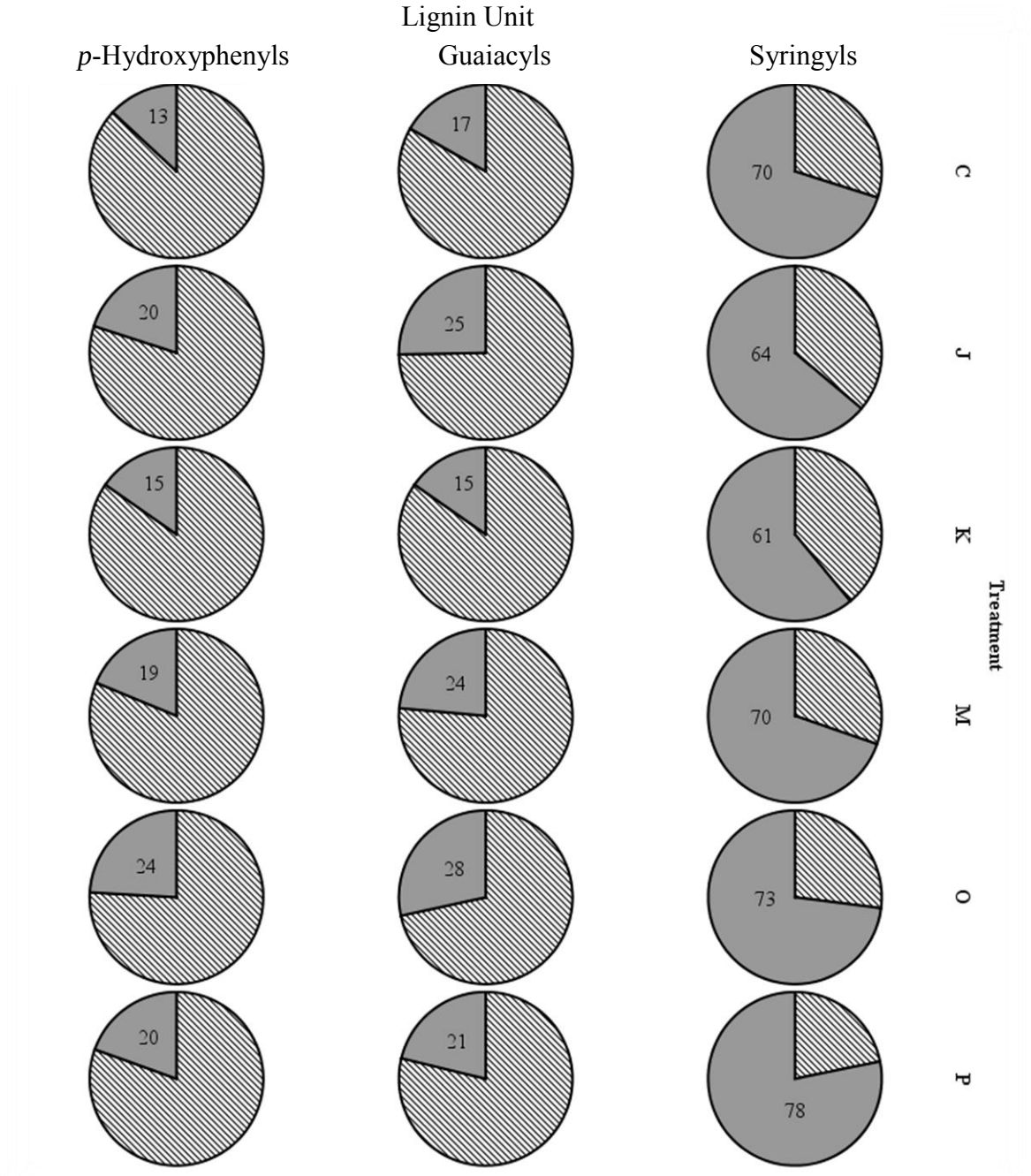


Figure 4.4 Distributions (%) of the total putative smoke taint compounds pool between the free (solid slices) and glycoconjugate (hashed slices) components shown by lignin unit source and treatment. Treatment fuels are: C, unsmoked control; J, jarrah fuel; K, karri fuel; M, marri fuel; O, oat fuel; and P, pine fuel

4.3.3 Influence of canopy size on accretion of lignin pyrolysis products in wine

The concentrations of several of the lignin pyrolysis products in wines from smoke exposed grapes showed consistent negative correlation with vine leaf area as well as with the leaf area per bunch (Figure 4.5).

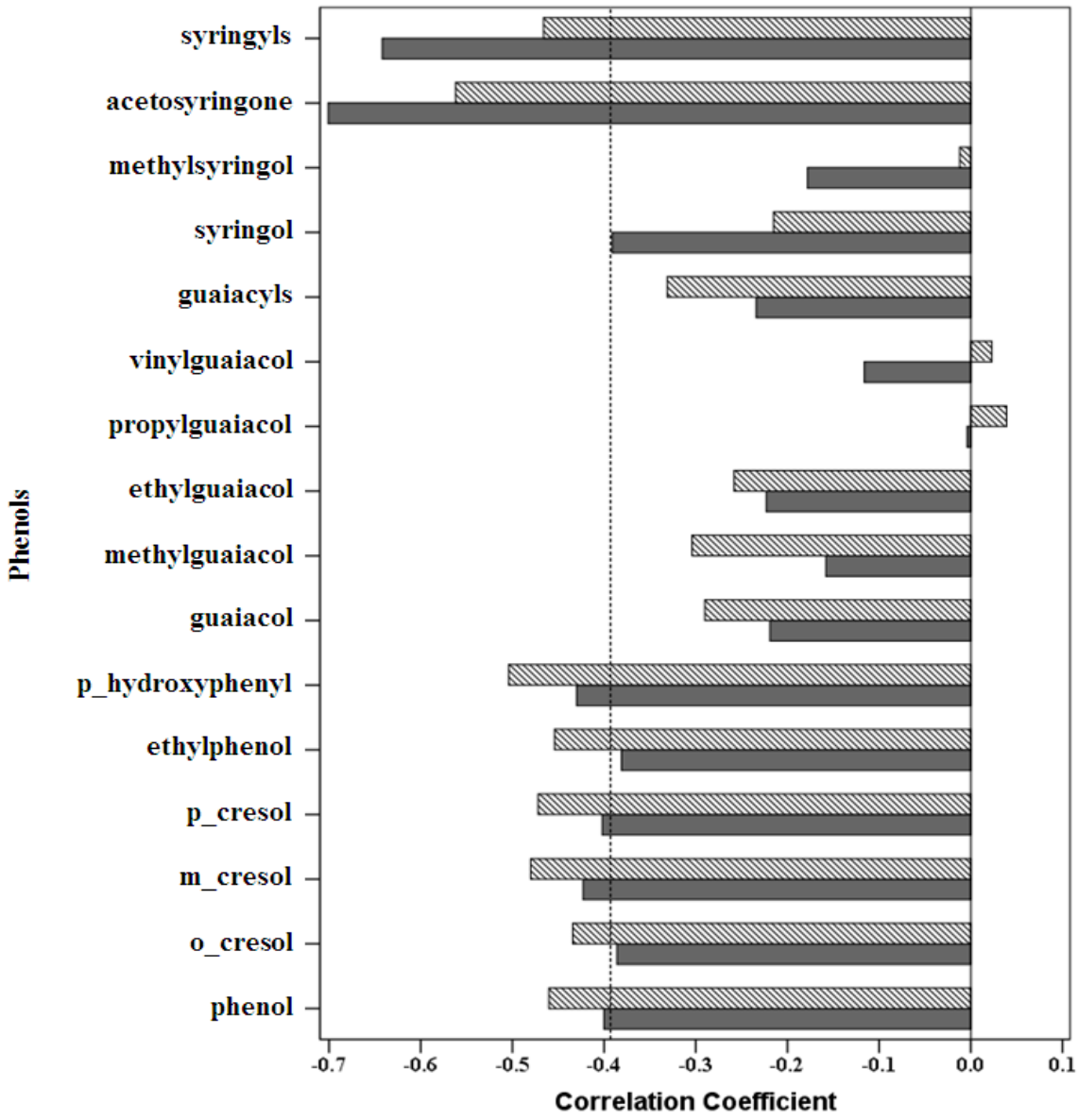


Figure 4.5 Correlations of totals of volatile and glycoconjugated phenols with leaf area per vine (solid bar) and the leaf area per bunch (hashed bar). Data shown are for individual phenols as well as sums by lignin units. The dashed horizontal line denotes the critical value (-0.394) at $p = 0.05$, thus coefficients below -0.394 or above 0.394 are significant at $p < 0.05$.

4.3.4 Analysis of metal concentrations in wine

The analysis of 39 elements found concentrations of 20 metals above the limits of quantification in the replicate wines. No element concentrations were found to be significantly elevated in the smoke treatment wines when compared to the unsmoked control wines.

4.3.5 Compounds not detected in control and smoke treatment wines

The wines were analysed for the lignin pyrolysate compounds, eugenol, isoeugenol, 4-allylsyringol and 4-propylphenol and also the essential oil, 1,8-cineole. None of these compounds were detected in the wines of the control or smoke treatments of this study.

4.4 Discussion

4.4.1 Putative smoke taint compounds in wine

Much of the earlier work into smoke taint in wines focussed on the accretion of guaiacyl lignin derivatives (Kennison et al. 2007, 2008, Sheppard et al. 2009, Singh et al. 2011). The results from this study demonstrate that wines made from smoke exposed grapes, compared to controls, can accrue significantly elevated levels of other phenols (Tables 4.1-4.3) that are emitted in smoke during pyrolysis of lignin (Table 3.5). This effect was generally invariant of the vegetation source of smoke. More recently, Hayasaka et al. (2010a) also reported increased accumulation of phenol, syringol and their substituted forms in bushfire affected wines. However, in this controlled smoke exposure experiment, as in the study of wines made from grapes exposed to wildfire generated smoke (Singh et al. 2012, Appendix 1), a broader range of lignin pyrolysis products are identified than has been reported hitherto. This is indicative of the possibility that more compounds may contribute to smoke taint aroma of smoke affected wines than the few volatile phenols implicated to date. This is consistent with the suggestion that sensory

descriptors of smoke affected wines are varied and more complex than those imparted by the commonly used smoke taint marker compounds, guaiacol and 4-methylguaiacol (Kennison et al. 2007, Hayasaka et al. 2010a).

This study also revealed that while the levels of the commonly reported smoke taint markers, guaiacol and 4-methylguaiacol were certainly the dominant components of the guaiacyl lignin-derived compounds, these represented only some 20% of the total pool of lignin-derived putative smoke taint compounds in wines. Instead, the quantitatively dominant contributors (>50%) were pyrolysis products of syringyl lignin, while phenol and substituted phenols were of broadly comparable abundance as guaiacol and substituted guaiacols (Table 4.1). Comparable proportions have been found in wines of several varieties exposed to wildfire smoke (Singh et al. 2012). However, some perspective is warranted on relative contributions of different phenol groups to the total pool. Firstly, the quantitative relative abundance of putative taint compounds does not necessarily indicate the respective compound's taint impact owing to differences in aroma and/or taste perception threshold concentrations. Thus, for example, while syringol was present at a far higher level than guaiacol (101 *vs*. 19 μg/L), its perception threshold concentration is also high (for example, in red wine 570 *vs*. 75 μg/L, cited in Petruzzi et al. 2010). Yet, in grapes exposed to high density bushfire smoke for an extended period, the total pool of syringol and substituted syringols can reach 25 times (~10 ppm) the levels found in this limited duration smoke exposure experiment (Singh et al. 2012), and hence, can far exceed their apparent high perception threshold. Secondly, the comparison above, of guaiacols *vs*. syringols, may be slightly skewed towards the latter. Here, the total of each of these compounds was estimated from the sum of the volatiles released during fermentation and those released from acid hydrolysis of the glycoside-bound phenols. According to Hayasaka et al. (2010a), however, yields of phenol, guaiacol and their substituted forms from acid hydrolysis of the respective glycoconjugates are considerably less (<10%) than that of glycoconjugated syringol and 4-methylsyringol (33%).

Wines made from smoke unexposed grapes (control treatment) contained a high total (241 μg/L) of endogenous volatile and glycoconjugated syringyl products. Given that grapevine lignin contains syringyl derivatives archetypal of a woody angiosperm (Guillén & Ibargoitia 1996) and that grape juice was fermented on skins (thus facilitating hydrolytic release of lignin units into wine (Loscos et al. 2009)), the presence of syringyl derivatives, albeit at high levels, is plausible (see also Singh et al. 2012). The observed significant increases in the concentrations of total syringols and substituted syringols in wines impacted by smoke of the angiosperm fuels (the three hardwoods and wild oat) may be attributable to exogenous uptake due to the presence of syringyl derivatives in fuels (Tables 3.4) and more significantly in their smoke emissions (Table 3.5). The provenance for the elevated levels of syringols in wines from pine smoke exposed grapes (Tables 4.1-4.3) however, cannot be similarly attributed since syringyl derivatives were expectedly lacking in this fuel and its smoke emissions. During smoke exposure of vines/grapes due care was taken to avoid smoke cross contamination from smoke of the angiosperm fuels. At any rate, if contamination was a mechanism, the control wines would have comparable levels to the pine smoke impacted wines. To understand the mechanism for the observed increase in syringyl products in wines from pine smoke exposed grapes further studies have been conducted and will be discussed in Chapter 5.

4.4.2 Relative abundances of volatile and glycoconjugated phenols in wine

Nineteen months after bottling, the distribution of putative smoke taint compounds in wines between the glycoconjugated and volatile pools differed according to lignin class. Regardless of the applied smoke treatments, pyrolysates of *p*-hydroxyphenyl and guaiacyl origin were predominantly (72-87%) present as glycoconjugates, with volatile forms making up the balance. By contrast, syringol and substituted syringols released after acid hydrolysis made up < 40% of the total pool of syringyl derivatives in wines (Figure 4.1). The predominance of glycoconjugated phenol, guaiacol and their substituted forms over the volatile forms is broadly consistent with previous findings (Singh et al. 2011, Hayasaka et al. 2010a&c, Wilkinson et al. 2011). The results for the

syringyl derivatives however, contrasts to those reported in Hayasaka et al. (2010a), who found levels of volatile syringol and 4-methylsyringol in both red and white wines were <4% of the total pool (i.e., total of volatiles after acid hydrolysis) for each of these compounds. The reason for this disparity is not clear. However, the wines in this study, unlike those of Hayasaka et al. (2010a), were analysed 19 months after bottling, the typical red wine maturation duration. The relatively long storage under the mildly acidic environment of wines coupled with syringols possibly having a weak glycoside bond (Hayasaka et al. 2010a) may have facilitated slow release of syringol and substituted syringols from their glycoconjugates, thus tilting the balance in favour of the volatile forms. Regardless of the mechanism, however, the observation that 19 months after bottling more than 70% of the phenol, guaiacol and their substituted forms exist as glycoconjugates presents a significant practical problem in smoke taint management. It also presents a challenge for efforts to remove volatile smoke taint compounds from wines through reverse osmosis filtration (Fudge et al. 2011) since the major 'taint' reservoir (glycoconjugates) still remains.

4.4.3 The accumulation of smoke borne metals in wine as taint

As wildfire smoke has been reported to contain a number of elements (Alves et al. 2010), the analysis was used to investigate if the metallic sensory descriptor reported by Ristic et al. (2011) and Parker et al. (2012) was caused by an accumulation of metals in smoke tainted wines. The results, however, produced no evidence that smoke exposure elevates concentrations of metal elements in wines (results not shown).

4.4.4 Influences of vine canopy size and type of lignin pyrolysed on concentrations of putative smoke taint in wine

In nearly all of the lignin pyrolysis products, the concentrations of phenols in wines were negatively correlated to vine canopy leaf area as well as the leaf area per bunch (Figure 4.5). The mechanism of accretion of smoke borne lignin pyrolysates in berries is not

well understood. Some of the possibilities include direct uptake of lignin products in smoke emissions by berries and/or foliar uptake and subsequent translocation and sequestration in berries. Tracer studies have shown leaves take up phenols (Beattie & Seibel 2007, Hayasaka et al. 2010b), although only trace quantities are subsequently translocated to berries (Hayasaka et al. 2010b). If the mechanism of uptake in berries were primarily through the leaves, then a positive relationship between canopy size (absorptive surface area) and taint concentration would be expected instead of the inverse relationship found in the current study. This suggests that direct uptake by berries may be a significant contributor to accumulation of phenols in berries. Phenol, guaiacol, syringol and their substituted forms from lignin pyrolysis are emitted in gas phase and particle phase, and particularly for some of the syringyl derivatives such as acetosyringone (e.g., Schauer et al. 2001). While canopy area may have minimal effect on uptake of emissions from gas phase, a denser canopy can intercept particulate phase emissions, and thus reduce contact with the surface of berries. The negative relationship we observed between leaf area per bunch and concentration of phenols (i.e., the more the grapes are sheltered by foliage the less the 'taint' concentration) is consistent with this explanation. Furthermore, the correlation was stronger for acetosyringone whose relative emission in particle phase is high compared to other lignin pyrolysis emissions (Schauer et al. 2001).

The vegetation fuels used in this study were chosen to investigate links between pyrolysis of different lignin sources and appearance of putative smoke taint compounds in wines. The results, particularly of wines from the pine smoke treatment, have revealed that the lignin pyrolysis products that accrue in wines do not necessarily discern the lignin composition of the pyrolysed fuel.

4.5 Conclusion

In Chapters 3 and 4 of this study vegetation fuels with varying lignin makeup were used to generate smoke and fumigate vines at the start of the berry ripening. The aim was to examine whether putative smoke taint compounds that accumulate in wines following

exposure of grapes to a bushfire event reflect the lignin composition of vegetation that is pyrolysed. The results showed a broader range of lignin pyrolysis products in wines than have been reported to date, suggesting more compounds are likely to contribute to the perceived smoke taint than have been implicated. The effect of vegetation fuel types was less about changes in the identity of compounds than their quantities. Thus, and more significantly, fuel lignin makeup does not appear to be a good indicator of the types of lignin pyrolysis products that become elevated in wines. This is predicated on the finding that radiata pine fuel which did not contain syringyl units in its lignin nor pyrolysis products of syringyl units in its smoke emission gave rise to significantly elevated levels of syringols and substituted syringols in wines made from grapes exposed to pine smoke.

This work also demonstrated that several phenols other than guaiacyl products (i.e. guaiacol and 4-methylguaiacol, the routinely used smoke 'taint' measures) are present in wines at similar or higher concentrations than the guaiacyl phenols. Significant among these are the *p*-hydroxyphenyl lignin degradation products, phenol, *o*-cresol, *m*-cresol, and *p*-cresol, and syringol. The cumulative levels of these 'other' phenols are considerably higher than those of the guaiacols. Since smoke tainted wines have sensory characteristics beyond those that could be attributed to guaiacols, the *p*-hydroxyphenyl products and syringols, which become elevated following smoke exposure, are possible candidates to account for the additional smoke taint characteristics in wines.

CHAPTER 5

THE ACCUMULATION OF SINAPYL PHENOLS FROM THE EXPOSURE OF GYMNOSPERM SMOKE EXPOSED GRAPES

5.1 Introduction

The composition of the smoke emission from the gymnosperm (pine) fuel was distinctly different from those of the four angiosperm fuels as it did not contain syringyl derivatives (Chapter 3). While most of the volatile lignin degradation products that accrued in the wines were also present in the smoke emissions, the wines made from vines exposed to the pine fuel emissions were an exception. The mechanism for the formation and accumulation of lignin-derived phenols in wine is therefore not due to a simple absorption and sequestration of smoke-derived phenols in fruit. This chapter is aimed at exploring possible mechanisms or factors that may explain accumulation of phenols in grapes and wine, particularly the syringyl phenols. In this respect, four possibilities were considered.

The first is a methoxylation of phenol or guaiacol derivatives by yeast or malo-lactic bacteria during primary or malo-lactic fermentation. While the pine treatment Merlot wines described in Chapter 4 were found to have syringyl derivatives, an examination of the fruit for syringol or syringyl derived glycoconjugates (Hayasaka et al. 2010a, Hayasaka et al. 2013) will reveal if sinapyl alcohol derived pyrolysate compounds were present in the fruit from pine smoke exposure.

The second possible mechanism is a plant response to an elevation in temperature. The trials described in Chapter 3 may have caused a minor elevation in air temperature inside the tent structure where hot smoke from high temperature pyrolysis was used to fumigate vines. Ford et al. (1979) have reported an increase in lignin production in tropical and temperate grasses grown at higher temperatures. Whether a similar response or change in lignin monomer composition occurs in wine grapes due to a short increase in plant temperature has not been examined. An experiment was therefore designed to

compare the fruit and wines of unheated control vines with those of vines exposed to elevated ambient temperature.

The third possible mechanism is a plant response to smoke where the formation of monolignols is altered in the fruit of vines exposed to emissions from carbohydrate pyrolysis products. At a cellular level, lignin accumulation can be induced by the interaction of pathogens (Matsumoto et al. 1978 as cited in Vance et al. 1980), wounding (Dean & Kuć 1987) and other stress factors (Whetten & Sederoff 1995), however lignin deposition or change in monolignol composition in response to smoke particles or smoke-derived chemicals has not been reported. The vegetation fuels described in Chapter 3 contained between 30% (jarrah) and 77% (wild oats) cellulose and hemicelluloses (Table 3.2). As the accumulation of lignin-derived phenols in the wines described in Chapter 4 showed no correspondence with the lignin composition of the pyrolysed fuels, a comparison of phenol accumulation in the fruit and wines made from exposure to smoke from a lignin source versus a carbohydrate source will reveal the influence, if any, of carbohydrate emissions.

The fourth possible mechanism is an *in planta* transformation (methoxylation) of the xenobiotically acquired hydroxy- and methoxy-phenols in smoke. While the absorption of phenol and *p*-cresol in maize has been reported (Beattie & Seibel 2007), where there is marked spatial distribution of the phenols upon absorption, the transformation products are not yet understood. Hydroxylation and methylation of coniferaldehyde and coniferyl alcohol by 3-O-methyltransferase is a major route for the production of sinapyl alcohol, the precursor of syringyl lignins (Osakabe et al. 1999). As the synthesis of lignin phenol monomers includes glycosylation of the phenolic hydroxyl group (Whetten & Sederoff 1995), the absorption and glycosylation of coniferyl derived phenols in gymnosperm smoke (for example guaiacol) may also undergo hydroxylation/ methylation to syringols. Although wine grapes exposed to smoke accumulate phenols as glycoconjugates (Hayasaka et al. 2010a), methoxylation of smoke borne phenols before or after glycosylation has not been reported. Therefore, a series of experiments were carried out to explore each of the possibilities described above.

Experiment 5.2 Assessment of possible sources of syringyl glycoconjugates in wines from pine smoke exposed grapes: are these derived from transformation of phenol and/or guaiacol during fermentation or are they present in pine smoke exposed grapes?

An analysis of the Merlot fruit and wine described in Chapter 4 using the methods of Hayasaka et al. (2010a, 2013) will uncover if the syringyl derivatives in the pine smoke treatment wines were also present in the fruit.

If, on the other hand, the control and pine smoke treated grapes have similar concentrations of syringyl glycoconjugates, then transformation and accumulation during fermentation will emerge as a likely mechanism for the elevated levels in wines of pine-smoke exposed fruit.

5.2.1 Materials and methods

5.2.1.1 Merlot smoke exposures and fruit analysis

The Merlot fruit from Chapter 4 were used for this section. The fruit was analysed for seven volatile lignin-derived phenols (Table 5.1) and fourteen glycoconjugated phenols (Table 5.2), as described by Hayasaka et al. (2013).

5.2.1.2 Statistical analysis

Data were analysed using SPSS 20 (IBM® SPSS® Statistics). Reported treatment effects are significant at $p < 0.05$.

5.2.2 Results

5.2.2.1 Effect of smoke exposure on volatile phenols in Merlot fruit

The total volatile phenols in Merlot fruit were significantly elevated from smoke exposure of vines 14 days after the onset of grape ripening (Table 5.1). Smoke exposure also significantly elevated the *o*-cresol and guaiacol concentrations. There was no significant difference in the effect of gymnosperm (pine) fuel compared to the angiosperm, wild oats fuel. The smoke exposed pine and wild oats replicates were not significantly different to the control replicates in syringol concentration.

Table 5.1 Effects of fuel type and smoke exposure on volatile phenol levels (μg/kg) in Merlot fruit.
Data are mean ±1 standard error (n=5); nd, not detected.

	Treatment		
Volatile phenols	Control	Pine	Wild Oats
o-Cresol	nd	2.8±0.9	3.6±0.7
m-Cresol	nd	nd	nd
p-Cresol	nd	nd	nd
Subtotal	nd	2.8±0.9	3.6±0.7
Guaiacol	nd	2.2±0.7	3.4±0.7
4-Methylguaiacol	nd	0.6±0.4	nd
Subtotal	nd	2.8±1.0	3.4±0.7
Syringol	4.0±1.3	3.2±0.4	2.6±1.1
4-Methylsyringol	nd	nd	nd
Subtotal	4.0±1.3	3.2±0.4	2.6±1.1
Total	4.0±1.3	8.8±1.7	9.6±1.9

5.2.2.2 Effect of smoke exposure on glycoconjugated lignin derivatives in Merlot fruit

Averaged across the two fuel types, the total pool of glycoconjugated lignin derivatives was more than 21 times the levels found in control fruit (Table 5.2). The largest response to smoke exposure occurred in the total concentration of *p*-hydroxyphenyl and guaiacyl derivatives (19 and 30 times respectively) with the least responsive being the syringyl products (15 times). Smoke exposure also increased the concentrations of each compound by up to 74 times (Table 5.2). There was no significant difference in the concentration of total *p*-hydroxyphenyl or guaiacyl derived glycoconjugates in the pine and oats smoked fruit, however the total glycoconjugated 4-methylguaiacol concentration in pine smoked fruit was 1.8 times than the oats fruit. Despite being significantly lower than the oats replicates in syringyl glycoconjugates (49.6 versus 127.6 μg/kg), the pine smoked fruit contained significantly higher syringyl glycoconjugates (49.6 versus 5.8 μg/kg) than unsmoked, control fruit (Table 5.2).

Table 5.2 Effects of fuel type and smoke exposure on glycoconjugated phenol levels (μg/kg) in Merlot fruit. *PG*: Pentosylglucoside, *RG*: Rhamnosylglucoside, *GG*: Glucosylglucoside. Data are mean ±1 standard error (n=5)

	Treatment		
Phenol glycosides	Control	Pine	Wild Oats
Phenol PG	1.3±0.1	29.4±4.2	33.0±4.6
Phenol RG	0.4±0.01	18.3±2.9	23.8±4.4
Cresol PG	8.5±0.9	118.7±25.5	115.8±18.9
Cresol RG	0.8±0.1	35.1±6.4	33.2±5.4
Subtotal	11.0±1.0	201.5±38.7	205.9±32.5
Guaiacol GG	0.8±0.1	30.7±6.1	45.3±9.3
Guaiacol PG	5.3±0.3	115.7±26.9	146.6±30.6
Guaiacol RG	0.4±0.01	15.7±3.2	21.7±4.0
4-Methylguaiacol GG	0.2±0.01	13.0±4.4	7.0±2.0
4-Methylguaiacol PG	1.1±0.1	34.4±12.2	18.7±4.2
4-Methylguaiacol RG	0.5±0.1	32.9±5.9	18.6±2.7
Subtotal	8.3±0.5	242.4±56.2	258.0±51.4
Syringol GG	2.1±0.2	34.0±11.4	100.2±28.5
Syringol PG	2.7±0.3	6.8±1.8	14.0±3.5
4-Methylsyringol GG	0.3±0.01	6.8±3.1	11.2±3.3
4-Methylsyringol PG	0.6±0.01	2.0±0.5	2.2±0.4
Subtotal	5.8±0.4	49.6±16.6	127.6±35.5
Total	25.0±1.7	493.6±110.1	591.5±113.4

Experiment 5.3 The accumulation of phenol glycoconjugates in wine grapes due to vine canopy heating

5.3.1 Materials and methods

5.3.1.1 Heating of vine canopies

The heating exposure experiment was set up as a completely randomised block design in a commercial vineyard containing 18 years old *Vitis vinifera L. cv*. Sauvignon Blanc vines, where a 2 kW fan forced electric heater was used to heat the air inside a purpose built tent as described in Chapter 4. Two electric fans were also used to ensure an even distribution of heated air across the replicate panel. The air temperature was found to rapidly increase to 50^{o}C and stabilise to within two degrees for the entire exposure. Two different sets of control replicates, one with the tent held over the vines for one hour and one with no tent coverage, were made to compare accumulation of phenols in the fruit. The tent was high pressure cleaned before the trials and left for eight weeks in full sunlight to eliminate any possible contamination from previous year's fuel pyrolysis trials. To minimise variability in experimental units within a block, each block was carefully selected for vines of uniform canopy size and crop load. The heating treatments and controls consisted of replicate panels of five vines. Exposure of the experimental vines was carried out 14 days post veraison (EL 36) for one hour with the first replicate starting at 9 am and the third finishing at 12 pm.

5.3.1.2 Grape and wine analysis

The fruit was analysed for seven volatile lignin-derived phenols and six phenol glycoconjugates (Table 5.3), as described by Hayasaka et al. (2013).

5.3.1.3 Statistical analysis

Data were analysed using SPSS 20 (IBM® SPSS® Statistics). Reported treatment effects are significant at $p < 0.05$.

5.3.2 Results

5.3.2.1 Effect of heat exposure on volatile phenols in Sauvignon Blanc fruit

The exposure of fruit bearing Sauvignon Blanc vines to an elevated air temperature of 50^{o}C, 14 days after the onset of fruit ripening, did not significantly elevate any of the volatile phenols analysed. No volatile phenols were detected (< 1 ppb) in the fruit samples of controls with tent exposure or the controls without tent exposure (data not shown).

5.3.2.2 Effect of heat exposure on glycoconjugated lignin derivatives in Sauvignon Blanc fruit

The exposure of fruit bearing Sauvignon Blanc vines, 14 days after the onset of fruit ripening, to an elevated air temperature of 50^{o}C did not significantly elevate any of the phenol glycoconjugates listed in Table 5.3.

Table 5.3 Effects of heat exposure on glycoconjugated phenol levels (µg/kg) in Sauvignon Blanc fruit. *RG*: Rhamnosylglucoside, *GG*: Glucosylglucoside. Data are mean ±1 standard error (n=3); nd, not detected.

	Treatment		
Phenol glycosides	Control no tent	Control with tent	Heat Treatment
Phenol RG	0.3±0.03	0.3±0.03	0.6±0.06
Cresol RG	1.5±0.2	0.9±0.1	1.2±0.12
Subtotal	1.8±0.23	1.2±0.13	1.8±0.18
Guaiacol RG	nd	nd	0.2±0.02
4-Methylguaiacol RG	0.2±0.02	0.2±0.02	0.3±0.03
Subtotal	0.2±0.02	0.2±0.02	0.5±0.05
Syringol GG	0.2±0.02	0.1±0.01	0.2±0.02
4-Methylsyringol GG	0.1±0.01	0.1±0.01	0.1±0.01
Subtotal	0.3±0.03	0.2±0.02	0.3±0.03
Total	2.3±0.2	1.6±0.16	2.6±0.26

Experiment 5.4 The exposure of vines to cellulose and hemicellulose pyrolysis emissions

5.4.1 Materials and methods

In Chapter 4, the exposure of vines to smoke from vegetation fuels with varying lignin, cellulose and hemicellulose makeup was described. To test if vines accumulate glycoconjugated phenols in fruit from an exposure to pyrolysed carbohydrate emissions, vines were exposed to the emissions of a lignin free, plant derived fuel source and compared to control and wheat straw exposed replicates. Paper made from cotton linters (Arches 88, Canson®, South Hadley MA, USA) is lignin free and comprises only cellulose and hemicellulose carbohydrates. The absence of lignin components in the cotton linter paper was first verified by *Py* GC-MS and the vineyard pyrolysis emissions examined by TD GC-MS.

5.4.1.1 Monolignol analysis by *Py* GC-MS

Samples of approximately 0.1 mg of Arches 88 paper were analysed as described in 3.2.5. The analysis was performed in triplicate.

5.4.1.2 Smoke analysis

Smoke emissions from the Arches 88 paper during vine exposure trials were sampled and analysed as described in section 3.2.6. Each smoking replicate of paper and wheat straw was performed in triplicate.

5.4.1.3 Vineyard trials

The smoke exposure experiment was set up as a completely randomised block design in a commercial vineyard containing 20 years old *Vitis vinifera L. cv.* Chardonnay vines. To minimise variability in experimental units within a block, each block was carefully

selected for vines of uniform canopy size and crop load. The treatments, for smoke generation and exposure, consisted of one kilogram of shredded Arches 88 paper and one kilogram of cured wheat straw, pyrolysed as described in Chapter 4, plus control replicates where the tent was held over the replicate panel without smoke exposure. Within each block, the treatments plus the controls were randomly allocated to experimental units and consisted of a panel of five vines. Experimental units were separated by at least two panels of vines to avoid smoke cross contamination. Each block containing the full treatment structure was replicated three times, thus there were a total of nine experimental units. Smoke exposure of the experimental vines was carried out 14 days post-veraison (EL 36), in a purpose built tent as described in section 4.2.2. The wheat straw was used to compare the accrual of lignin-derived phenols from the smoke exposure of a lignin source. The wheat straw was collected as cured header tails immediately after harvest from a farm in Kojonup, Western Australia (33°50'S, 117°09'E).

5.4.1.4 Winemaking

The fruit from each replicate panel was harvested separately at commercial maturity, total soluble solids ~ 23°Brix, (Reichert AR 200 digital refractometer, Reichert Inc., New York, U.S.A.) six weeks after smoking treatments. Each lot was de-stemmed and pressed with a 100 ppm potassium metabisulphite (Chem Supply AR grade, Gillman S.A. Australia) addition. The must was inoculated with 300 mg/l Saccharomyces cerevisiae EC1118 (Lallemand Inc., Montreal, Canada) and 100 mg/l diammonium phosphate (Sigma- Aldrich, Sydney, Australia) added as a ferment nitrogen supplement. Each replicate was fermented in 25 litre glass demijohns to dryness (<1g/l residual sugars), racked from gross lees with the addition of 60 mg/l potassium metabisulphite and cold stabilised at - 4°C for 21 days. The wines were filtered to 0.2 μm (Sartorius Sartopure 2 Maxicap, Sartorius, Gottingen, Germany) and bottled under food grade nitrogen with stelvin closures.

5.4.1.5 Fruit and wine analysis

The fruit and wine were analysed for seven volatile lignin-derived phenols (Table 5.4 and Table 5.6) as described by Hayasaka et al (2013). The fruit was analysed for eight phenol glycoconjugates (Table 5.5) and the wine for fourteen phenol glycoconjugates (Table 5.7), as described by Hayasaka et al (2013).

5.4.1.6 Statistical analysis

Data were analysed using SPSS 20 (IBM® SPSS® Statistics). Reported treatment effects are significant at $p < 0.05$.

5.4.2 Results

5.4.2.1 Paper lignin composition

Minor amounts of phenol (~1% of total pyrogram area count) and *o*-cresol (~0.1% of total pyrogram area count) were found as pyrolysis products of the paper fuel from the *Py*-GC-MS analysis (data not shown). No other lignin-derived phenols were detected in the pyrograms.

5.4.2.2 Volatile phenols in smoke emissions

Consistent with the *Py*-GC-MS results of the fuels, the TD-GC-MS analyses of smoke emissions from the pyrolysis of paper during the vine smoke exposure experiment contained minor amounts of phenol (~1% of total pyrogram area count) and *o*-cresol (~0.1% of total pyrogram area count, data not shown). No other lignin-derived phenols were detected in the pyrograms.

5.4.2.3 Effect of smoke exposure on volatile phenols in Chardonnay fruit

Smoke exposure of vines 14 days after the onset of grape ripening significantly elevated the *o*-cresol in the fruit of paper and straw exposed Chardonnay vines, albeit at minor levels (1.7 and 4.3 µg/kg respectively). The *o*-cresol content of fruit from straw exposed vines was also significantly higher than the paper exposed vines (Table 5.4). All other volatile phenols were below detection levels.

Table 5.4 Effects of fuel type and smoke exposure on volatile phenol levels (µg/kg) in Chardonnay fruit. Data are mean ±1 standard error (n=3); nd, not detected.

	Treatment		
Volatile phenols	Control	Paper	Straw
o-Cresol	nd	1.7±0.1	4.3±0.1
m-Cresol	nd	nd	nd
p-Cresol	nd	nd	nd
Subtotal	nd	1.7±0.1	4.3±0.1
Guaiacol	nd	nd	nd
4-Methylguaiacol	nd	nd	nd
Subtotal	nd	nd	nd
Syringol	nd	nd	nd
4-Methylsyringol	nd	nd	nd
Subtotal	nd	nd	nd
Total	nd	1.7±0.1	4.3±0.1

5.4.2.4 Effect of smoke exposure on glycoconjugated phenols in Chardonnay fruit

The exposure of Chardonnay vines to the emission products of lignin free paper fuel, 14 days after the onset of veraison, significantly elevated the phenol and cresol rhamnosylglucoside concentrations compared to the unsmoked control fruit (Table 5.5). The straw exposed fruit was significantly higher than the control and paper exposed fruit in each lignin-derived glycoconjugated phenol.

Table 5.5 Effects of fuel type and smoke exposure on glycoconjugated phenol levels (µg/kg) in Chardonnay fruit. *PG*: Pentosylglucoside, *RG*: Rhamnosylglucoside, *GG*: Glucosylglucoside. Data are mean ±1 standard error (n=3)

	Treatment		
Phenol glycosides	Control	Paper	Straw
Phenol *RG*	0.7±0.1	17.3±0.1	23.2±1.5
Cresol *RG*	2.5±0.1	24.1±0.6	49.5±3.5
Subtotal	3.2±0.2	41.4±0.7	72.7±5.0
Guaiacol *PG*	15.6±0.6	18.9±0.8	58.7±3.3
Guaiacol *RG*	0.7±0.1	1.6±0.1	8.7±0.8
4-Methylguaiacol *PG*	5.6±0.1	6.7±0.2	17.0±1.1
4-Methylguaiacol *RG*	2.8±0.1	3.6±0.1	10.2±0.5
Subtotal	24.7±0.8	30.8±1.2	94.6±5.5
Syringol *GG*	3.1±0.1	5.0±0.2	26.6±2.9
4-Methylsyringol *GG*	1.4±0.1	2.0±0.1	6.5±0.7
Subtotal	4.5±0.1	7.0±0.2	33.1±3.6
Total	32.4±0.9	79.2±2.1	200.4±13.2

5.4.2.5 Effect of smoke exposure on volatile phenols in Chardonnay wine

As found in the fruit analysis, only *o*-cresol was found to be elevated at comparable levels to the straw treatment wines (Table 5.6).

Table 5.6 Effects of fuel type and smoke exposure on volatile phenol levels (µg/l) in Chardonnay wine. Data are mean ±1 standard error (n=3); nd, not detected.

	Treatment		
Volatile phenols	Control	Paper	Straw
o-Cresol	nd	1.7±0.1	2.2±0.1
m-Cresol	nd	nd	nd
p-Cresol	nd	nd	nd
Subtotal	nd	1.7±0.1	2.2±0.1
Guaiacol	nd	nd	nd
4-Methylguaiacol	nd	nd	nd
Subtotal	nd	nd	nd
Syringol	nd	nd	nd
4-Methylsyringol	nd	nd	nd
Subtotal	nd	nd	nd
Total	nd	1.7±0.1	2.2±0.1

5.4.2.6 Effect of smoke exposure on glycoconjugated phenols in Chardonnay wine

The analysis of fourteen phenol glycoconjugates in wine made from paper exposed Chardonnay vines has found only glycoconjugated phenol and cresol (a sum of *ortho*, *meta* and *para* cresol pentosylglucosides) at significant elevations compared to the unsmoked control wines (Table 5.7). The wines made from paper exposed fruit contained significantly less guaiacyl and sinapyl glycoconjugate concentrations (Table

5.7) than the straw exposed wines and were not significantly different in guaiacyl and sinapyl glycoconjugate concentrations to the wines made from control, unexposed fruit.

Table 5.7 Effects of fuel type and smoke exposure on glycoconjugated phenol levels (µg/l) in Chardonnay wine. *PG*: Pentosylglucoside, *RG*: Rhamnosylglucoside, *GG*: Glucosylglucoside. Data are mean ±1 standard error (n=3); nd, not detected.

	Treatment		
Phenol glycosides	Control	Paper	Straw
Phenol *PG*	4.2±0.1	15.0±0.5	15.8±0.5
Phenol *RG*	0.2±0.1	4.4±0.2	7.6±0.5
Cresol *PG*	6.6±0.5	21.9±0.7	37.5±2.1
Cresol *RG*	0.3±0.1	2.9±0.1	8.1±0.5
Subtotal	11.3±0.3	44.2±0.5	69.0±3.1
Guaiacol *GG*	0.1±0.01	0.2±0.01	0.1±0.01
Guaiacol *PG*	4.9±0.3	5.2±0.1	17.7±0.9
Guaiacol *RG*	0.2±0.01	0.4±0.1	2.4±0.1
4-Methylguaiacol *GG*	nd	nd	nd
4-Methylguaiacol *PG*	1.1±0.1	1.1±0.1	3.0±0.3
4-Methylguaiacol *RG*	0.3±0.01	0.4±0.1	1.7±0.2
Subtotal	6.6±0.2	7.3±0.3	24.9±1.2
Syringol *GG*	0.8±0.1	1.2±0.2	4.4±0.3
Syringol *PG*	1.2±0.01	1.2±0.1	2.4±0.2
4-Methylsyringol *GG*	0.1±0.01	0.1±0.01	0.3±0.1
4-Methylsyringol *PG*	0.1±0.01	0.1±0.01	0.3±0.01
Subtotal	2.2±0.1	2.6±0.3	7.4±0.4
Total	20.1±0.3	54.1±0.4	101.3±4.4

Experiment 5.5 The transformation (methoxylation) of phenols by plants after exposure to gaseous phenols

Two separate trials were designed to investigate a possible transformation of phenol, guaiacol and syringol after absorption by fruit bearing plants. In the first trial, the transformation of 2-methoxyphenol (guaiacol) to 2,6-dimethoxyphenol (syringol) was examined. By exposing fruit bearing, potted tomato plants to guaiacol vapour in an enclosed environment, the uptake and glycosylation of guaiacol and/or transformation of guaiacol to syringol in the fruit was investigated. The second trial exposed Sauvignon Blanc vines at veraison to gaseous phenol, guaiacol and syringol to investigate transformation of each pure compound in the fruit and wine made from the exposed fruit.

5.5.1 Materials and methods

5.5.1.1 Exposure of tomatoes to gaseous guaiacol

Seedling plants of Super Sweet Cherry Tomato (*Solanum lycopersicum* var. *cerasiforme*) were grown in a hot house and regularly pruned to restrict vertical growth and the development of more than two fruiting bunches. Two stages of fruit ripeness were used for guaiacol exposure: at the start of fruit ripening when the green fruit began to show the first stages of softening and when the fruit was fully ripe. Three replicates of green fruited tomatoes and three replicates of ripe fruited tomatoes were watered and sealed in six 30cm by 40 cm by 50 cm glass chambers as control (unexposed) replicates. Three replicates of green fruited tomatoes and three replicates of ripe fruited tomatoes were watered and sealed in six 30cm by 40 cm by 50cm glass chambers with 10 ml of pure guaiacol (Sigma Chemicals, Sydney, Australia) in a 20 ml glass beaker. Six tomato plants were used in each replicate. The glass chambers were inverted over the pots with the bottom sealed onto a sheet of glass with glass putty and the plants were enclosed in the chambers for ten days, after which time the pure guaiacol solutions had decreased by approximately 8 ml. The glass chambers were kept in the same hot house used for propagating the seedlings. No guaiacol aroma outside of the glass aquariums was detected during the exposure period and the control replicates were sealed and unsealed before the treatment replicates to avoid possible guaiacol vapour exposure.

5.5.1.2 Exposure of gaseous phenol, guaiacol and syringol to Sauvignon Blanc fruit

The liquid phenols vapour exposure experiment was set up as a completely randomised block design in a commercial vineyard containing 18 years old *Vitis vinifera L. cv*. Sauvignon Blanc vines. To minimise variability in experimental units within a block, each block was carefully selected for vines of uniform canopy size and crop load. The treatments, for exposure, consisted of replicate panels for each of the three pure compounds: phenol, guaiacol and syringol. Two control treatments were also made to verify no phenol residue contamination occurred as a result of using the same tent to contain the compounds in each treatment. In the first control, the tent was placed over the replicate panels without compound exposure and in the second control, the replicate panels were not covered by the tent. Each experimental unit consisted of a panel of five vines. Experimental units were separated by at least three panels of vines to avoid cross contamination and the blocks were arranged with the prevailing southerly winds to avoid residual exposure from each treatment type by the following order: the control treatments with no tent coverage were furthest to the south, followed by the controls with tent exposure, the phenol replicates, the guaiacol replicates and the syringol replicates furthest north. Each block containing the full treatment structure was replicated three times, thus there were a total of 15 experimental units. Exposure of the experimental vines was carried out 14 days post veraison (EL 36), with the three phenol replicates on the first day, followed by the guaiacol replicates and syringol replicates on sequential days. Each exposure was for one hour with the first replicate starting at nine am and the third finishing at 12 pm. For each exposure, 100 g of pure compound was placed on aluminium trays with a total surface area of 2 m^2. The solid phenol was found to liquefy totally within five minutes and the guaiacol was applied as a liquid, however the syringol could only be liquefied by placing the trays over hot (~ 90 ^oC) water changed at 15 minute intervals. A purpose built tent, as described in Chapter 4, was used to contain the vapours of each pure compound and an electric fan was used to blow across the trays of compounds to increase evaporation. A second fan was used to ensure an even distribution of vapours across the replicate panel. The tent was high pressure

cleaned before the trials and left for eight weeks in full sunlight to eliminate any contamination from previous year's trials. The tent was also high pressure cleaned after the last exposure from each day's trials and left in full sunlight for six hours to minimise residual contamination. The mass of pure compound after each trial was recorded to allow comparisons of each compound's loss. The control replicates with tent coverage were completed the day after the last compound exposure with the same cleaning process used between compounds.

5.5.1.3 Tomato fruit analysis

The tomato fruit were analysed for nine volatile lignin-derived phenols (Table 5.8) and 16 phenol glycoconjugates (Table 5.9), as described by Hayasaka et al (2013).

5.5.1.4 Sauvignon Blanc fruit analysis

The Sauvignon Blanc fruit was analysed for seven volatile lignin-derived phenols (Table 5.11) and six phenol glycoconjugates (Table 5.12), as described by Hayasaka et al (2013).

5.5.1.5 Statistical analysis

Data were analysed using SPSS 20 (IBM® SPSS® Statistics). Reported treatment effects are significant at $p < 0.05$.

5.5.2 Results

5.5.2.1 Effect of guaiacol exposure on volatile phenols in tomatoes

The exposure of fruit bearing tomato plants to guaiacol vapours significantly elevated the volatile guaiacol in the fruit (Table 5.8). The guaiacol exposed green fruit contained over 7 times the concentration of volatile guaiacol than the exposed ripe fruit. No volatile phenol or syringol derivatives were detected in the fruit.

Table 5.8 Effects of guaiacol exposure on volatile phenol levels (µg/kg) in tomato fruit. Data are mean ±1 standard error (n=3); nd, not detected.

	Treatment			
Volatile phenol	Control Green	Control Ripe	Treatment Green	Treatment Ripe
Phenol	nd	nd	nd	nd
o-Cresol	nd	nd	nd	nd
m-Cresol	nd	nd	nd	nd
p –Cresol	nd	nd	nd	nd
4-Ethylphenol	nd	nd	nd	nd
Subtotal	nd	nd	nd	nd
Guaiacol	nd	nd	1331±80.0	176.0±7.0
4-Methylguaiacol	nd	nd	nd	nd
4-Ethylguaiacol	nd	nd	nd	nd
Subtotal	nd	nd	1331±80.0	176.0±7.0
Syringol	nd	nd	nd	nd
Subtotal	nd	nd	nd	nd
Total	nd	nd	1331±80.0	176.0±7.0

5.5.2.2 Effect of guaiacol exposure on glycoconjugated lignin derivatives in tomatoes

Significant accumulations of guaiacol GG and PG were found in tomato fruit exposed to guaiacol vapours. The mature (red) fruit contained significantly higher (3.7 times) total guaiacol glycoconjugate concentrations than the green exposed fruit (Table 5.9). The exposure to guaiacol vapours did not significantly elevate the phenol or syringol

glycoconjugates in the fruit, although slight increases in phenol-PG, cresol-PG and cresol-GG were apparent (Table 5.9).

Table 5.9 Effects of volatile guaiacol exposure on glycoconjugated phenol levels (µg/kg) in tomato fruit. *PG*: Pentosylglucoside, *RG*: Rhamnosylglucoside, *GG*: Glucosylglucoside. Data are mean ±1 standard error (n=3); nd, not detected.

	Treatment			
Phenol glycosides	Control Green	Control Ripe	Treatment Green	Treatment Ripe
Phenol *GG*	nd	nd	nd	nd
Phenol *PG*	0.3±0.03	0.2±0.02	1.5±0.1	1.6±0.1
Phenol *RG*	nd	0.1±0.01	0.4±0.02	0.4±0.03
Cresol *GG*	0.4±0.02	0.1±0.01	1.2±0.3	1.4±0.3
Cresol *PG*	0.3±0.02	0.2±0.03	1.5±0.2	1.6±0.2
Cresol *RG*	nd	nd	nd	nd
Subtotal	1.0±0.07	0.6±0.07	4.6±0.5	5.0±0.5
Guaiacol *GG*	3.7±0.2	2.6±0.2	10.7±0.7	27.2±2.1
Guaiacol *PG*	2.4±0.1	3.5±0.3	68.1±5.2	235.4±20.2
Guaiacol *RG*	0.1±0.01	0.1±0.01	0.5±0.03	0.3±0.02
4-Methylguaiacol *GG*	0.2±0.01	0.8±0.06	1.0±0.1	0.1±0.01
4-Methylguaiacol *PG*	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.01
4-Methylguaiacol *RG*	nd	nd	nd	nd
Subtotal	6.5±0.3	7.1±0.6	70.4±6.2	263.1±21.1
Syringol *GG*	0.4±0.02	1.5±0.2	1.2±0.1	0.3±0.02
Syringol *PG*	0.1±0.01	0.8±0.05	0.6±0.04	0.2±0.02
4-Methylsyringol *GG*	nd	nd	nd	nd
4-Methylsyringol *PG*	nd	nd	nd	nd
Subtotal	0.5±0.02	2.3±0.2	1.8±0.14	0.5±0.03
Total	8.0±0.4	10.0±0.9	76.8±8.2	268.6±21.4

5.5.2.3 Pure compound volatilisation in Sauvignon Blanc vineyard exposures

The average mass and number of moles volatilised into the tent for each Sauvignon Blanc exposure are shown in Table 5.10. The number of moles of phenols volatilised was inversely proportional to molecular size: i.e. phenol loss was two and four times that of guaiacol and syringol, respectively (Table 5.10).

Table 5.10 Average mass (g) of pure phenol used in Sauvignon Blanc trials and calculated average number of moles. Data are mean ±1 standard error (n=3).

	Treatment		
	Phenol	Guaiacol	Syringol
Mass of phenol used	100.00±0.01	100.00±0.01	100.00±0.01
Average mass of phenol remaining	86.43±0.39	92.03±0.38	94.68±0.39
Average total mass volatilised	13.57±0.39	7.97±0.38	5.32±0.38
Average number of moles volatilised	0.144±0.04	0.064±0.003	0.034±0.002

5.5.2.4 Effect of pure compound exposure on volatile phenols in Sauvignon Blanc fruit

The exposure of fruit bearing Sauvignon Blanc vines to guaiacol vapours significantly elevated (532.0 µg/kg) the volatile guaiacol compared to the tent covered controls (Table 5.11). Surprisingly, the guaiacol exposed fruit also contained *o*-cresol and the phenol exposed vines were also found to contain a significant, albeit small (2 µg/kg), increase in guaiacol compared to the covered and non-covered controls. No treatment fruit was found to contain any volatile syringol, including the fruit exposed to syringol vapours. Interestingly, however, measurable levels of guaiacol were present in syringol exposed fruits. No residual guaiacol or syringol was found to occur in the tent covered control replicates.

Table 5.11 Effects of phenol exposures on volatile phenol levels (µg/kg) in Sauvignon Blanc fruit.
Data are mean ±1 standard error (n=3); nd, not detected.

	Treatment				
Volatile phenol	Control without tent	Control with tent	Phenol	Guaiacol	Syringol
o-Cresol	nd	nd	nd	9.0±0.9	nd
m-Cresol	nd	nd	nd	nd	nd
p –Cresol	nd	nd	nd	nd	nd
Subtotal	nd	nd	nd	9.0±0.9	nd
Guaiacol	nd	nd	2.0±0.2	532.0±53	2.0±0.2
4-Methylguaiacol	nd	nd	nd	nd	nd
Subtotal	nd	nd	2.0±0.2	532.0±53	2.0±0.2
Syringol	nd	nd	nd	nd	nd
4-Methylsyringol	nd	nd	nd	nd	nd
Subtotal	nd	nd	nd	nd	nd
Total	nd	nd	2.0±0.2	541.0±54	2.0±0.2

5.5.2.5 Effect of pure compound exposure on glycoconjugated lignin derivatives in Sauvignon Blanc fruit

Phenol exposure of vines 14 days after the onset of grape ripening significantly increased the total concentration of phenol-RG in the fruit (Table 5.12). Phenol exposure also significantly elevated the guaiacol-RG concentration compared to the control. Guaiacol exposure significantly elevated guaiacol-RG as well as phenol-RG and cresol-RG concentrations compared to the control replicates. Syringol vapour exposure also significantly increased fruit syringol concentration (Table 5.12). However, the syringol uptake was approximately 10% of the phenol and guaiacol uptake. The fruit from syringol exposed vines contained a small but significant concentration of phenol and guaiacol rhamnosylglucosides compared to the control replicate fruit. Only trace levels (up to 1.5 μg/kg) of phenol glycoconjugates were present in the control replicates. No elevation in syringyl glycoconjugates was found from an exposure to phenol or guaiacol vapours.

Table 5.12 Effects of phenol exposures on glycoconjugated phenol levels (µg/kg) in Sauvignon Blanc fruit. *RG*: Rhamnosylglucoside, *GG*: Glucosylglucoside. Data are mean ±1 standard error (n=3); nd, not detected.

	Treatment				
Phenol glycoconjugates	Control without tent	Control with tent	Phenol	Guaiacol	Syringol
Phenol *RG*	0.3±0.03	0.3±0.03	3763.8±376	19.0±1.9	3.6±0.4
Cresol *RG*	1.5±0.2	0.9±0.1	1.4±0.14	15.6±1.6	1.4±0.14
Subtotal	1.8±0.2	1.2±0.13	3765.2±376	34.6±3.5	5.0±0.5
Guaiacol *RG*	nd	nd	8.3±0.8	3946.3±395	7.1±0.7
4-Methylguaiacol *RG*	0.2±0.02	0.2±0.02	0.3±0.03	0.3±0.03	0.3±0.02
Subtotal	0.2±0.02	0.2±0.02	8.6±0.8	3946.6±395	7.4±0.7
Syringol *GG*	0.2±0.02	0.1±0.01	0.2±0.02	0.2±0.02	314.2±31
4-Methylsyringol *GG*	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.01	0.1±0.01
Subtotal	0.3±0.03	0.2±0.02	0.3±0.03	0.3±0.03	314.3±31
Total	2.3±0.2	1.6±0.16	3774.1±377	3981.5±398	326.7±32.6

5.6 Discussion

5.6.1 Syringyl phenol derivatives in gymnosperm smoked Merlot fruit

The pine smoke exposed Merlot fruit described in Chapter 4 was examined to investigate if exposure to smoke from gymnosperm fuels causes an accumulation of sinapyl derived phenols. Investigations into the accrual of putative taint phenols by other researchers (Hayasaka et al. 2010a, Kennison, et al. 2008 and Sheppard et al. 2009) have considered the mechanism of smoke taint in grapevines to be a simple absorption and glycosylation of the phenols present in smoke. The observed significant increase in the concentrations of syringol glycoconjugates in fruit impacted by smoke of the angiosperm, wild oats fuel, may be attributable to exogenous uptake due to the presence of syringyl derivatives in the fuel (Table 3.4) and more significantly in wild oats smoke emissions (Table 3.5). The source of elevated levels of syringols in fruit from pine smoke exposed fruit (Tables 5.1-5.2), however, cannot be similarly attributed since syringyl derivatives were expectedly lacking in this fuel and its smoke emissions. Despite a large difference in total lignin pyrolysed (445 to 78 g respectively), there was no significant difference in the total *p*-hydroxyphenyl, guaiacyl or syringyl derived glycoconjugates present in the pine smoked and oats smoked fruit. As found in Chapter 4, the lignin pyrolysis products that accrue in fruit did not necessarily discern the lignin composition of the pyrolysed fuel. The presence of glycoconjugated syringyl derivatives in the pine smoked fruit demonstrates that the mechanism of accumulating these putative taint compounds is not yeast or malo-lactic bacteria derived but occurs in the fruit at some point during or after the exposure.

5.6.2 Accumulation of putative taint glycoconjugates in grapes from heat exposure

The smoke generation and entrapment trials described in Chapter 4 may have increased the vine canopy temperature for a short duration in some exposures. Ford et al. (1979) found an increase in lignin production in grass species grown at higher temperatures and

angiosperm grapevines may have the same temperature response. If monolignol production and glycosylation (Whetten & Sederoff 1995) is increased in fruit exposed to a temperature increase, there may also be an increase in lignin-derived phenol glycoconjugates in the fruit. The heating of replicated Sauvignon Blanc panels to 50^{o}C for one hour did not produce any changes in volatile or glycoconjugated (Table 5.3) phenols in the fruit and any possible increase in canopy temperatures during smoke exposures (Chapter 4) did not therefore contribute to the accumulation of taint phenols.

5.6.3 Grapevine exposure to cellulose and hemicellulose emissions

The fuels used for smoke exposure trials in Chapter 4 contained high percentages (30-70%) of carbohydrates as cellulose and hemicelluloses. Pyrolysis of these carbohydrates generates the highest proportion of flammable gases (Browne 1958) as well as trace levels of phenol and *o*-cresol (Wodley 1971). This experiment assessed whether some of the phenols, including syringol, that accumulated in grapes could originate from the combustion of the carbohydrate fractions of the fuels used in smoke exposure trials.

The Arches 88 paper used for pyrolysis is manufactured from cotton linters which are biologically unique in being almost pure cellulose (van Soest & McQueen 1973). The *Py*-GC-MS analysis was found to contain phenol (~1% of total pyrogram area count) and *o*-cresol (~0.1% of total pyrogram area count) consistent with cellulose pyrolysis where these compounds can occur in minor amounts from carbohydrate rearrangement reactions (Almendros et al. 1997, Pastorova et al. 1994, Pouwels et al. 1989).

The TD GC-MS analysis of Arches 88 paper pyrolysis, used in Chardonnay smoking trials, also contained minor amounts of phenol (~1% of total pyrogram area count) and *o*-cresol (~0.1% of total pyrogram area count), consistent with the findings of Tóth and Potthast (1984). The pyrolysis temperatures recorded in our trials replicated wildfire temperatures (Gould et al. 2007) and both the flaming period and extended smouldering period covered the temperatures where phenol and *o*-cresol are produced from carbohydrate rearrangement reactions (Tóth & Potthast 1984).

The paper exposed Chardonnay vines were found to have significantly elevated phenol and cresol glycoconjugates compared to the unexposed control vines. Despite very low phenol and *o*-cresol levels in paper emissions compared to wild oats emissions (*cf* Table 3.5- Chardonnay paper and wild oats TD GC-MS, data not shown) the fruit of paper exposed Chardonnay vines were not significantly different in phenol rutinoside levels compared to the oats exposed fruit (17.3 vs 23.2 µg/kg). Although not the purpose of this experiment, it highlights the possibility of sequestering large amounts of putative taint phenol glycoconjugates from a small concentration of phenol in smoke for a short duration. The exposure of grapevines to the pyrolysis of the cellulose and hemicellulose fractions did not increase the formation of putative taint phenols (other than those present in the emissions) in fruit or wines. Thus, while cellulose smoke exposure can elevate phenol and *o*-cresol, it appears unlikely that the increased syringyl derived phenols in smoke exposed fruit/wine originated from the carbohydrate fraction of the pyrolysed fuels.

5.6.4 Phenol glycoconjugate accumulation in fruit after pure phenol exposure

A minor concentration of phenol and *o*-cresol in smoke was found to elevate phenol and *o*-cresol in Chardonnay fruit (Table 5.4 and 5.5), indicating that only a small exposure of phenols may be needed to significantly elevate putative taint phenols. Although these minor phenol and *o*-cresol exposures were not found to significantly elevate coniferyl or sinapyl derived phenol glycoconjugates in Chardonnay fruit, tomatoes and Sauvignon Blanc vines were exposed to pure phenols to test if higher levels of exposures could produce phenol transformations.

Both tomato and Sauvignon Blanc fruit accumulated significantly higher glycoconjugates of the aglycone used for exposure. In Sauvignon Blanc fruit the phenol glycoconjugates from phenol and guaiacol compound exposure were over 25 times that found from smoke exposure (*cf.* Tables 5.12 and 6.2). Despite the high concentration of phenol and guaiacol rhamnosylglucoside in the phenol and guaiacol exposed fruit, *in*

planta transformation of the xenobiotically acquired hydroxyl- and methoxy-phenols into dimethoxy-phenol was not observed.

5.7 Conclusion

While the presence of syringol derivatives in fruit exposed to gymnosperm emissions has confirmed they are derived from grapevine exposure to vegetation pyrolysis, the mechanism for the formation of these derivatives remains unresolved. The series of experiments from this chapter have also shown that phenols taken up from smoke/gas/vapour are largely sequestered as glycoconjugates or stored as such, with only limited transformation.

CHAPTER 6

VARIETAL DIFFERENCES IN THE ACCUMULATION OF LIGNIN-DERIVED SMOKE TAINT PHENOLS

6.1 Introduction

Wine grapes exposed to smoke from wildfires and prescribed burns produce wines with elevated levels of volatile and glycoconjugated phenols (Kennison et al. 2007, Hayasaka et al. 2010a, 2013, Singh et al. 2012, Kelly et al. 2012), which impart unpleasant characters to wines including burnt, smoky, medicinal and dirty aromas and flavour attributes (Kennison et al. 2009, Ristic et al. 2011, Parker et al. 2012). Expectedly, such wines have low consumer acceptance and smoke exposure can cause significant negative economic impact on grape growers and winemakers (Whiting & Krstic 2007). It is highly probable that future climate change will increase the risk of wildfires in Australia (Pitman et al. 2007) and the economic impact of smoke taint will likely also increase. Strategies to mitigate the risk may include planting varieties that sequester less smoke-derived phenols as taint, however from the work reported to date it is unclear whether different wine grape cultivars accumulate different levels of smoke-borne phenols under comparable smoke exposure conditions. Putative cultivar differences in levels of phenols from wildfire smoke exposed fruit have been reported (Singh et al. 2012, Dungey et al. 2011, Hayasaka et al. 2010a), but determination of cultivar differences from such data is difficult due to variations in smoke exposure (intensity and duration) as well as in the occurrence of a smoke exposure event vis-à-vis vine phenology (Kennison et al. 2009).

To determine if cultivar differences in uptake and accumulation of smoke-borne phenols in wine grapes occurs, three cultivars, Chardonnay, Sauvignon Blanc and Merlot, were exposed to smoke in vineyard trials. To minimise potential confounding factors and idiosyncratic outcomes, the smoke exposure experiments were replicated with identical exposure conditions (*i.e.*, fuel composition, mass, pyrolysis of fuel) and smoked at the same phenological stage (14 days post-veraison) in commercial vineyards.

6.2 Materials and methods

6.2.1 Fuel types and fuel compilation

The experiments reported here were carried out in vineyards situated in the Margaret River wine region (33º57′S, 115º01′E) in the south west of Western Australia. Two fuels, the softwood species radiata pine (*Pinus radiata* D. Don) and a pasture grass, wild oats (*Avena fatua* L.), were compiled and prepared as described in Chapter 3 to compare uptake of smoke-borne phenols between cultivars.

6.2.2 Grapevine smoke exposure

The smoke exposure experiments were designed and executed as detailed in Chapter 3. Briefly, the experiments were set up as randomised block designs in commercial vineyards. Three cultivars were chosen for exposure: *Vitis vinifera* L. cv. Sauvignon Blanc, Chardonnay and Merlot. The vineyard exposure trials were set up following the methodology described in Chapters 4 and 5.

6.2.3 Chemical analysis

The fruit from each replicate panel was harvested separately at commercial maturity, total soluble solids ~23°Brix, six weeks after application of smoking treatments. Grape samples were analysed for seven volatile phenols and fourteen phenol glycoconjugates, as described by Hayasaka et al. (2013). Volatile and glycoconjugated phenols were analysed on five and three replicate samples, respectively.

6.2.4 Statistical analysis

Data were analysed using SPSS 20 (IBM® SPSS® Statistics). Reported treatments effects are significant at $p < 0.05$.

6.3 Results

6.3.1 Accretion of smoke-borne phenols in grapes

a) Volatile phenols

Volatile phenols are expected to occur, albeit at very low concentrations, constitutively in lignin-bearing plants, or parts thereof. Accordingly, for all three cultivars, the concentrations of volatile phenols in grapes from the unsmoked control vines were either below the limit of quantitation of the analytical method used (< 2.5 nmoles/kg) or present at trace levels. Smoke exposure early in the grape ripening phase significantly elevated concentrations of many of the volatile phenols, particularly in Sauvignon Blanc grapes (Table 6.1). Nonetheless, the total volatile phenols concentrations in smoke exposed grapes were ≤ 331 nmoles/kg. In Chardonnay, only *o*-cresol was present at quantifiable levels. Generally, cultivar responses to smoke exposure in terms of the levels of individual volatile phenols and/or their total pools in grapes were of the order: Sauvignon Blanc >> Merlot > Chardonnay. Where smoke exposure increased the total pool of volatile phenols, the major contributors were the cresol isomers, guaiacol and syringol. While smoke exposure affected the levels of volatile phenols in grapes, there was no consistent effect of fuel type (smoke source) across cultivars or phenol types.

Table 6.1 Effects of fuel type and smoke exposure on volatile phenol levels (nmoles/kg) in fruit of three cultivars.

Data are mean ±1 standard error (n=5, except Sauvignon Blanc Wild Oats and Chardonnay where n=3); nd, not detected.

	Cultivar							
	Sauvignon Blanc			Chardonnay		Merlot		
Volatile phenols	Control	Pine	Wild Oats	Control	Wild Oats	Control	Pine	Wild Oats
o-Cresol	nd	136.9±15.9	175.7±14.1	nd	20.0±1.5	nd	25.9±8.0	33.3±6.3
m-Cresol	nd	24.0±2.3	24.7±3.1	nd	nd	nd	nd	nd
p-Cresol	nd	20.3±1.8	nd	nd	nd	nd	nd	nd
Subtotal	nd	181.2±19.1	200.4±17.2	nd	20.0±1.5	nd	25.9±8.0	33.3±6.3
Guaiacol	nd	59.6±14.3	83.2±11.7	nd	nd	nd	17.7±5.3	27.4±5.5
4-Methylguaiacol	nd	31.8±5.4	14.5±0.1	nd	nd	nd	4.3±2.9	nd
Subtotal	nd	91.4±19.7	97.7±11.7	nd	nd	nd	22.0±7.5	27.4±5.5
Syringol	nd	40.2±11.5	32.4±23.4	nd	nd	25.9±8.5	20.8±2.4	16.9±7.3
4-Methylsyringol	nd	nd	nd	nd	nd	nd	nd	nd
Subtotal	nd	40.2±11.5	32.4±23.4	nd	nd	25.9±8.5	20.8±2.4	16.9±7.3
Total	nd	312.8±36.2	330.5±19.8	nd	20.0±1.5	25.9±8.5	68.7±14.2	77.6±14.9

b) Glycoconjugated phenols

Depending on cultivar, grapes from the unsmoked vines contained up to 175 nmol/kg endogenous total glycoconjugated phenols including glucosylglucosides (GG), pentosylglucosides (PG) and rhamnosylglucosides (RG). Smoke exposure, averaged across fuel types and cultivars, increased the total pool of grape glycoconjugated phenols by >14-fold (96 *vs*. 1392 nmol/kg, Table 6.2). The source of smoke (fuel type) however had no significant effect. Similarly, there were no significant cultivar effects on the total glycoconjugated phenols of grapes at commercial harvest, nor were there significant cultivar by fuel type interactions.

Table 6.2 Effects of fuel type and smoke exposure on glycoconjugated phenol levels (nmoles/kg) in fruit of three cultivars. *PG*: Pentosylglucoside, *RG*: Rhamnosylglucoside, *GG*: Glucosylglucoside.
Data are mean ±1 standard error (n=5, except Sauvignon Blanc Wild Oats and Chardonnay where n=3); nd, not detected.

	Cultivar							
	Sauvignon Blanc			Chardonnay		Merlot		
Phenol glycosides	Control	Pine	Wild Oats	Control	Wild Oats	Control	Pine	Wild Oats
Phenol *PG*	6.1±0.3	178.2±20.2	167.1±29.0	25.3±0.2	302.1±20.2	3.3±0.4	75.5±10.7	84.8±11.9
Phenol *RG*	3.4±0.5	135.8±16.2	203.9±44.4	1.7±0.1	57.7±3.7	0.9±0.1	45.4±7.3	59.2±10.9
Cresol *PG*	12.8±0.2	273.4±42.1	301.6±52.9	53.7±1.2	443.6±25.2	21.2±2.3	294.6±63.3	287.4±47.0
Cresol *RG*	5.4±0.3	183.9±28.3	180.2±19.9	6.1±0.3	118.8±8.5	1.9±0.3	84.1±15.3	79.7±13.0
Guaiacol *GG*	0.7±0.01	55.6±11.2	95.8±8.7	2.7±0.1	5.6±0.3	1.8±0.3	68.4±13.7	101.0±20.8
Guaiacol *PG*	8.7±0.5	211.7±38.4	276.4±31.9	39.1±0.5	140.2±7.8	12.6±0.7	276.1±64.2	350.0±73.0
Guaiacol *RG*	1.9±0.1	100.5±19.7	158.9±6.7	1.7±0.2	20.0±1.9	0.9±0.1	36.3±7.3	50.1±9.3
4-Methylguaiacol *GG*	0.2±0.01	33.3±7.5	19.1±2.3	1.4±0. 1	1.3±0.2	0.3±0.01	28.2±9.4	15.0±4.2
4-Methylguaiacol *PG*	1.5±0.1	83.5±15.1	34.4±1.3	13.0±0.2	39.3±2.6	2.5±0.3	79.4±28.1	43.3±9.6
4-Methylguaiacol *RG*	2.0±0.2	106.3±19.4	52.9±2.4	6.2±0.2	22.9±1.1	1.2±0.1	73.6±13.1	41.6±6.0
Syringol *GG*	4.3±0.3	51.9±14.7	168.6±19.9	6.5±0.1	55.5±6.2	4.3±0.5	71.0±23.9	209.2±59.5
Syringol *PG*	5.1±0.2	15.5±3.0	35.9±3.7	11.5±0.2	31.0±1.8	6.1±0.7	15.2±4.1	31.1±7.8
4-Methylsyringol *GG*	0.6±0.01	7.6±1.8	14.9±3.0	2.8±0.1	13.3±1.3	0.7±0.1	13.9±6.2	22.7±6.8
4-Methylsyringol *PG*	1.3±0.1	3.0±0.5	4.3±0.9	2.6±0.1	6.7±0.4	1.4±0.1	4.2±1.1	4.8±0.9
Total	54.0±2.2	1440.2±206.0	1714.0±174.5	174.3±1.9	1258.0±74.4	59.1±4.0	1165.9±258.1	1379.9±260.7

6.4 Discussion

The total concentration of volatile phenols in smoke exposed Chardonnay and Merlot grapes observed in this study were comparable to those reported by Hayasaka et al. (2010a) in bushfire-exposed grapes of Chardonnay and Cabernet Sauvignon. This similarity from contrasting smoke exposure conditions suggests that once the smoke-borne volatile, toxic and reactive phenols (Whetten & Sederoff 1995) are absorbed into berries, these are sequestered into physiologically compatible complexes by binding with mono-, di-, and oligo-saccharides (Hayasaka et al. 2010a). However, the presence of up to 331 nmoles/kg of total volatile phenols observed in Sauvignon Blanc grapes suggests plasticity exists among cultivars in volatile phenol accumulation in grapes. Earlier work suggested cultivar sensitivity in the accumulation of smoke-borne phenols (Whiting & Krstic 2007), although it was not clear whether the cultivars were at a similar stage of berry development when the smoke exposure event occurred. This is an important consideration in determining cultivar differences since the uptake of smoke-borne phenols changes markedly throughout berry development (Kennison et al. 2009). The results of this study show that when smoke exposure events occur at a comparable stage of berry development (in this case, 14 days post-veraison), there is little difference among the cultivars in the accumulation of total glycoconjugated phenols. These observations underscore the importance of standardising smoke exposure conditions (duration, intensity as well as timing in relation to grape development) and further suggest that the apparent differential cultivar sensitivities of earlier reports may reflect more ontogenetic and exposure conditions than intrinsic cultivar differences.

Although cultivar and fuel type had little influence on the concentrations of total glycoconjugated phenols in grapes, both treatments affected the composition. For example, while the white cultivars accrued equivalent levels of total phenol and total cresols, the levels of these phenols in the red cultivar, Merlot, were significantly lower (i.e., SB = CH > M). This apparent red *vs*. white cultivar dichotomy in phenol uptake is clearly not universal since accretion of total guaiacol was similar between Sauvignon Blanc and Merlot (~445 nmoles/kg), which was higher than the ~≤ 166 nmoles/kg

observed in Chardonnay. Further quantitative cultivar differences were also evident when composition was considered by the glycone moieties of glycoconjugated phenols. While a clear cultivar pattern was not apparent across all the glycoconjugate types and all phenols, for all the rhamnosylglucoside conjugates (RG), the following cultivar ranking was evident: Sauvignon Blanc > Chardonnay = Merlot. Clearly, in glycosylated form, these phenols are aroma and perhaps flavour inactive. Whether such differential prevalence of diglycosides, which require sugar-specific exoglycosidases for cleavage of the sugar-sugar bonds that makes the resultant phenolic monoglucoside amenable to attack by a glucosidase and release of sensorially potent volatile phenols (Sarry & Günata 2004), influences the extent to which smoke taint can evolve is unclear.

The glycoconjugated phenols that responded to the fuel source of smoke were 4-methylguaiacol–PG, 4-methylguaiacol–RG, syringol–GG and syringol–PG. These responses, in part, reflected the lignin composition of the fuels. Thus, for example, grapes exposed to the smoke of the pine fuel, whose lignin contains relatively high 4-methylguaiacol compared to the oat fuel (Table 3.4, also see Kelly et al. 2012), accrued significantly higher levels of 4-methylguaiacol–PG and 4-methylguaiacol –RG than oat-smoke exposed grapes. The reverse was the case for the syringol diglycosides. Grapes exposed to smoke of oat fuel, which has a high level of syringols in its lignin compared to pine fuel (Table 3.4, also see Kelly et al. 2012), contained significantly higher levels of syringol–GG and syringol–PG than pine-smoke exposed grapes (Table 6.2). The detection of elevated concentrations (~7 times the background levels, Table 6.2,) of syringol diglycosides in Sauvignon Blanc grapes exposed to pine smoke, also shows that the accretion of phenols in white grapes does not bear a stoichiometric relationship to the phenol composition of the smoke's fuel source as reported in Chapter 4. Across cultivars and fuel types, the dominant (70-85%) contributors to the total glycoconjugated phenol pool were the diglycosides of phenol, cresol and guaiacol (Table 6.2). Interestingly, glycoconjugates of syringol and 4-methylsyringol made up $\leq$ 20% of the total smoke-derived phenol glycoconjugates in grapes. These results contrast with those reported in Hayasaka et al. (2010a, 2013), in which syringol-GG was the single most dominant contributor to the total phenolic diglycosides in grapes of a

range of cultivars exposed to bushfire smoke. The source of this variance for the relative contributions is not clear, apart from methodological differences in smoke generation (experimental *vs*. wildfire smoke) as well as intensities and durations of exposure. For the three cultivars evaluated here, when exposures were timed to occur at a comparable stage of berry development (early in the berry ripening phase), no significant cultivar sensitivity was observed in accretion of total phenols in grapes, although the phenol composition was variable.

CHAPTER 7

EFFECT OF WINEMAKING METHODS ON EXTRACTION OF SMOKE-DERIVED PHENOLS FROM GRAPES INTO WINES

7.1 Introduction

When vineyards are impacted by smoke, viticulturists and winemakers endeavour to determine the organoleptic impact the smoke event will have on finished wines. While no significant cultivar sensitivity to the uptake of putative smoke taint phenols has been found (Chapter 6, Kelly et al. 2014), our current understanding of transformations and estimates of the expected levels of phenols in finished wines due to differences in traditional winemaking (white and red) is incomplete. Recently, there have been advances in the analysis of phenols in smoke affected fruit and resultant wines (Hayasaka et al. 2013), however estimates of the expected proportions of volatile phenols and glycoconjugated phenols extractable from grapes into wines (that can be used as industry guidelines) may not be fully inferred from these reports, either due in part to the use of non-standard winemaking (Hayasaka et al. 2010a) or to the limited range of glycoconjugated phenols reported (Ristic et al. 2011). In smoke affected grapes, a high proportion of glycoconjugated phenols are sequestered in the skin (Dungey et al. 2011) and skin maceration and contact during winemaking may affect the extraction of phenol glycoconjugates into wine. It is therefore imperative to understand the likely extraction levels of a comprehensive range of glycoconjugated phenols from smoke impacted grapes under the commonly used white and red winemaking practices.

The organoleptic impact of smoke taint in wine is influenced by the distribution of volatile and glycoconjugated phenols (Parker et al. 2012). Red wine ferments are often subjected to malolactic fermentation (MLF) by inoculation with lactic acid bacteria and the metabolic activity of lactic acid bacteria can influence wine aroma complexity by hydrolysing wine aroma glycoconjugates (Ugliano et al. 2003, D'Incecco et al. 2004).

The effect of MLF on the distribution of glycoconjugated phenols however, is yet to be reported.

The smoke exposed fruit described in Chapter 6 has been processed with three different traditional red and white winemaking techniques, including when fruit is de-stemmed, crushed and pressed and when fruit is whole bunch pressed, to determine the likely proportions of glycoconjugated phenols that are extracted from grapes into finished wines. It is expected that results may provide guidelines for expected smoke-derived phenols in wines from different winemaking styles based on levels determined in affected grapes. Additionally, a paired comparison of MLF Merlot wines (described in Chapter 4) and non-MLF Merlot wines will test whether the glycosidase activity of lactic acid bacteria contributes significantly to hydrolysis of glycoconjugated phenols to volatile phenols.

7.2 Materials and methods

7.2.1 Fuel types, fuel compilation and smoke exposures

The Chardonnay, Sauvignon Blanc and Merlot fruit used for this chapter are as described in Chapter 6.

7.2.2 Winemaking

The fruit from each replicate panel was harvested separately at commercial maturity, total soluble solids ~23°Brix (Reichert AR 200 digital refractometer, Reichert Inc., New York, U.S.A.), six weeks after the application of smoking treatments. The Chardonnay and Sauvignon Blanc wines were made by traditional white winemaking methods where there was minimal skin contact before commencement of fermentation. The Sauvignon Blanc replicates were separately de-stemmed, crushed and pressed, while the Chardonnay replicates were whole bunch pressed, each with the addition of 100 ppm

potassium metabisulphite (Chem Supply AR grade, Gillman S.A. Australia). For both varieties the must was inoculated with *Saccharomyces cerevisiae* EC1118 (Lallemand Inc., Montreal, Canada) at 300 ppm and supplemented with 100 ppm diammonium phosphate (Sigma- Aldrich, Sydney, Australia). Each replicate was fermented in 25 litre glass demijohns to dryness (<1 g/l residual sugars), racked from gross lees with the addition of 60 ppm potassium metabisulphite and cold stabilised at -4°C for 21 days. The wines were filtered through a 0.2 μm pore size cartridge (Sartorius Sartopure 2 Maxicap, Sartorius, Gottingen, Germany) and bottled under food grade nitrogen with stelvin closures.

The Merlot wines were made by traditional red winemaking methods as described in Chapter 4. After the ferments progressed to dryness (<1g/l residual sugars), the wines were racked from gross lees, and divided into two equal halves by volume. The first half (non-MLF wines) were cold stabilised at -4°C for 21 days with the addition of 60 ppm potassium metabisulphite and the second half (MLF wines) were inoculated with *Oenococcus oeni* (Viniflora CH 16, CHR Hansen, Denmark) at 10 ppm to initiate malolactic conversion. The MLF replicates were kept at 23°C until malic acid concentrations dropped to <0.1 g/l (19 - 60 days) and subsequently cold stabilised at -4°C for 21 days with the addition of 60 ppm potassium metabisulphite. Both the non-MLF and MLF wines were filtered as described above.

7.2.3 Chemical analysis

Wine samples were analysed for seven volatile phenols and fourteen phenol glycoconjugates, as described by Hayasaka et al. (2013). Volatile and glycoconjugated phenols were analysed on five and three replicate samples, respectively 18 to 24 months after bottling.

7.2.4 Statistical analysis

Data were analysed using SPSS 20 (IBM® SPSS® Statistics). Reported treatment effects are significant at $p < 0.05$.

7.3 Results

7.3.1 Influence of winemaking techniques on wine phenols

a) Volatile phenols

The traditional methods of winemaking are different for the three cultivars used in this study and influenced the wine volatile phenol levels (Table 7.1). For both control and smoke exposure treatments, Chardonnay wines made from whole bunch pressed juice had no measurable concentrations (<2.5 nmoles/kg) of volatile phenols, as was generally the case in the grapes (Table 6.1).

Table 7.1 Effects of fuel type and smoke exposure on volatile phenol levels (nmoles/kg) in wine of three cultivars. Data are mean ±1 standard error (n=5, except Sauvignon Blanc Wild Oats and Chardonnay where n=3); nd, not detected.

	Cultivar							
	Sauvignon Blanc			Chardonnay		Merlot		
Volatile phenols	Control	Pine	Wild Oats	Control	Wild Oats	Control	Pine	Wild Oats
o-Cresol	nd	11.1±3.5	12.3±8.2	nd	nd	nd	77.7±18.2	88.8±11.9
m-Cresol	nd	27.7±9.2	27.7±16.0	nd	nd	nd	33.3±8.6	33.3±4.7
p-Cresol	nd	9.2±4.1	nd	nd	nd	nd	27.7±7.2	20.3±1.8
Subtotal	nd	48.0±14.1	40.0±22.2	nd	nd	nd	138.7±33.7	142.4±17.2
Guaiacol	nd	43.5±9.7	64.4±20.3	nd	nd	40.3±2.5	178.8±30.9	207.8±25.1
4-Methylguaiacol	nd	13.0±4.2	nd	nd	nd	nd	60.8±15.6	34.7±4.8
Subtotal	nd	56.5±13.8	64.4±20.3	nd	nd	40.3±2.5	239.6±46.2	242.5±29.8
Syringol	nd	15.6±4.4	45.4±9.9	nd	nd	122.0±8.0	131.0±6.9	205.0±25.2
4-Methylsyringol	nd	nd	nd	nd	nd	nd	nd	nd
Subtotal	nd	15.6±4.4	45.4±9.9	nd	nd	122.0±8.0	131.0±6.9	205.0±25.2
Total	nd	120.1±28.0	149.8±51.7	nd	nd	162.3±9.0	509.3±81.4	589.9±58.6

Wines from the control treatments of Sauvignon Blanc and Merlot grapes had no measurable (<2.5 nmoles/kg) volatile phenols except guaiacol and syringol in the Merlot wines which were fermented on skins (Table 7.1). In contrast to the Chardonnay wines from whole bunch pressed juice, Sauvignon Blanc and Merlot wines from the smoke exposed, crushed and de-stemmed grapes had elevated levels of six volatile phenols (Table 7.1). Between these two latter groups, however, significantly higher volatile phenols were present in wines that were de-stemmed, crushed and fermented on skins (Merlot) than in wines made from de-stemmed, crushed and pressed grape juice (Sauvignon Blanc) (Table 7.1). Interestingly, the volatile phenol levels were comparable in smoke affected Sauvignon Blanc grapes and the resultant wines, whereas in Merlot, higher levels were found in wines than in grapes (*cf*. Table 6.1 and 7.1).

b) Glycoconjugated phenols

The total glycoconjugated phenol concentrations significantly differed between Chardonnay (whole bunch pressed without crushing), Sauvignon Blanc (de-stemmed, crushed and pressed) and Merlot (de-stemmed, crushed and fermented on skins) wines in approximately 2:5:10 ratios, respectively (Table 7.2).

Table 7.2 Effects of fuel type and smoke exposure on glycoconjugated phenol levels (nmoles/kg) in wine of three cultivars. *PG*: Pentosylglucoside, *RG*: Rhamnosylglucoside, *GG*: Glucosylglucoside. Data are mean ±1 standard error (n=5, except Sauvignon Blanc Wild Oats and Chardonnay where n=3); nd, not detected.

	Cultivar							
	Sauvignon Blanc			Chardonnay		Merlot		
Phenol glycosides	Control	Pine	Wild Oats	Control	Wild Oats	Control	Pine	Wild Oats
Phenol *PG*	6.0±0.6	141.1±21.5	114.5±24.4	10.8±0.4	41.3±1.3	4.9±0.5	164.6±24.6	147.1±15.7
Phenol *RG*	1.4±0.2	59.7±3.6	60.8±16.7	0.6±0.2	19.0±1.2	1.1±0.1	74.9±7.3	90.1±8.7
Cresol *PG*	9.7±0.6	170.8±28.4	148.3±24.4	16.6±1.1	94.5±5.3	22.3±2.3	265.4±36.1	266.0±23.5
Cresol *RG*	3.1±0.6	76.8±9.3	55.1±7.0	0.8±0.1	19.8±1.3	2.7±0.3	105.7±9.9	121.0±12.5
Guaiacol *GG*	0.3±0.01	0.6±0.2	1.6±0.7	0.2±0.01	0.2±0.01	2.0±0.5	7.0±0.8	8.2±1.4
Guaiacol *PG*	6.2±0.5	113.0±17.3	128.5±8.7	11.8±0.7	42.8±2.2	14.2±1.2	319.2±52.7	387.8±42.8
Guaiacol *RG*	1.0±0.1	35.3±8.3	38.0±2.9	0.4±0.01	5.7±0.2	1.1±0.1	46.9±5.7	76.1±9.4
4-Methylguaiacol	nd	0.2±0.01	0.1±0.01	nd	nd	0.2±0.01	2.7±0.8	2.2±0.3
4-Methylguaiacol *PG*	0.9±0.1	52.2±8.2	21.1±1.1	2.6±0.2	7.1±0.6	3.2±0.5	108.5±19.4	66.6±9.9
4-Methylguaiacol *RG*	0.8±0.1	25.4±3.2	11.0±0.8	0.6±0.1	3.8±0.5	1.6±0.2	70.5±6.0	54.1±5.9
Syringol *GG*	1.1±0.1	6.0±1.4	19.9±3.7	1.8±0.3	9.4±0.6	2.7±0.4	49.3±13.6	129.6±25.9
Syringol *PG*	1.8±0.2	4.2±0.8	6.8±0.6	2.7±0.1	5.3±0.4	8.6±0.6	20.0±2.9	38.8±6.0
4-Methylsyringol *GG*	0.1±0.01	0.6±0.1	1.2±0.2	0.1±0.01	0.6±0.01	0.2±0.01	4.8±1.4	10.3±2.6
4-Methylsyringol *PG*	0.3±0.01	0.5±0.1	0.6±0.1	0.3±0.01	0.7±0.01	10.4±0.6	16.6±2.1	15.9±0.9
Total	32.7±2.2	686.4±76.6	607.5±85.5	49.3±0.8	250.2±10.9	75.2±3.9	1256.1±173.9	1413.8±139.1

Fruit processing and winemaking methods also had significant influence on the concentrations of all 14 glycoconjugated phenols (Table 7.2). With the exceptions of syringol-PG and 4-methylsyringol-PG, the ranking of the concentrations of the remaining 12 glycoconjugated phenols among the three wines was the same as that for the total glycoconjugated phenols, i.e., Merlot > Sauvignon Blanc > Chardonnay. Of the total pool, the diglycosides of cresol and phenol contributed the largest components (ranging from 44% in Merlot wines to 70% in Chardonnay). The second largest class of phenol contributors were the diglycosides of guaiacol, accounting for between 20% (Chardonnay wines) and 33% (Merlot wines). Collectively, the phenol, cresol and guaiacol diglycosides made up 78% (Merlot) and 89% (Chardonnay and Sauvignon Blanc) of the total phenolic glycoconjugates in these wines. The contributions of the syringol and 4-methylsyringol glycoconjugates to the total pool were only ~10% or less. The relative abundance of the different phenol classes in wines is broadly comparable to the respective proportions observed in grapes (data not shown but compare Table 6.2 and 7.2). Averaged across cultivars, wines from smoke exposed grapes had more than 16 times the total phenol glycoconjugate concentration than the control wines (Table 7.2). The fuel source of smoke had no influence on the total phenol glycoconjugates. Of the individual phenol glycoconjugates, however, pine smoke exposure generally tended to produce higher concentrations of phenols of the *p*-hydroxyphenyl and guaiacyl–lignin origin, although the fuel effects were significant only for 4-methylguaiacol–PG and 4-methylguaiacol–RG. By contrast, wines made of wild oats smoke exposed fruit had significantly higher concentrations of phenols of the syringyl-lignin provenance (particularly, syringol–GG and syringol–RG) than the wines from the pine smoke treatment (Table 7.2). These differences broadly mirror the fruit glycoconjugate results.

7.3.2 Effect of winemaking method on extraction of grape glycoconjugated phenols into wines

The extraction of total glycoconjugated phenols from grapes into wines significantly varied among the three wines (Figure 7.1). Merlot wines, which were made according to standard red winemaking practices (i.e., fermented on skin until dryness), extracted 88%

of the grape glycoconjugated phenols. The extraction rates for the white wines, which did not involve skin contact, were considerably lower (averaging roughly 25% of the grape total glycoconjugated phenols). However, this average masks effects of different fruit processing/handling methods that are customarily used in white winemaking. Sauvignon Blanc wines, made following crushing of fruit prior to pressing, extracted 39% of fruit total glycoconjugated phenols, approximately twice the extraction rates of Chardonnay wines (~18%) from whole bunch pressed must without crushing.

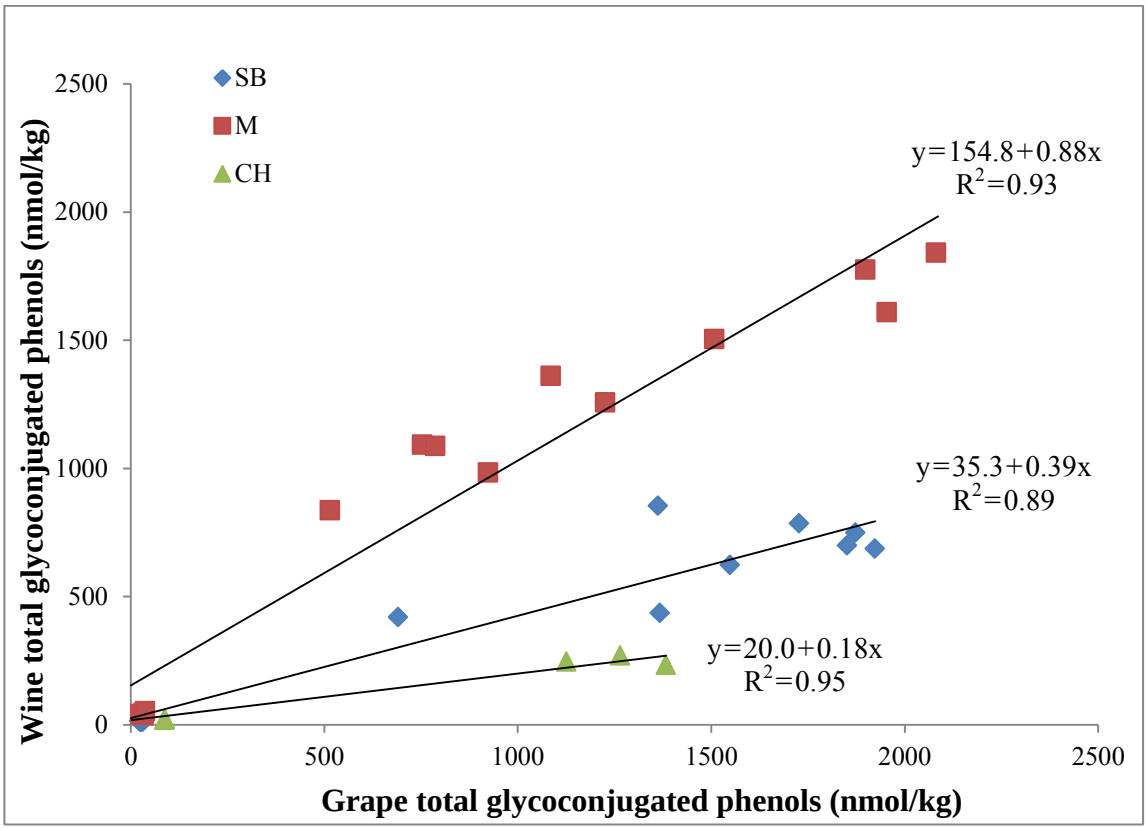


Figure 7.1 Apparent extraction rates of total phenol glycoconjugates from grapes to wine using whole bunch pressing (CH), crushing, de-stemming and pressing (SB) and fermentation on skins (M).

The extraction rates of the grape glycoconjugated phenols into wines were also different for different phenol classes and wines. In whole bunch pressed Chardonnay, the extraction of glycoconjugated phenols of the *p*-hydoxyphenyl, guaiacyl and syringyl classes were 14, 19 and 11%. In Sauvignon Blanc, the corresponding extraction rates were 39, 27 and 11%. By comparison, extraction rates of 90, 75 and 74% were observed for Merlot wines. Differential extraction rates also occurred between glycoside type and phenol glycoside type. For example, guaiacol glucosylglucoside had very low extraction (≤ 7%) regardless of winemaking style and a low extraction (~ 10%) was observed for syringol glucosylglucoside in both Sauvignon Blanc and Chardonnay wines (Tables 7.3)

Table 7.3 Effects of smoke exposure on extraction (%) of glycoconjugated phenols (nmoles/kg) from fruit into wine. Extraction values (%) are calculated on an aqueous content of 70% present in fruit irrespective of variety. *PG*: Pentosylglucoside, *RG*: Rhamnosylglucoside, *GG*: Glucosylglucoside. Data are mean ±1 standard error (n=10, except Sauvignon Blanc Wild Oats and Chardonnay where n=6); nd, not detected.

Phenol glycosides	Sauvignon Blanc	Chardonnay	Merlot
Phenol *PG*	51.8±3.0	9.6±0.5	139.8±9.0
Phenol *RG*	27.8±3.2	23.4±2.7	119.3±8.9
Cresol *PG*	40.5±2.7	15.0±0.9	68.4±4.6
Cresol *RG*	27.5±3.1	11.9±1.6	102.9±6.8
Guaiacol *GG*	0.9±0.2	2.7±0.8	7.1±0.7
Guaiacol *PG*	36.6±2.1	21.6±2.3	87.3±7.1
Guaiacol *RG*	22.9±3.2	20.2±2.3	107.6±9.1
4-Methylguaiacol *GG*	0.6±0.2	nd	9.9±1.7
4-Methylguaiacol *PG*	45.1±3.6	12.9±1.8	114.9±8.8
4-Methylguaiacol *RG*	16.9±1.7	11.8±1.9	83.4±6.4
Syringol *GG*	9.7±1.5	12.3±1.9	63.3±16.1
Syringol *PG*	17.4±2.1	12.3±1.6	102.5±9.5
4-Methylsyringol *GG*	5.9±1.1	3.1±0.8	39.2±7.8
4-Methylsyringol *PG*	11.1±0.9	7.0±0.4	289.8±35.3
Total	31.0±2.8	14.0±1.1	80.1±5.9

7.3.3 Effect of MLF on extraction and hydrolysis of glycoconjugated phenols in Merlot wines

Comparisons of Merlot wines with and without MLF showed no significant changes in the concentration of total volatile phenols, total glycoconjugated phenols or of the phenol components (Figure 7.2).

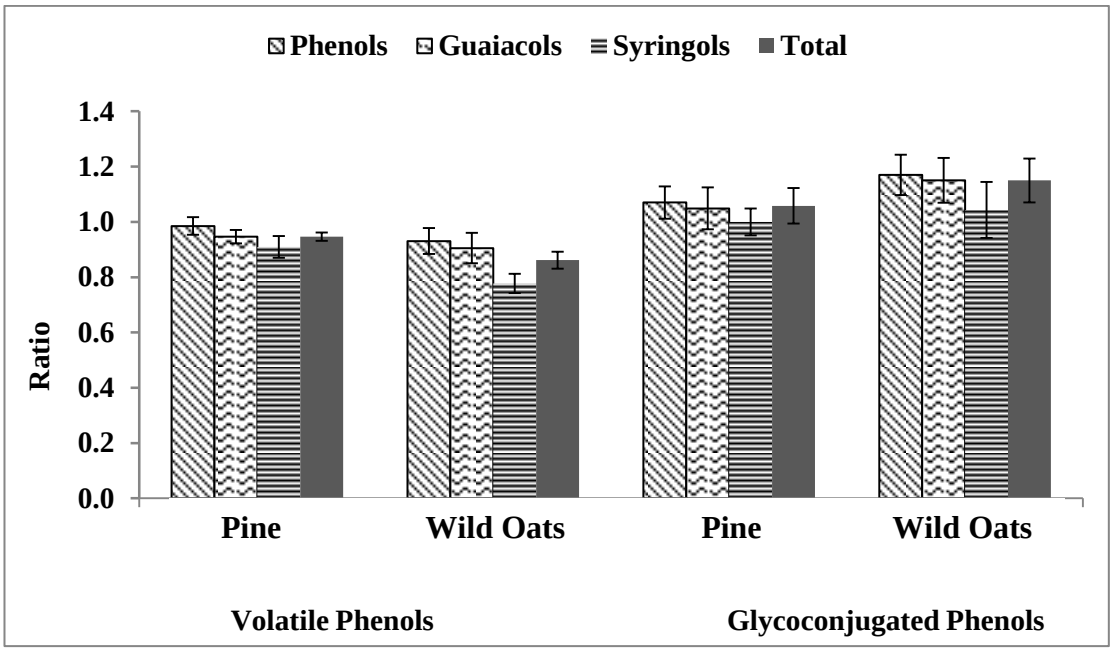


Figure 7.2 Ratios of non-MLF to MLF wines for volatile and glycoconjugated phenols shown by fuel type. Data are means ±1 standard error. For reference, the data for the MLF wines is shown in Tables 7.1 and 7.2.

7.4 Discussion

The traditional methods of winemaking are different for the three cultivars used in this study. Thus, cultivar effects on volatile and glycoconjugated phenols are necessarily subsumed in the winemaking method effects. Although smoke exposed grapes generally contained low concentrations of volatile phenols (Table 7.1), the apparent absence of volatile phenols in the Chardonnay wines was unexpected. This is indicative of a low overall extraction of volatile phenols into whole bunch pressed juice and subsequent negligible hydrolysis of the diglycoside bound phenols during

and/or post-fermentation. These findings contrast with high concentrations of volatile phenols observed in bushfire smoke exposed Chardonnay fruit fermented on skins (Hayasaka et al. 2010a) or in wines made after crushing and pressing Chardonnay juice (Singh et al. 2012) and highlight the effects of processing and/or winemaking methods on extraction of phenols.

Wines made with minor skin contact from de-stemmed crushed and pressed grape juice (Sauvignon Blanc) contained volatile phenols comparable to the levels found in smoke affected grapes, whereas fermentations with extended skin contact (Merlot) produced wines with higher levels than were found in smoke affected grapes (*cf*. Tables 6.1 and 7.1). The relative increase in volatile phenols from the three winemaking methods suggests the extended skin contact may have facilitated hydrolysis of glycoside-bound phenols, as observed earlier (e.g., Kennison et al. 2008).

Since the concentrations of the total phenol diglycosides in grapes (Table 6.2) were comparable across cultivars and fuel types, the differences between wines (Table 7.2) primarily reflected the effects of the fruit processing and/or winemaking methods that are traditionally used for these cultivars. These results highlight that extraction of phenol diglycosides not only differs between the red wine (made with skin contact) and white wine (made without skin contact) making methods, but also between white grape processing and/or winemaking practices – grape crushing before pressing releases considerably more (~2.5-fold) glycoconjugated phenols than whole bunch pressing without crushing. Winemaking style did not alter the relative distribution of the phenol classes with the ratio of classes in wines broadly comparable to the respective proportions observed in grapes (Tables 6.2 and 7.2). The low contributions of syringol glycoconjugates (<10% of total) observed in this study contrast to results for wines from bushfire smoke affected fruit in which syringol glycoconjugates were the single largest components (Hayasaka et al. 2010a, 2013, Singh et al. 2012). While the reason for this variance is unclear, given the similarity of the relative proportions in fruit and wine in the current study, the low values here suggest differences are probably related to accumulation in grapes (i.e. exposure intensity and duration).

Winemaking practices significantly altered the extraction of total glycoconjugated phenols from grapes into wines (Figure 7.1). Fermentations with extended skin contact (Merlot wines, which were fermented on skin until dryness) extracted about 85% of the grape glycoconjugated phenols, which is comparable to results for skin fermented Cabernet Sauvignon and Chardonnay (Hayasaka et al. 2010a). This high ratio of extraction was more than double that of wines made with limited skin contact (Sauvignon Blanc wines, where fruit was crushed prior to pressing extracted 39% of fruit total glycoconjugated phenols) and more than four times the extraction ratio observed from whole-bunch pressed fruit (Chardonnay, ~18% of total glycoconjugated phenols).

It appears thus that white and red grape cultivars exposed to an identical bushfire smoke exposure will have markedly different levels of putative smoke taint compounds in wines under typical winemaking conditions. However, whether these differences translate into sensory differences (i.e., less negative impact in white wines than in red wines) is not clear since sensory impacts may be modulated by the red *vs*. white wine matrix effects (Boidron et al. 1988). For white wine production where skin contact is not essential, the marked reduction in extraction of total glycoconjugated phenols from whole bunch pressing white fruit (~18%) compared to crushing then pressing fruit (39%) provides a real advantage for winemakers to minimise extraction.

Differences in winemaking also influence the ratio of glycoconjugated phenols with a much higher proportion of syringyl glycoconjugates extracted in red winemaking (74%) compared to white winemaking (11%). These differences reflect the localisation of a high proportion of the total grape glycoconjugated phenols in skins (Dungey et al. 2011). Differential extraction rates between glycoside type and phenol glycoside also occurred. Guaiacol glucosylglucoside had very low extraction (≤ 7%) regardless of winemaking style (cultivar) as also reported in Ristic et al. (2011) for Shiraz and Grenache wines. While a similarly low extraction (~ 10%) was observed for syringol glucosylglucoside in both Sauvignon Blanc and Chardonnay wines, a very high extraction of syringol glucosylglucoside was found in Merlot (63%) which compares to Cabernet Sauvignon and Chardonnay wines fermented on skins (>70%, Hayasaka et al. 2010a). While the low apparent extraction rates for the white

varieties can be attributed to winemaking styles, the low extraction rate of guaiacol glucosylglucoside compared to syringol glucosylglucoside in wines fermented on skins is unclear.

Red wines are normally put through malolactic fermentation (MLF) by inoculation with lactic acid bacteria (LAB), often after the completion of alcoholic fermentation. Although MLF is primarily used for de-carboxylating malate to acetate, the metabolic activity of LAB can also modify wine aroma complexity by transforming a range of compounds including hydrolysis of glycoconjugates (Ugliano et al. 2003, D'Incecco et al. 2004) and potentially releasing volatile phenols. The odour and flavour sensory profile of smoke affected wines is largely dependent on the concentrations of volatile phenols in wines although some deconjugation of glycoconjugated phenols (at least of monoglucosides) can occur in the mouth (Parker et al. 2012). It can thus be expected, that LAB mediated hydrolysis of glycoconjugates that alters the distribution of volatile and glycoconjugated phenols can also alter the sensory profile of smoke affected wines. However, it is not clear that LAB are capable of significant hydrolysis of glycoconjugated phenols. The glycoconjugated phenols in smoke affected wines were mostly present as diglycosides. It is unclear whether the nature/form of the glycoconjugates present in wines contributed to the apparent lack of hydrolysis of glycoconjugates in the LAB inoculated wines. The hydrolysis and release of aglycones from diglycosides either can occur at once by actions of diglycosidases or sequentially by cleavage of the sugar-sugar link by sugar-specific exoglycosidases followed by release of volatile phenols by glucosidases (Sarry & Günata 2004). While LAB contain the complementary suite of enzymes (exoglycosidase and glucosidase) that may make sequential hydrolyses possible (Bodio et al. 2002, D'Inecco et al. 2004), presence of a diglycosidase in LAB is yet to be shown (Sarry & Günata 2004). If LAB are capable of releasing aglycone moieties from their diglycoside conjugates through sequential hydrolyses, then the lack of response here may be a strain specific response (Ugliano et al. 2003) and further evaluation of other LAB strains is warranted to gain a fuller picture of LAB capacity on release of sensorially potent aglycones from their phenolic diglycosides.

7.5 Conclusion

The methods of fruit processing and winemaking markedly influence the amount and/or proportions of grape phenols that are released into wines. While red winemaking methods that involve skin contact release considerably higher proportions (≥ 80%) of grape phenols than white winemaking methods (no skin contact, average 25%), there is also significant difference in phenol extraction between different grape processing methods for white winemaking: crushing before pressing releases ~40% of grape phenols compared to ~18% for whole bunch pressing without crushing. An understanding of how phenol grape extraction changes as a function of fruit processing and winemaking techniques may aid in mitigating and managing smoke taint in bushfire smoke affected grapes.

The results from this work found no evidence that malolactic fermentation, that is often used in red winemaking, increases extraction and hydrolysis of glycoconjugated phenols. Thus, at least for *Oenococcus oeni* (Viniflora CH 16), in wines suspected of containing elevated levels of glycoconjugated phenols, the decision to use MLF should not significantly alter the concentrations or distributions of volatile and glycoconjugated phenols of the resultant wines.

CHAPTER 8

THE EFFECT OF PHENOL COMPOSITION ON THE SENSORY PROFILE OF SMOKE AFFECTED WINES

8.1 Introduction

Wines produced from vineyards exposed to bushfire smoke often have unpalatable levels of smoke related attributes including smoky, metallic, bitter, ash and medicinal flavour and aroma attributes (Høj et al. 2003, Parker et al. 2012). A large number of volatile and glycoconjugated phenols are present in wines made from smoke exposed grapes (Kelly et al. 2012, 2014, Hayasaka et al. 2010a, 2013) and although the sensory profile of smoke taint is reportedly closely related to the volatile phenol composition, glycoconjugated phenols may be deconjugated to the volatile forms in tasting smoke affected wine (Parker et al. 2012). The detailed analysis of Merlot wines described in Chapter 4 has been used to explore relationships between fuel lignin composition and sensory descriptors associated with smoke tainted wine.

8.2 Materials and Methods

The Merlot wines used for this study were as described in Chapter 4. Wines made from karri, pine and oats smoke exposed fruit and non-smoked control wines were selected for sensory assessment. The sensory assessments of wines were conducted in a well ventilated room purpose built for wine sensory analysis. Sixteen respondents were randomly chosen from oenology students who had completed two years of sensory instruction as part of Curtin University's oenology program. The sensory instruction included wine fault identification and descriptors of wine faults including all of the wine descriptors used in this trial. Wine samples of 50 ml were presented to respondents in random order, in clear, coded, XL5 glasses at ambient room temperature (21°C). All wines were expectorated after tasting and respondents were instructed to rinse their mouths by tasting and expectorating a 50 ml solution of rain water mixed with 5% pure lemon juice, followed by a 50ml rinse of rain water. The sensory trial was done in compliance with Curtin University's Ethics

Committee. The sensory attributes selected for aroma and taste were as reported for the sensory assessment of smoke tainted wines by Parker et al. (2012). Five descriptors of overall fruit, ash, solvent, medicinal and smoke aroma were recorded for each wine by smell alone and the seven descriptors of overall fruit, smoke, sourness, metallic, bitter, ashy aftertaste and drying were recorded by taste. The aroma scores for each wine were determined before the tasting of wines began. Respondents recorded sensory intensity of each attribute on a continuous 15 cm line with points marked 'low' and 'high' (Appendix 4). For multivariate analysis the phenols quantified in Chapter 4 were screened by wine concentration (Tables 4.2 and 4.3) and their reported sensory thresholds (Wasserman 1966, Chua 2010, Parker et al. 2012, Kennison et al. 2007).

The wines were analysed by Fourier Transform Infra-Red Spectroscopy (Oeno Foss Type 4101, Foss, Hillerød, Denmark) for alcohol (%w/v), residual sugars (sum of glucose and fructose), malic acid concentration (g/l) and volatile acidity (acetic acid g/l equivalent). Titratable acid (tartaric acid g/l equivalent) and pH were determined as described by Iland et al. (2004).

8.2.1 Statistical analysis

Data were analysed using SPSS 20 (IBM® SPSS® Statistics). Reported treatments effects are significant at $p < 0.05$.

8.3 Results

The sensory scores (aroma and taste) of wines made from smoke treatment were significantly different in every attribute compared to the unsmoked control wines regardless of fuel type. (Figures 8.1 and 8.2).

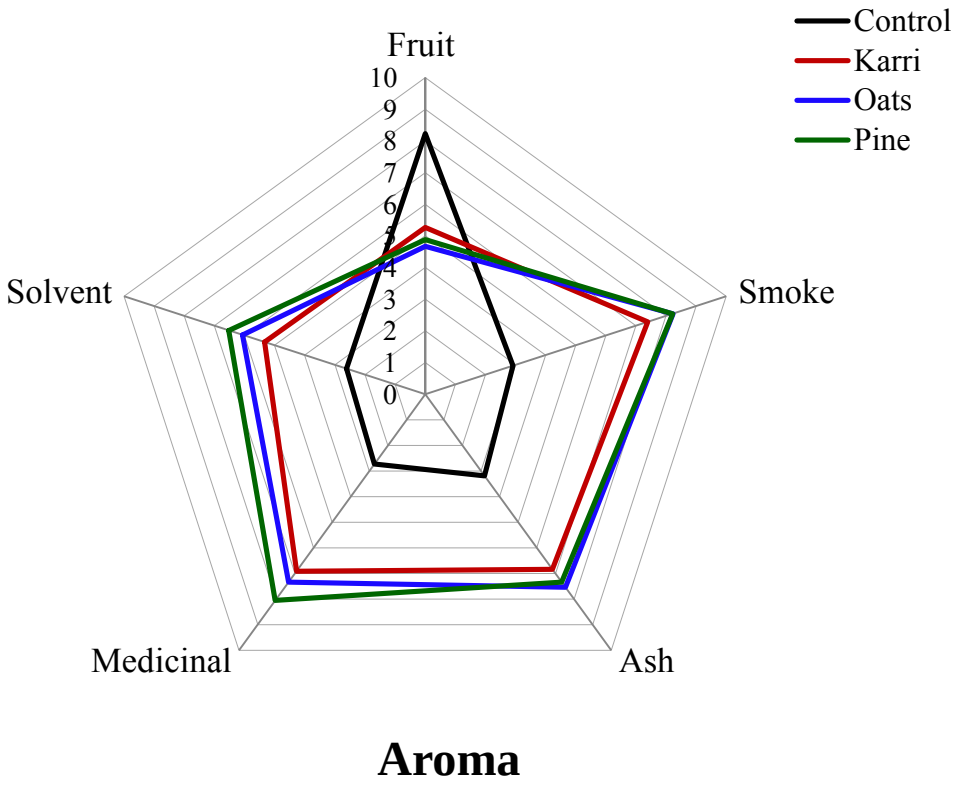


Figure 8.1 Radar plot of Merlot wine mean aroma intensity ratings.

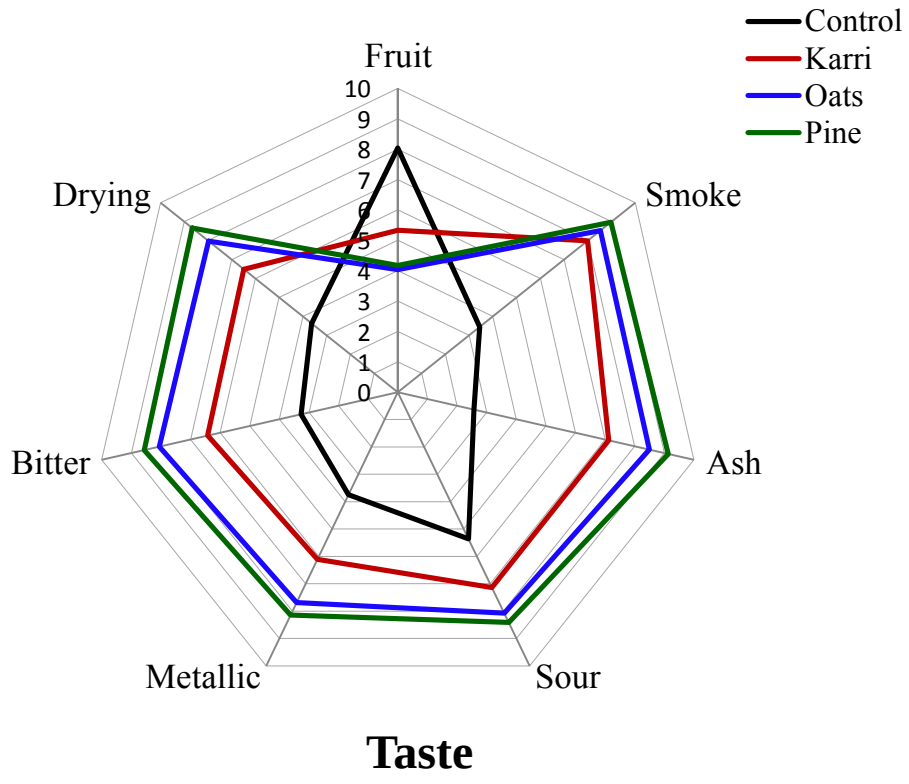


Figure 8.2 Radar plot of Merlot wine mean taste intensity ratings.

Smoke treatment significantly increased the detractive aroma and taste scores and decreased overall fruit aroma and taste scores compared to the control wines (Figures 8.1 and 8.2). Although significantly greater than the control wines in each negative sensory descriptor of taste and aroma, the karri treatment wines were not significantly different in aroma scores compared to the oats and pine treatment wines (except solvent aroma where they were significantly less than the pine treatments, Figure 8.1). The karri smoked wines were significantly higher in fruit taste compared to the oats and pine smoked wines and significantly lower in each negative taste descriptor except smoke taste for which all three fuels had similar impact (Figure 8.2). Across each of the twelve sensory descriptors there was no significant difference in the oats and pine treatments.

Analysis of the control and smoke treatment wines found similar acidity, residual sweetness, volatile acidity and ethanol concentration (Table 8.1)

Table 8.1 Chemical parameters of sensory wines.

Data are mean ±1 standard error (n=5); nd, not detected. Total Acidity (TA) is grams per litre equivalent of tartaric acid and Volatile Acidity is grams per litre equivalent of acetic acid. Alcohol is weight per volume percentage of ethanol.

Treatment	pH	TA g/l	Alcohol %	Residual Sugars g/l	Malic acid g/l	Volatile Acidity g/l
Control	3.6± 0.03	4.7± 0.3	13.8±0.12	nd	nd	0.3 ± 0.002
Karri	3.6± 0.01	4.6± 0.1	13.9±0.07	nd	nd	0.3 ± 0.002
Oats	3.6± 0.02	4.7± 0.1	13.6±0.05	nd	nd	0.3 ± 0.002
Pine	3.6± 0.02	4.4± 0.1	13.8±0.04	nd	nd	0.3 ± 0.002

Principal components analysis of the twelve sensory descriptors revealed that the variation embodied in the twelve sensory attributes could be effectively summarised by a single axis of variation (95.6% of total variance, $p < 0.0001$), representing a fruity-smoky sensory spectrum (Figure 8.3 a,b). Correspondingly, the smoking treatment wines were segregated on fruit and non-fruit aroma and taste attributes. The control wines were characterised by high fruit taste/aroma and low smoke-related sensory attributes. By contrast, the smoke affected wines, particularly of oat and pine fuel, were strongly and similarly associated with low fruity characters and high smoke-attributes (Figure 8.3 a,b). The karri smoke affected wines, falling intermediate along the fruit-smoke sensory attributes spectrum, were distinct from both the control and oats and pine treatments.

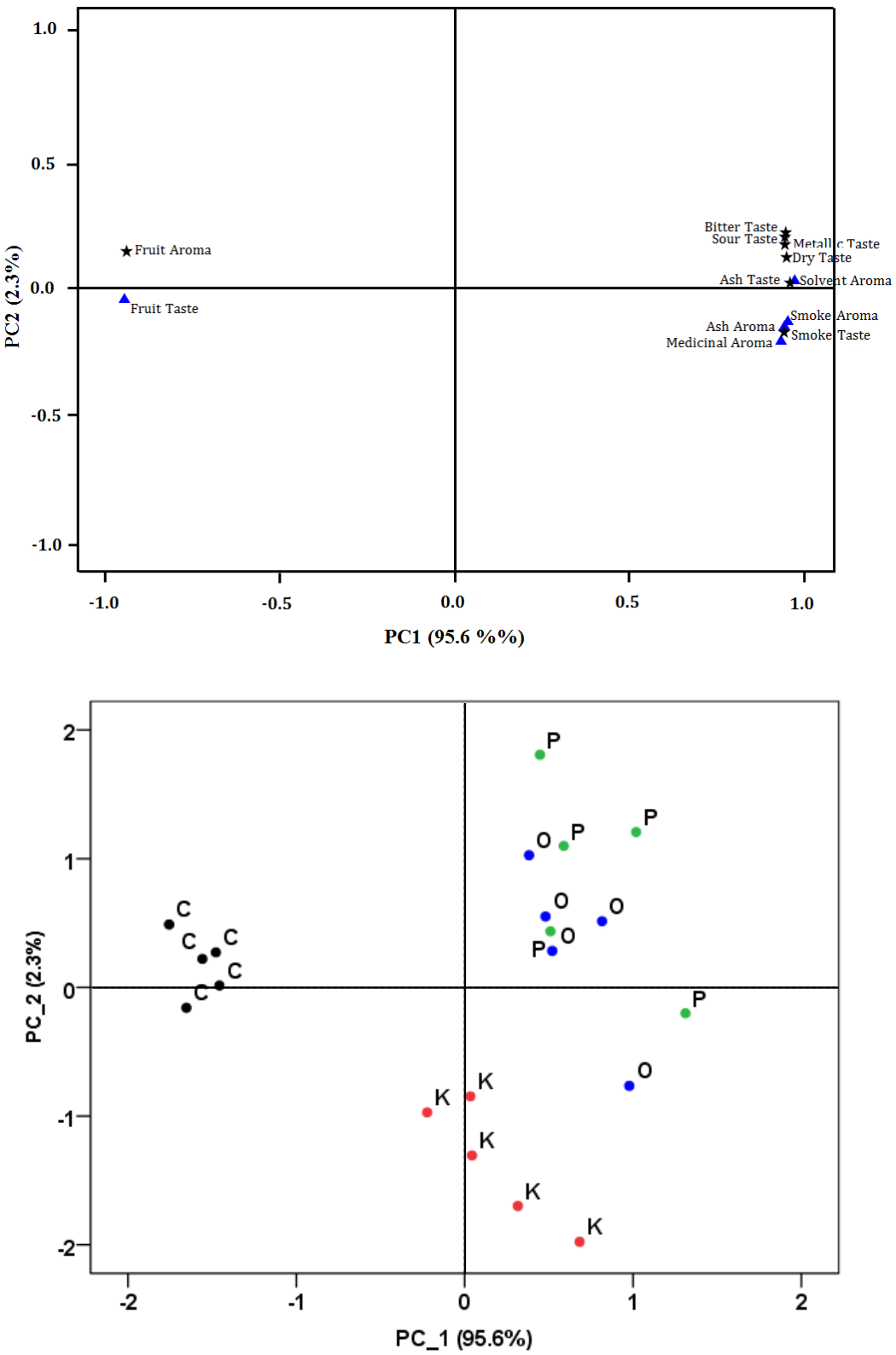


Figure 8.3 Differentiation of wines made from grapes exposed to smoke of different fuel types based on principal component analysis of sensory attributes: plots of (a) the sensory attributes vector loadings and (b) the resultant scores of the fuel treatment shown on the first two principal axes of variation.

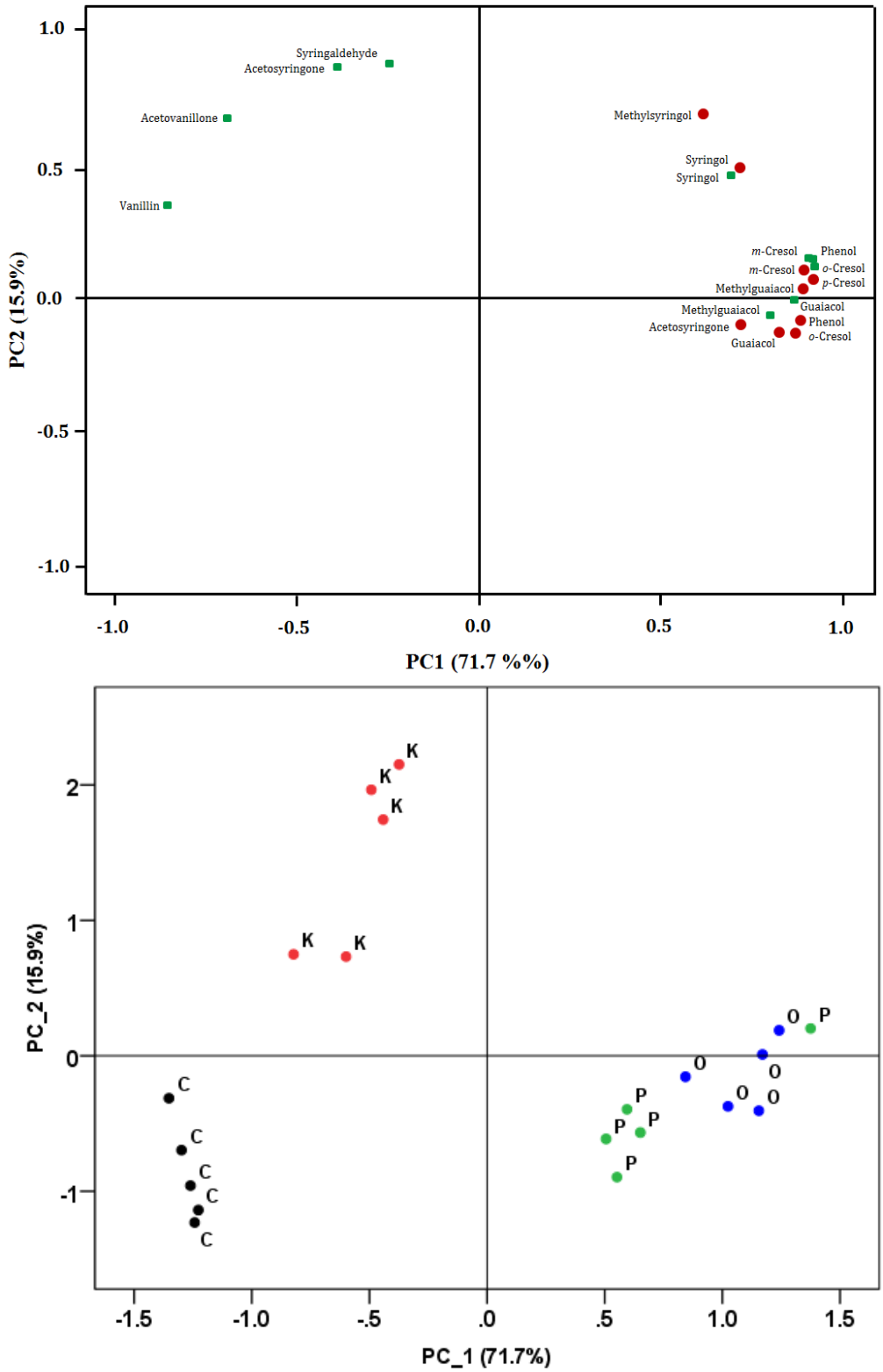


Figure 8.4 Separation of wines made from grapes exposed to smoke of different fuel types based on principal component analysis of wine volatile and glycoconjugated phenol composition: plots of (a) the vector loadings of the volatile (red circles) and bound phenols (green squares) and (b) the resultant fuel treatment scores displayed on the first two principal axes of variation.

A principal component analysis of the volatile and glycoconjugated phenols (Chapter 4) showed the first and second components jointly extracted 87.6% of the total variance (Figure 8.4a,b). The first PC (~72%) was strongly and positively correlated with volatile and glycoconjugated phenols except the glycoconjugates of vanillin, acetovanillone, acetosyringone and syringaldehyde with which it was negatively associated (Figure 8.4a). As such, PC1 represented an index of intensity of 'smokiness'. The second component accounting for about 8% of the variance had moderate to strong positive associations with glycoconjugates of syringaldehyde, acetosyringone, acetovanillone, vanillin, and volatile 4-methylsyringol and syringol (Figure 8.4a). The resultant principal component scores of the fuel smoke treatments are displayed in Figure 8.4b. The first PC clearly differentiated the treatments based on overall smoke index: from low level in the controls to intermediate in the karri and high for the oat and pine fuel treatments. Along the second dimension, the fuel effects are further differentiated on the basis of their relative contents of glycoconjugates of acetosyringone, syringaldehyde, acetovanillone, vanillin, syringol and volatile syringol and 4-methylsyringol. Thus, the karri fuel smoke impacted wines (which had relatively high concentrations of acetosyringone, syringaldehyde, acetovanillone, vanillin, syringol and volatile syringol and 4-methylsyringol) were further differentiated from the pine and oat smoke affected wines. The relative proximity of the karri smoke affected wines to the control wines was driven by their similarity in the levels of the glycoconjugates of vanillin and acetovanillone (cf. Figures 8.4a and b) however separation is also seen from the oats and pine treatment wines from higher levels of glycoconjugates of acetosyringone and syringaldehyde (Figure 8.5, panel 2 and Table 8.2).

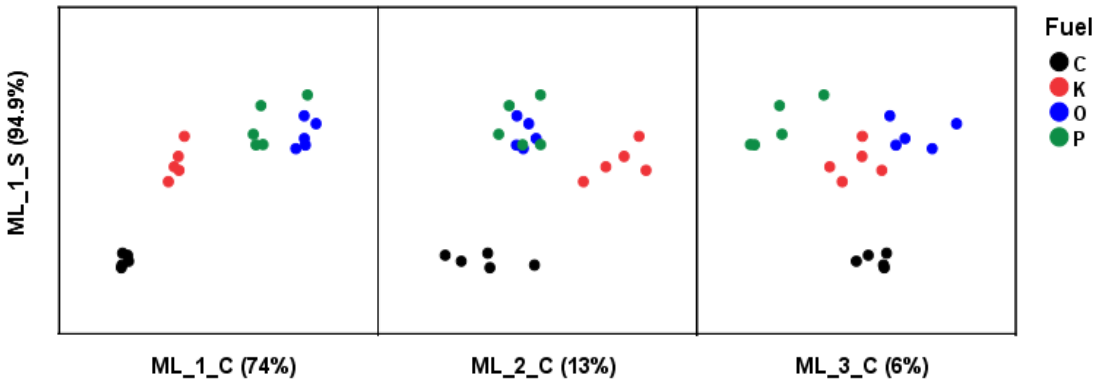


Figure 8.5 Relationships between principal component scores of wine volatile and non-volatile phenol composition (x-axis) and aroma and taste sensory attributes (y-axis). Maximum Likelihood (ML_1_C, ML_2_C, ML_3_C) denote principal component scores for phenol composition and sensory variables.

Table 8.2 Effects of smoke exposure and fuel type on volatile and glycoconjugated vanillin, acetovanillone, syringaldehyde and acetosyringone levels (µg/l) in Merlot wines. Data are mean ±1 standard error (n=5)

	Phenols	Control	Karri	Pine	Oats
Volatile	Vanillin	86.7±10.7	107.6±14.3	89.9±6.5	90.0±9.3
	Acetovanillone	234.4±10.4	273.6±6.9	307.3±12.7	312.1±12.6
	Acetosyringone	96.8±5.7	119.2±7.5	207.7±8.6	192.1±10.6
Bound	Vanillin	165.5±11.1	163.0±5.3	76.3±3.8	87.6±6.5
	Acetovanillone	41.7±4.8	65.6±3.1	15.4±1.3	17.2±1.5
	Syringaldehyde	48.8±2.0	86.2±2.3	52.3±2.7	47.9±2.4
	Acetosyringone	13.7±0.7	24.0±1.6	9.6±0.8	0.9±0.5

The following significant differences were observed in concentrations:

Volatile acetovanillone: oats = pine > karri > control

Volatile acetosyringone: oats = pine > karri = control

Bound vanillin: oats = pine > karri = control

Bound acetovanillone: karri > control > oats = pine

Bound syringaldehyde: karri > control = oats = pine

Bound acetosyringone: karri > control = oats = pine

There were no significant differences in volatile vanillin concentration and volatile syringaldehyde was not detected in control or smoke treatment wines.

Partial least squares regression analysis of volatile phenols, glycoconjugated phenols, negative aroma descriptors and negative taste descriptors (Figure 8.6) shows the relationship between wine phenol composition and sensory attributes with 96% of the variance in phenol composition and 84% of the variance in sensory attributes accounted for in the first two dimensions.

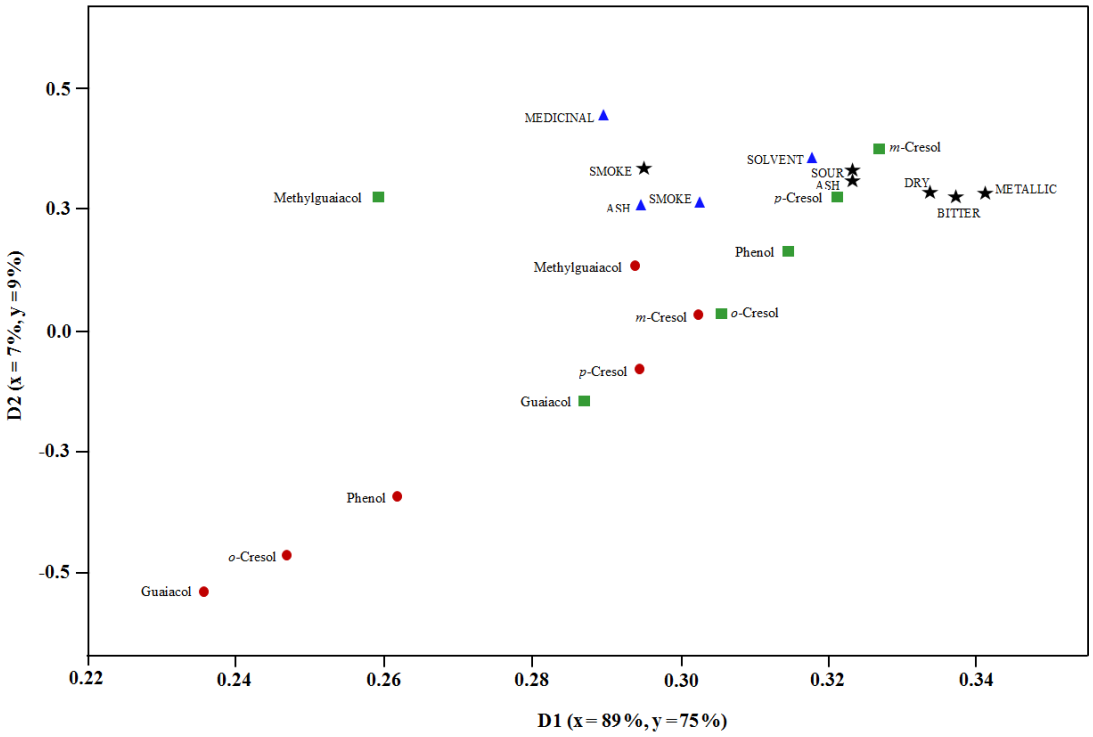


Figure 8.6 Partial least square regression of wine phenol composition and negative sensory attributes. (Wine phenol composition (x axis) with glycoconjugated phenols [green squares] and volatile phenols [red circles]. Wine negative sensory attributes (y axis) with aroma attributes [blue triangles] and taste attributes [black stars]).

The glycoconjugates of *m*-cresol, *p*-cresol and phenol have the closest association to solvent aroma and each of the taste descriptors except smoke taste (Figure 8.6). Volatile 4-methylguaiacol has the closest association to ash aroma and smoke aroma and taste and volatile guaiacol has a large separation from smoke aroma and taste. Bound *m*-cresol, *p*-cresol and phenol were highly correlated to the metallic, ash, sourness, drying and bitter taste descriptors (Appendix 5). In each case the correlation of phenol and cresols concentration to each of the taste descriptors was similar for the glycoconjugated forms than the volatile forms.

8.4 Discussion

Merlot wines made from grapes exposed to smoke emissions from the five vegetation fuels in this study were significantly higher in putative taint phenols than unsmoked control wines (Chapter 4). Karri, pine and oats treatment wines and

unsmoked control wines have been evaluated here to determine the influence wine phenol composition has on the sensory attributes of smoke affected wine. Clear differences in aroma (Figure 8.1) and taste (Figure 8.2) were found in wines from smoking applications. The control wines were significantly higher in fruit aroma and taste and significantly lower in each of the negative sensory aroma and taste attributes compared to the karri, pine and oats treatment wines (Figures 8.1 and 8.2). Differences were also apparent between angiosperm *Eucalypt* karri treatment and the pine and oats treatment wines. While karri treatment wines were significantly different to the control wines in each aroma and taste attribute they also had significantly more fruit taste and were lower in each negative taste attribute compared to both pine and oats treatments, except for smoke taste where there was no difference (Figure 8.2). This was reflected by significantly lower volatile and glycoconjugated phenol concentrations in the karri treatment wines compared to the pine and oats treatment wines (Tables 4.1, 4.2 and 4.3) as well as relatively higher acetovanillone, acetosyringone and syringaldehyde which confer soft and sweet undertones (Sterckx et al. 2011) and thus possibly partly mitigating the negative sensory impact of the other wine phenols. The sensory profile of the pine treatment wines were closely aligned to the oats treatment wines with no significant differences in any of the aroma or taste sensory scores. Smoke application had no significant effect on acidity, residual sweetness, alcohol or volatile acidity in the wines (Table 8.1). The differences in sensory attributes were therefore reflective of smoking treatments and not changes in these basic wine chemical profiles.

While each phenol quantified in Chapter 4 has a characteristic sensory threshold in wine, a synergistic effect is expected to occur where a combination of phenols below their respective threshold concentrations have a significant effect on the sensory properties of a wine (Keast & Breslin 2002). Nineteen phenols in volatile and glycoconjugated forms were quantified in the wines (Tables 4.1, 4.2 and 4.3). The glycoconjugated forms have no direct aroma properties as they are non-volatile (Parker et al. 2012) whereas volatile phenol contribution to aroma is mainly governed by a compound's solubility in a wine matrix and pure compound volatility (Voilley & Souchon 2006). Consideration was made to screen the phenols quantified in Chapter 4 based on sensory threshold (Wasserman 1966, Chua 2010, Parker et al.

2012, Kennison et al. 2007) and wine concentration (Tables 4.2 and 4.3) to reduce the number of volatile and glycoconjugated phenols for multivariate analysis.

The lignin makeup of fuels was not found to be a good indicator of phenol composition in smoke affected wines (Chapter 4) and the karri treatment wines have distinct differences in taste profile (Figure 8.2) and phenol composition (Tables 4.1, 4.2 and 4.3) from the oats and pine treatments. The relationship between the principal component scores of wine volatile and glycoconjugated phenols and sensory attributes (Figure 8.5) found the same separation of karri treatments from oats and pine treatment wines. The drivers for separation in sensory profile in the karri treatments are a closer alignment to glycoconjugates of acetosyringone, syringaldehyde, vanillin and acetovanillone. Vanillin and acetovanillone are usually associated with extraction from oak barrels during wine aging (de Revel et al. 2005) and may also form from transformation of ferulic acid and 4-vinylguaiacol by malo-lactic bacteria (Bloem et al. 2006). The winemaking here did not include contact with oak products. The apparent high levels of vanillin and acetovanillone in the control and karri treatment wines thus suggests that the high levels of phenols in the pine and oat treatment wines restricted bacterial transformation of ferulic acid and 4-vinylguaiacol to vanillin and acetovanillone by malo-lactic bacteria. This is worthy of further investigation. Vanillin and acetovanillone impart a powerful aroma and flavour characteristic of vanilla (Bloem et al. 2006) and acetosyringone and syringaldehyde impart soft, sweet undertones (Sterckx et al., 2011) which in comparison to every other phenol examined is considered a positive sensory attribute.

Toth and Potthast (1984) describe phenol, the cresols, guaiacol, 4-methylguaiacol and 4-ethylguaiacol as having a hot bitter taste and this is consistent with the pattern of association seen in Figure 8.6. The harsh ash, sour, metallic, bitter and drying tastes of smoke tainted wines (Parker et al. 2012) were closely associated and highly correlated with glycoconjugates of phenol, *m*-cresol and *p*-cresol in the smoke treatment wines (Figure 8.6) and these phenol glycoconjugates are therefore most likely the driver for the harsh taste of smoke affected wines. Parker et al. (2012) found *m*-cresol and guaiacol β-D-glucosides impart undesirable flavours in smoke affected wine by deconjugation in the mouth to release volatile phenols. A large

number of glycoconjugated phenols were associated with negative smoke taint flavour descriptors in this study. This supports the findings of Parker et al. (2012) and suggests several phenols and their glycoconjugates may also contribute to the complex palate of taste descriptors described in this sensory assessment. The amelioration of wine by reverse osmosis (Fudge et al. 2011) may remove the sensory influence of volatile phenols, however the negative taste descriptors associated with the phenol glycoconjugates are expected to remain unchanged.

Earlier work has focussed on volatile guaiacol concentrations as an indicator of smoke taint in wine (Kennison et al. 2007, 2008, Sheppard et al. 2009) and smoke taste and aroma descriptors have been reportedly due to volatile guaiacol concentrations (Kennison et al. 2009, Parker et al. 2012). In this study, the volatile 4-methylguaiacol concentration was found to have a closer association to smoke aroma than guaiacol concentration despite volatile guaiacol concentrations being up to four times the 4-methylguaiacol concentrations in smoke treatment wines. Wasserman (1966) calculated the flavour and aroma index of 4-methylguaiacol in water as being significantly higher (~13 times) than guaiacol and the partial least square regression (Figure 8.6) shows volatile 4-methylguaiacol and not guaiacol to be closely aligned to both smoke aroma and taste. Interestingly, the karri treatment wines were significantly less in both volatile and glycoconjugates of guaiacol and 4-methylguaiacol (Tables 4.2 and 4.3) than the oats and pine treatment wines and yet there were no significant differences in the descriptor of smoky aroma or smoky flavour between the three treatments. Syringol also contributes to smoke aroma and taste (Wasserman 1966) but has a lower taste and odour index than guaiacol (~1/4) and although syringol concentrations in all smoking treatments were well below aroma and taste thresholds (1.85 and 1.65 ppm respectively, Wasserman 1966) the synergistic influence of syringol may be important in contributing to smoke taste and aroma.

Although earlier work has focussed on volatile guaiacol concentrations as an indicator of smoke taint in wine (Kennison et al. 2007, 2008, Sheppard et al. 2009), this study has found the glycoconjugates of phenol, *m*-cresol and *p*-cresol more closely aligned to the harsh smoke taint descriptors (Parker et al. 2012) associated with taint in wine made from smoke exposed grapes.

CHAPTER 9

GENERAL DISCUSSION

9.1 Introduction

The accumulation of smoke taint phenols in wine grapes from an exposure to bushfire smoke poses a large risk to Australian grape and wine producers. Wines made from smoke exposed grapes often exhibit harsh, metallic, bitter, drying and smoky characteristics with low consumer acceptance (Whiting & Krstic 2007) and as such, investigations into smoke taint in wine are important for the wine industry. The smoke-borne compounds responsible for imparting the harsh organoleptic properties of smoke taint are thought to originate from the pyrolysis of the lignin component of vegetation fuels (Hayasaka et al. 2010a, Singh et al. 2012). Large variations in lignin are found in fuel vegetation types and the effects of exposure of grapes to smoke of vegetation with varying lignin composition are unknown. For grape and wine producers there are no methods available to circumvent phenol accumulation in smoke exposed grapes and no methods available for ameliorating smoke taint in wine. In an attempt to reduce the impact of smoke taint on the grape and wine industry, several key questions have been addressed in this thesis. Firstly, an examination has been made to investigate the effect of vegetation type on the formation of lignin derived phenols that accumulate in grapes and wine. Previous research has been limited to examining smoke taint from experimental fires using model vegetation fuels or exposure to wildfires where the vegetation is unknown (Kennison et al. 2008, Sheppard et al. 2009, Hayasaka et el. 2010a, Singh et al. 2012) and have therefore not expounded differences in taint accumulation due to vegetation type. Industry has also sought research on cultivar sensitivity to accumulate taint, however differences reported in previous research (Singh et al. 2012, Dungey et al. 2011, Hayasaka et al. 2010a) have been unreliable as variations in smoke exposure conditions and vine phenology have occurred. A comparison of taint accumulation in three cultivars has been made in this thesis where smoke exposure and vine phenology have been standardised and from these cultivars, an examination of taint extraction from grapes has been made using traditional winemaking methods. With recent developments in grape and wine phenol analysis (Singh et al. 2012, Hayasaka

et al. 2010a) a large number of phenols and phenol glycoconjugates can now be quantified. While this has increased the number of compounds thought to contribute to smoke taint, this thesis has examined firstly the ratio of phenols extracted in winemaking and secondly the organoleptic impact the compounds have in tainting wine. While these findings have not provided preventative or ameliorative solutions for industry, they are important in understanding the accumulation, extraction and sensory impact of smoke taint.

9.2 The effects of lignin composition on the accretion of lignin derived phenols in wine.

This thesis has used taxonomically distinct groups of vegetation fuels to examine the influence of lignin makeup on potential smoke taint compounds that accrue in wine as a result of exposure of grapes to smoke. Five fuels from the Margaret River region were compiled from component proportions consumed in a decadal fire event. Each of the fuel's cellulose, hemicelluloses, lignin and monolignol percentages were quantified with fuel phenol emissions generated at pyrolysis temperatures that imitated wildfire pyrolysis. The phenol emissions indicated broadly comparable results for the quantitatively significant lignin derivatives. Sampling of karri, marri and wild oats prescribed burning found broad similarity to the smoke generated in vineyard trials, confirming the emissions used to fumigate vines in this thesis successfully simulated wildfire/prescribed burning emissions. This research differed from other studies where sample analysis of wildfires or prescribed burning had not been taken in close proximity to the fire front, allowing for a number of chemical changes to occur over time from atmospheric interaction (Kamens et al. 1985, 1988). Analysis of the emissions found a much higher proportion of *p*-hydroxyphenyl derived phenols present in smoke than previously reported. The control and analysis of vineyard fuel pyrolysis allowed for valid comparisons of vegetation fuels and cultivars which have otherwise been absent in smoke taint research.

A detailed analysis of the phenols found in this study's fuel emissions, indicated the possibility more compounds are present in smoke affected wines than had been reported in previous research (Kennison et al. 2007, 2008, Sheppard et al. 2009,

Singh et al. 2011). Quantification of a large number of phenols in wine (Singh et al. 2012, Appendix 1) significantly expanded the known compounds elevated from smoke exposure. In the wines produced in this study, the commonly reported smoke taint markers, guaiacol and 4-methylguaiacol, although dominant components of the guaiacyl lignin-derived compounds, represented only some 20% of the total pool of lignin-derived putative smoke taint compounds in wines. Phenol and substituted phenols were found in comparable abundance to the guaiacyl lignin-derived compounds, however the quantitatively dominant contributors (>50%) were pyrolysis products of syringyl lignins. Previous work has suggested the descriptors of smoke tainted wine are more complex than those imparted by guaiacol and 4-methylguaiacol alone and the broad range of phenols quantified in wines in this thesis allows an exploration of compositional influence on the sensory characteristics of smoke taint.

The influence of vine fruit load and canopy size on phenol accumulation was examined to explore the mechanism of accretion of smoke borne lignin pyrolysates in wine grapes. Nearly all phenol concentrations were negatively correlated to vine canopy leaf area and leaf area per bunch suggesting direct uptake by berries may be a significant contributor to accumulation of smoke borne phenols in wine grapes.

9.3 The accumulation of sinapyl phenols from the exposure of gymnosperm smoke exposed grapes.

Unexpectedly, grapes exposed to gymnosperm emissions were found to accrue syringols and substituted syringols, a class of compounds not present in gymnosperm smoke. Several pathways for syringol accrual were considered. The first was a methoxylation of phenol or guaiacol derivatives in wine grapes to syringols by yeast or malo-lactic fermentation, however an analysis of pine smoked grapes found the presence of syringyl glycoconjugates indicating the mechanism for syringyl formation occurred during or after exposure and was not due to winemaking processes.

The pathway of lignin synthesis in plants includes the production, methoxylation and glycosylation of phenols (Whetten & Sederoff 1995). Phenol glycoconjugate accrual in grapevines in response to temperature elevation and carbohydrate pyrolysis emissions were examined as the second possible mechanism. The heating of vines to 50^{o}C for one hour was not found to alter volatile or glycoconjugated phenols in the fruit.

The third mechanism tested the exposure of vines to carbohydrate emissions. As there was no correlation between the pyrolysed fuel lignin and lignin derived phenol taint accrual in smoke treatment wines and as the fuels used in this study contained high percentages (30-77%) of carbohydrates as cellulose and hemicelluloses, exposures of vines to the pyrolysis of a pure carbohydrate source was examined. Paper made from cotton linters was examined by *Py* GC-MS and pyrolysis emissions analysed by TD GC-MS, confirming no lignin or lignin pyrolysis compounds were present other than minor phenol and *o*-cresol concentrations which were attributable to carbohydrate rearrangement reactions (Tóth & Potthast 1984). The fruit and wine of vines exposed to cotton linter pyrolysis had no volatile or glycoconjugated phenols other than phenol and *o*-cresol which were present in the smoke. The accrual of syringyl derived phenols in grapes from an exposure to the carbohydrate fraction is therefore unlikely. An unexpected outcome of this experiment was the accumulation of relatively high phenol and *o*-cresol glycoconjugates from minor amounts of these compounds in the carbohydrate emissions. This suggests grapevines are particularly sensitive to gaseous phenols or at least phenol and *o*-cresol accrual.

The fourth mechanism investigated was an *in planta* transformation (methoxylation) of xenobiotically acquired hydroxy- and methoxy-phenols. While an exposure of vines to minor concentrations of phenol and *o*-cresol was not found to significantly elevate coniferyl or sinapyl derived phenol glycoconjugates, exposures of higher levels of pure phenol, guaiacol and syringol were studied. Tomato plants exposed to pure guaiacol were found to accumulate significant concentrations of guaiacol glycoconjugates, demonstrating the accrual of phenols from vapour exposures is not limited to grapevines, however syringol derivatives were not found to accumulate from the guaiacol exposure in tomatoes. Sauvignon Blanc vines were exposed to

vapours of pure phenol, guaiacol and syringol and significantly higher glycoconjugates of the aglycone used from exposure were found in the fruit (> 25 times found in smoke exposures), however *in planta* transformations were not observed.

The presence of syringol derivatives in fruit exposed to gymnosperm emissions was therefore confirmed as being due to vegetation pyrolysis exposure, however the mechanism for the accrual of sinapyl derived lignin derivatives remains unresolved. Further experiments will be designed to continue this work.

9.4 Cultivar differences in the accumulation of lignin-derived smoke taint phenols.

It is likely that future impacts of climate change will increase the risks of wildfire in Australia (Pitman et al. 2007). Smoke from wildfires can cause significant financial damage to the Australian wine industry (Whiting & Krstic 2007) and management strategies to mitigate the risk may include planting varieties less susceptible to smoke taint. Earlier research indicated cultivar differences to the accumulation of putative taint phenols in wine grapes, however in these studies the smoke exposure conditions were either not known or not standardised (Hayasaka et al. 2010a, Singh et al. 2012, Dungey et al. 2011). Two fuels were chosen from the Merlot study to examine phenol accumulation in Chardonnay, Sauvignon Blanc and Merlot. Little difference was found in the accumulation of total phenols among the cultivars from smoke exposure at a comparable stage of berry development. This finding underscored the importance of standardising smoke exposure conditions in research. Apparent differences in taint uptake reported in previous work are most likely a result of ontogenetic and exposure conditions and the findings of this thesis suggest there is no benefit in replacing one cultivar for another in seeking to minimise the impact of fire events on wine grape production. While cultivar and fuel type were not found to significantly influence the total concentrations of phenol glycoconjugates, differences were found between cultivars in some glycoconjugate types and phenols, however a clear cultivar pattern was not apparent. Some differences were also observed in part to reflect the lignin source of the smoke. The degree to which these phenols and phenol glycoconjugates remain in finished wine due to wine processing method was therefore examined. The detection of elevated concentrations of syringol

diglycosides in Sauvignon Blanc grapes exposed to pine smoke, confirmed the accrual of syringol derivatives is not limited to Merlot as described in Chapter 4.

9.5 Extraction of smoke-derived taint from standard winemaking procedures.

The production of wine is an expensive process that is only rewarded through the sale of a fault free, structurally sound product. When vineyards are exposed to a smoke event the sensory evaluation of grapes cannot be used to predict evidence of smoke taint. The analysis of volatile and glycosidically bound phenols in smoke exposed fruit gives an indication to growers that taint may be present, however the percentage extraction of these compounds into the finished wine has not been previously reported. This thesis has used common winemaking techniques to investigate the extraction of taint from smoke exposed grapes into finished wine. Marked differences in the extraction of phenols due to fruit processing and winemaking methods were found. The extraction was highest (~ 88%) in red winemaking where there was extended skin contact and in white wines made by crushing and pressing before fermentation, the extraction was significantly reduced (~ 39%). A further significant reduction was found in white winemaking when the fruit was whole bunch pressed (~ 18%). These findings allow viticulturists to estimate the phenol levels in wines made from smoke affected fruit and demonstrate a clear reduction in phenol extraction for whole bunch pressing white wines. Minor cultivar differences in volatile and glycoconjugated phenols in the grapes were subsumed in the winemaking methods effects. Winemaking method also affected the extraction and subsequent hydrolysis of phenol glycoconjugates. Smoke exposed Chardonnay grapes contained low levels of volatile phenols and whole bunch pressing produced wines with an apparent absence of volatile phenols. This compared to wines made by crushing before pressing with equivalent levels of volatile phenols and fermentation with extended skin contact producing wines with higher volatile phenols than were found in the smoke exposed grapes. It is apparent that while there will be marked differences in levels of volatile and glycoconjugated phenols as putative taint in wines made from white and red cultivars in an identical smoke event, how these differences translate organoleptically in the different wine matrixes is unclear. An examination of wines made with malolactic fermentation found no change in phenol concentration or profile compared to wines made without

malolactic fermentation. This finding reassures winemakers the decision to inoculate wines for malolactic fermentation should not significantly change the taint profile of smoke affected wines.

9.6 The effect of phenol composition on the sensory profile of smoke affected wines.

The comprehensive phenol analysis of Merlot wines in this thesis has facilitated an extensive sensory evaluation to explore the underlying drivers of smoke taint in wine. Wines made from grapes exposed to smoke emissions from the five vegetation fuels in this study were significantly higher in putative taint phenols than unsmoked control wines. Sensory analysis of control, karri, pine and oats treatment wines has established differences in aroma and taste from smoke exposure were not limited to an elevation in a range of detractive descriptors but also a masking of positive fruit descriptors. Unsmoked control wines were significantly higher in fruit aroma and taste and significantly lower in each of the negative aroma and taste descriptors compared to the smoke treatment wines. Sensory differences due to fuel type were driven by phenol composition and concentration and vanillin, acetovanillone, syringaldehyde and acetosyringone concentrations were influential in separating karri treatment wines from pine and oats treatments. The karri treatment wines were significantly higher in fruit taste and significantly lower in each negative taste descriptor, except smoke taste, when compared to the pine and oats treatment wines.

Correlations of sensory aroma and taste descriptors to phenol composition for smoke tainted wine were explored to examine the phenol drivers of smoke taint. Putative taint phenols are predominantly glycosidically bound in smoke tainted wine and while they are aroma inactive, they may deconjugate in the mouth to impart harsh flavour descriptors (Parker et al. 2012). In this thesis a number of glycoconjugated phenols were closely correlated to ash, metallic, solvent, bitter and drying taste descriptors, indicating a large number of phenol diglycosides may deconjugate in the mouth when tasting smoke affected wine. Phenol and the cresols appeared in each correlation analysis to be more influential than guaiacol and 4-methylguaiacol in imparting the detractive sensory properties of smoke taint except for smoke taste and

aroma which was driven by 4-methylguaiacol concentration. The harsh, metallic taste in smoke treatment wines was highly correlated to glycoconjugated *m*-cresol concentration. The grape and wine industry in Australia uses volatile guaiacol concentration as a smoke taint indicator even though phenols are predominantly glycosidically bound in smoke exposed fruit. From the findings of this thesis, glycosidically bound phenol and cresols concentrations in fruit are the best indicator of potential smoke taint.

9.7 Conclusion

This thesis has investigated lignin derived phenol accumulation in wine grapes from exposures to the pyrolysis of taxanomically different vegetation fuels. Fuel lignin makeup was not found to be a good indicator of the types of lignin pyrolysis products that become elevated in fruit and wines. Unexpectedly, grapes were found to accumulate syringyl derived phenols when exposed to gymnosperm emissions despite an absence of syringol derived pyrolysates in gymnosperm smoke. The likely mechanisms for this occurance were investigated but remain unclear. When smoke exposure occured at a comparable stage of berry development, there was no difference among cultivars in the accumulation of total taint, a finding that has significance for growers seeking to minimise the impact of smoke taint. Significant differences were found in the extraction of taint due to traditional winemaking methods and taint extraction guidelines have been developed from the work of this thesis. In finding that a large number of phenols found in pyrolysis emissions were elevated in smoke exposed grapes, a sensory evaluation of smoke tainted wines in this study has revealed some compounds moderate the harsh organoleptic descriptors of smoke taint. Glycoconjugates of phenol, *m*-cresol and *p*-cresol were found to be the most likely drivers for the negative flavour descriptors in smoke affected wine.

Further work is required in smoke taint research to prevent or ameriolate the accumulation of phenols in grapes and wine. Although there is currently no solution to smoke taint for wine grape growers and oenologists, the industry requires greater understanding of the sensory limitations for each cultivar in volatile and glycoconjugate phenol concentrations. This would empower grape growers to better assess the suitability of smoke exposed grapes for wines before the costly process of

harvest and winemaking commence. The synergistic sensory effect of phenols and phenol glycoconjugates in differing wine matrices is a complex issue in assessing smoke taint. While no cultivar differences in accruing phenols as taint was reported in this thesis, at least for the three cultivars described in Chapter 7, there may be cultivar/wine style advantages in masking the sensory effects of smoke taint in wine.

The mechanism for the formation of phenol glycosides, particularly the syringol derivatives, in wine grapes from an exposure to smoke is also warranted of further investigation. This anomaly may not be unique to grapevines and uncovering the mechanisms of plant response to phenol exposure may reveal a greater adaptive response in plants to phenols in wildfire emissions.

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APPENDICES

APPENDIX 1. Singh, D., Zerihun, A., Kelly, D., Cain, N., Nankervis, P. and Downey, M. (2012). A GC-MS based analytical method for detection of smoke taint associated phenols in smoke affected wines. Current Bioactive Compounds, 8, 190-199.

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APPENDIX 4. Respondent sensory assessment results sheet.

APPENDIX 5. Correlations of volatile and glycosidically bound phenols to aroma and taste descriptors of Merlot wines.

APPENDIX 6. List of abbreviations.

APPENDIX 7. Copyright clearance statements.

Appendix 1. Singh, D., Zerihun, A., Kelly, D., Cain, N., Nankervis, P., and Downey, M. (2012). A GC-MS based analytical method for detection of smoke taint associated phenols in smoke affected wines. Current Bioactive Compounds, 8, 190-199.

A GC-MS Based Analytical Method for Detection of Smoke Taint Associated Phenols in Smoke Affected Wines

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Abstract: Guaiacol and 4-methylguaiacol are routinely used as markers to determine extent of smoke impact on winegrapes and wines. However, smoke contains a complex group of compounds that may contribute to smoke taint in winegrapes and wine. In this study, a gas chromatography-mass spectrometry (GC-MS) based analytical method was developed and validated for the profiling of various smoke taint compounds in wines made from smoke affected fruit. A total of 22 analytes were separated and identified in the GC-MS chromatogram, all of which were selected to evaluate the samples and precision of the method. The GC-MS method showed good repeatability/reproducibility with intra- and inter-day relative standard deviation (RSD) of ±14%. The method was used to demonstrate that the smoked grapes and resultant wines, compared to unsmoked wines, contained significantly enhanced levels of guaiacol and 4-methylguaiacol along with other lignin derived phenols such as cresols and syringol. In smoke affected grapes and young wines, volatile phenols exist as glyco-conjugates (potential taint), which hydrolyse slowly leading to unacceptable levels of taint accumulation in wine during storage. The GC-MS method reported here, in conjunction with the optimised acid hydrolysis of phenol glyco-conjugates, was successfully used to determine potential levels of smoke taint compounds in wines. Thus, the method can be used for screening smoke exposed grapes for potential taint levels prior to wine making. The results presented here highlight the need to include an array of smoke derived phenols to develop a complete picture of smoke taint and associated aroma in affected grapes and wines.

Keywords: Acid hydrolysis, gas chromatography-mass spectrometry, glycosides of phenols, lignin, smoke taint, solid phase extraction, volatile phenols, wine.

INTRODUCTION

Research conducted in the last five years has found that smoke affected winegrapes and wines produced from these grapes have “smoke taint” aroma [1-5]. Common descriptors of smoke taint aroma in wines are smoky, dirty, earthy, burnt, smoked meat, bacon, damp fire, plastic, ashtray and band aid characters. These unpleasant characteristics in the wines, prepared from smoke affected fruit, have resulted in low consumer appeal and financial loss to the wine grape industry [3].

The vegetative biomass consumed in bushfires and fuel reduction burning is primarily composed of cellulose (40-45%), hemicelluloses (20-35%) and lignin (18-35%) compounds [6]. It is widely believed that the pyrolysis of lignin in a fuel releases phenols that give smoke its distinctive smell and these compounds are normally associated with the tastes and smells of smoke cured foods [7, 8]. However, production and concentration of these compounds in the smoke depend upon oxidative combustion conditions such as temperature, moisture content and fuel type [9-11].

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Guaiacol and 4-methylguaiacol, which are thermal degradation products of lignin, have been widely used as indicator compounds in assessing smoke taint levels and the degree to which fruit and wines have been affected by smoke [2-4]. However, concentrations of guaiacol and 4-methylguaiacol are not always a reliable indicator of the extent of smoke exposure. In some cases these compounds were not detected, or detected at low levels, in the fruit while high levels were subsequently identified during or after winemaking or storage [12-14]. This discrepancy was attributed to the presence of glycosidic conjugates of volatile phenols in the grapes, which were thought to evolve into smoke taint during fermentation and wine making. Later research involving high pressure liquid chromatography mass spectrometry (HPLC-MS/MS) and hydrolysis under acid or enzymatic conditions confirmed the presence of glycosidic conjugates in grapes and wine [1, 5, 15].

Pyrolysis of smoke produced from the combustion of vegetative biomass contains several other volatile and semi-volatile phenols [10, 11, 16], which can contribute to smoke taint and hence, to the overall sensory properties of smoke affected fruit and wine. Recently, elevated levels of free phenols and their glycosides such as cresols, syringol and syringol derivatives have been reported in smoke affected fruit and wine [1, 17] indicating that identification and quantification of guaiacol and 4-methylguaiacol may not present the complete picture of smoke taint and associated aroma in

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fruit and wine. Additionally, individual concentrations of these phenols may be well below sensory thresholds but their combined concentrations may result in a perceived sensory effect. Therefore, it is important to investigate whether different phenols contribute to smoke taint.

The present paper describes the development (optimisation and validation) of a gas chromatography-mass spectrometry (GC-MS) based analytical method to identify and quantify the characteristic organic compounds (i.e. volatile phenols) emitted during pyrolysis of wood (or lignin) in wines prepared from smoke affected fruit. The method involved solvent extraction and a subsequent capillary GC-MS detection and determination of volatile phenols in wine made from fruit exposed to smoke. Glycoside bound phenols were extracted from the wine using solid-phase extraction (SPE) before acid hydrolysis to generate aglycones followed by solvent extraction and GC-MS analysis.

MATERIALS AND METHODS

Chemicals

HPLC grade acetonitrile, methanol, ethanol, sulphuric acid, and sodium hydroxide were purchased from Merck and Co. Inc. (Darmstadt, Germany). Standards for phenol, *o*-, *m*- and *p*-cresol, 4-ethylphenol, 4*n*-propylphenol, 4-ethyl-2-methoxyphenol (4-ethylguaiacol), 4*n*-propyl-2-methoxyphenol (4*n*-propylguaiacol), 2-methoxy-4-vinylphenol (4-vinylguaiacol), 2,6-dimethoxyphenol (syringol), 2,6-dimethoxy-4-methylphenol (4-methylsyringol), 4-allyl-2,6-dimethoxyphenol (4-allylsyringol) and syringaldehyde were acquired from BioScientific Pty Ltd. (Gymea, NSW, Australia). Eugenol, isoeugenol, guaiacol, 4-methylguaiacol, vanillin, acetovanillone, acetosyringone standards, ethylacetate and *n*-hexane (GC grade) were obtained from Sigma-Aldrich (St. Louis, MO, USA). 2-methoxy-d_3-phenol (d_3-G) was purchased from CDN isotopes (Pointe-Claire, QB, Canada). Purity of all standards was verified by GC-MS before preparation of stock solutions. Deionised water was obtained through a MilliQ system (Milli-RX Analytical-Grade Water Purification System, Millipore, Billerica, MA, USA).

Wine Samples

Wines were made from *Vitis vinifera* L. cv. Chardonnay, Merlot, Shiraz, Sangiovese and Cabernet Sauvignon fruit collected from the King Valley wine region of north eastern Victoria (36°42' South, 146°25' East), Australia. Fruit was collected in March 2007 following bushfire events in December 2006 and January 2007 [5]. To meet quarantine regulations, fruit was frozen at -20 ℃ for at least seven days prior to shipping to a small scale winery for winemaking. Wines were made according to a standardised methodology [18]. For comparison, wines were made from smoke unexposed grapes of Chardonnay, Shiraz and Cabernet Sauvignon varieties (2006 and 2009 vintage) from the Mildura region (34°42' South 142°28' East) and analysed for both free and bound forms of volatile phenols. The Mildura region had no bushfire activity in 2005-06 and 2008-09.

Sample Preparations

Free forms of phenols were measured by extracting 5 mL of the wine samples with 2 mL of ethylacetate:*n*-hexane (1:1, v/v) after spiking with 10 µL of d_3-G and adding 1.05 g NaCl. The samples were vortexed for 1 min followed by incubation at room temperature for 60 min. The incubation at the room temperature was continued for another 1-2 h after addition of 2 mL of ethylacetate:*n*-hexane (1:1, v/v) and vortexing for 1 min. A 1 mL portion of the organic phase, obtained after spinning at 2,469 x g for 5 min, was transferred to a 2 mL GC autosampler vial, capped and analysed for various phenols using the GC-MS method described below.

For analysis of glyco-conjugated phenols, the following sample preparation, extraction and acid hydrolysis procedures were performed prior to GC-MS analysis. A 20 mL aliquot of the wine to be analysed was frozen in liquid nitrogen and dried using a freeze dryer (Freezone, Labconco Corporation, Kansas City, MO, USA) at -75 ℃. The dried samples were redissolved in 10 mL of deionised water. 1.5 mL of 10 M NaOH added and the solution filtered through a 0.45 µm polypropylene syringe filter (Whatman, Kent, UK).

Solid-phase extraction (SPE) was utilised to extract bound forms of phenols from freeze dried wine samples and to remove non-phenolic substances (sugars, organic acids, proteins and pigments), which can interfere with the chromatographic separation. An Oasis® HLB Plate 96-well plate (Waters Corporation, Milford, MA, USA) was used for SPE as reported previously [1, 5, 19-21]. Solid-phase plates were conditioned with 0.5 mL methanol followed by a rinse with 0.5 mL deionised water. One mL of wine samples were loaded into 8 wells and the liquid was removed under vacuum. The wells were rinsed three times with 1 mL aliquots of deionised water.

The solid-phase plate columns were eluted under vacuum with 0.17 mL ethanol (99.9%) and rinsed with 0.33 mL deionised water into a clean 2 mL 96 well plate. One mL of each sample was then transferred in three replicates to 20 mL GC-MS head-space autosampler vials. To this was added 4 mL of 5 N H_2SO_4 (pH1.0). The sealed autosampler vials were incubated for 1 h at 100 ℃. Samples were cooled on ice and transferred to Kimble tubes (PYREX® Corning, New York, NJ, USA) containing 1.05 g NaCl. These samples were spiked with 10 µL of internal standard (1 mg/L d_3-G in ethanol) and extracted with 2 mL of ethylacetate:*n*-hexane (1:1, v/v) as described above for the analysis of free forms of volatile phenols.

Gas Chromatography Mass Spectral Analysis of Various Phenols

Grape and wine samples were analysed for various phenols using an Agilent 7890A gas chromatograph and 5975 mass spectrometer (Agilent Technologies, Palo Alto CA, USA) equipped with a fused silica capillary column (AT-5MS, 0.25 mm I.D. x 30 m length and 0.25 µm film thickness, GRACE, Deerfield, IL, USA). Helium (ultra purity grade, BOC Gases, Adelaide, SA, Australia) was used as a carrier gas with an average linear velocity of 37 cm/s and a flow rate of 1 mL/min. Liquid sample (1 µL) was injected using a CTC-PAL autosampler (CTC Analytics AG, Zwingen, Switzerland) into the GC inlet injector at 240 ºC fitted with a 4 mm id liner (Agilent Technologies, Palo Alto, CA, USA). The GC injector (inlet 1) was operated in the

pulsed/splitless mode with a pulsed pressure of 40 psi for 0.5 min followed by a split flow of 100 mL/min for 1 min. Oven temperature started at 50 ºC and was increased by 15 ºC/min until reaching 280 ºC and was held at 280 ºC for 1 min. Under this temperature program the elution order was phenol, cineole, *o*-cresol, *m*-cresol, *p*-cresol, guaiacol, 2,4-dimethyphenol, 4-ethylphenol, 4-methylguaiacol, 4*n*-propylphenol, 4-ethyl-guaiacol, 4-vinylguaiacol, syringol, eugenol, 4*n*-propyl-guaiacol, vanillin, 4-methylsyringol, isoeugenol, acetovanillone, allylsyringol, syringaldehyde, acetosyringone, and d_3-G Fig. (**1**).

The MS ion source temperature was 230 ºC and the GC-MS transfer line temperature was 220 ºC. A solvent delay of 3 min was set up and data acquisition mode was set to Selective Ion Monitoring (SIM) mode. The ions monitored are detailed in Table **1** (Souirce: National Institute of Standards and Technology virtual library). The selected ions were monitored for 50 ms each. Samples were analysed in triplicate. The detector showed good linear response for each of the 22 analytes ($r^2 \geq 0.99$).

Calibration Standards and Method Validation

Solutions containing 1000, 500, 250, 100, 50, 25, 10, 5, 2.5 and 1.0 µg/L phenol, cineole, *o*-cresol, *m*-cresol, *p*-cresol, guaiacol, 2,4-dimethyphenol, 4-ethylphenol, 4-methylguaiacol, 4*n*-propylphenol, 4-ethylguaiacol, 4-vinylguaiacol, syringol, eugenol, 4*n*-propylguaiacol, vanillin, 4-methylsyringol, isoeugenol, acetovanillone, allylsyringol, syringaldehyde and acetosyringone were prepared in ethylacetate:*n*-hexane (1:1, v/v). The calibration standard curves were prepared by transferring 1.0 mL of a solution containing all the 22 compounds to a 2 mL vial and adding 10 µL of d_3-G internal standard solution. A typical chromatogram from a standard solution containing the 22 analytes is shown in Fig. (**1A**).

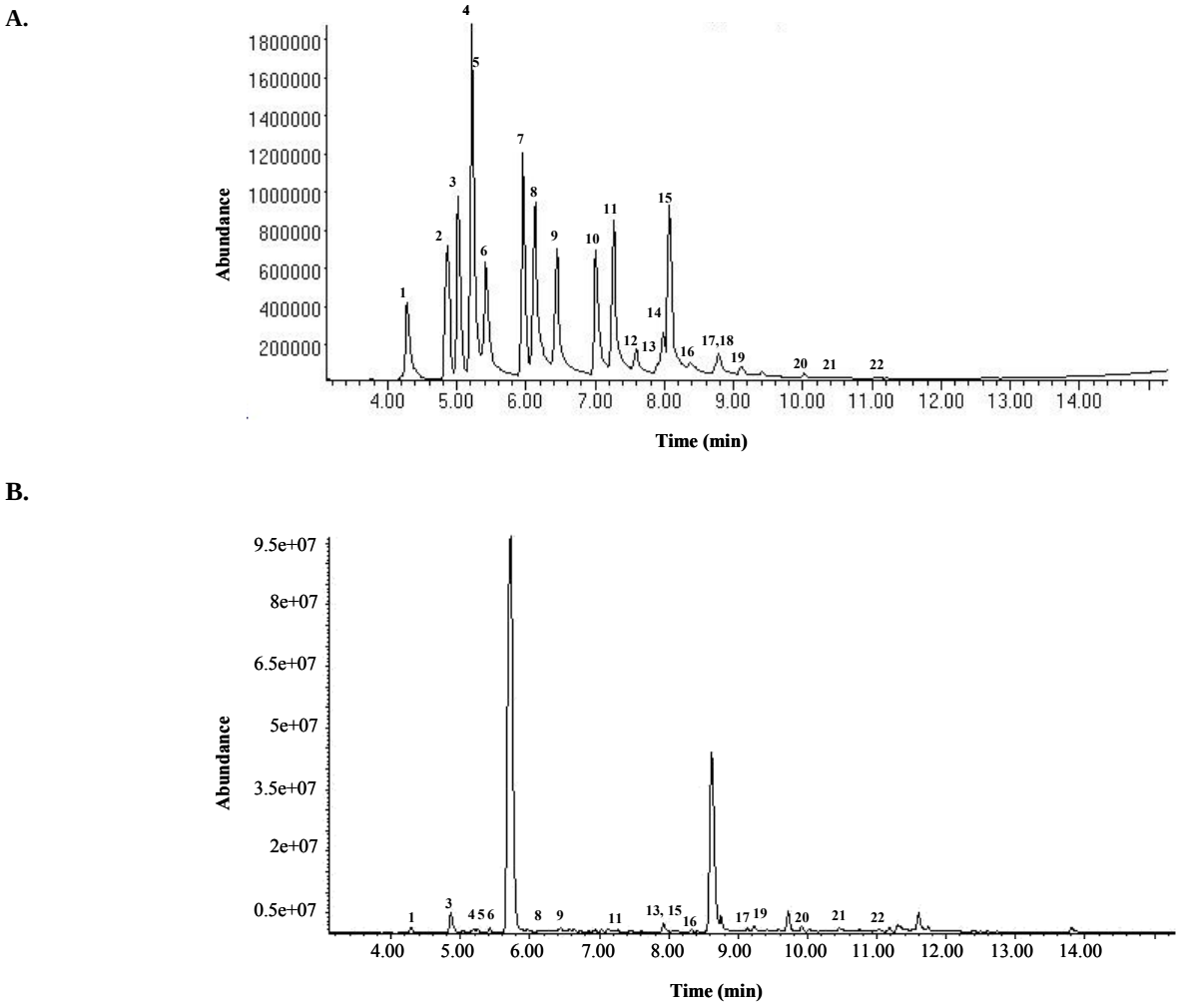

Fig. (1). SIM chromatograms showing retention times (min) of various phenolic standards (**A**) and ethyl acetate: n-hexane (1:1) extract of wine (**B**) on GC-MS AT-5MS silica capillary column (GRACE, Deerfield, IL; 30 m, 0.25 mm id and 0.25 µm film thickness). The retention times (min): 1. phenol (4.284); 2. cineole (4.856); 3. *o*-cresol (5.022); 4. *m*-cresol (5.198); 5. *p*-cresol (5.23); 6. guaiacol (5.409); 7. 2,4-dimethyphenol (5.962); 8. 4-ethylphenol (6.122); 9. 4-methylguaiacol (6.439); 10. 4*n*-propylphenol (7.009); 11. 4-ethylguaiacol (7.263); 12. 4-vinylguaiacol (7.588); 13. syringol (7.909); 14. eugenol (7.985); 15. 4*n*-propylguaiacol (8.027); 16. vanillin (8.371); 17. 4-methylsyringol (8.746); 18. isoeugenol (8.795); 19. acetovanillone (9.118); 20. allylsyringol (10.025); 21. syringaldehyde (10.488); and 22. acetosyringone (11.039).

The specificity, precision and validation of the analytical method were determined by spiking a series of standards to red wine (Shiraz). The wines were spiked in triplicate with 0, 10, 20, 40, 80, and 160 μg/L of mixed standards to determine analyte recoveries.

The limit of detection (LOD) and limit of quantification (LOQ) of guaiacol and 4-methylguaiacol were determined by using statistical procedures described previously [22].

RESULTS AND DISCUSSION

Calibration and Performance Characteristics

Lignins are primarily polymers of three monolignols i.e. para-coumaryl, coniferyl and sinapyl alcohols which differ in their degree of methoxylation [23]. A total of 22 compounds, covering different chemical families of lignin were studied (Table **1**). Eight *p*-coumaryl alcohols (phenol, cineole, *o*-, *m*- and *p*-cresols, 4-ethylphenol, 2,4-dimethylphenol and 4*n*-propylphenol), nine coniferyl alcohols (guaiacol, 4-methylguaiacol, 4-ethylguaiacol, eugenol, isoeugenol, 4*n*-propylguaiacol, 4-vinylguaiacol, vanillin and acetovallinone) and five sinapyl alcohols (syringol, 4-methylsyringol, allylsyringol, syringaldehyde and acetosyringone) were selected for the method development and validation. The representative compounds for each monolignol class (Table **1**) were chosen by considering published data on composition of smoke from lignin pyrolysis and liquid smoke flavourings [6-8, 24-27]. The presence of some of these compounds was also confirmed in the smoke from prescribed burns [1]. This further highlighted the risk of exposure of grape vines to various volatile phenols in smoke from bushfire or prescribed burns and development of smoke taint in grapes and wine.

Ethylacetate has been used to extract non-flavonoid phenols in wines [28] and maple products [29] for analyses by HPLC with very good reproducibility and without any chemical modifications. Previous researchers have observed that the mean percentage recovery for all phenolic and furfural compounds using different methods of extraction was, in decreasing order: ethyl acetate (87.6%) > Sep-Pak (82.2%) > lyophilization (62.9%) > ether (44.3%) > Supelclean (41.8%). Recently, Hayasaka *et al.* [1] used ethylacetate:*n*-pentane (1:1, v/v) for extraction of volatile phenols from grape juice and wines without reporting any artefacts. Preliminary trials substituting *n*-pentane with *n*-hexane showed improved baseline on chromatographs (Fig. (**1**) and data not shown); thus in this study we used ethylacetate:*n*-hexane (1:1, v/v) for extraction of volatile phenols from unsmoked (control) and smoke affected wines. The parameters of the method were optimised by using standards of various phenols (Table **1**) in ethylacetate:*n*-hexane (1:1, v/v). For each standard, ten concentrations (1-1000 μg/L) were tested in 5-10 replicates; these concentrations covered the concentration ranges expected for these compounds in wine. Total ion chromatogram and retention times of all the studied compounds are shown in Fig. (**1A**, **1B**) and Table **1**. Target ion and one or two qualifier ions were used to calculate the (volatile compound/internal standard) ion peak area ratio for each studied volatile compound (Table **1**). For all the compounds examined, the relationships between ion peak area ratios and analyte concentration ratios were linear over the entire calibration range (1-1000 μg/L). The coefficients of determination (r^2) were ≥ 0.99.

The limits of detection and quantitation of the analytes were low enough (Table **2**) to detect and/or quantitate these compounds in wine samples from grapes unaffected by smoke. The LOQ values determined in this work were close to the lowest concentration of the calibration range and are comparable to those published elsewhere [1].

Accuracy, Recovery, Repeatability and Reproducibility

In order to calculate the accuracy of the method, a recovery study was carried out. Known concentrations of the volatile phenols were spiked in triplicate into a smoke unaffected wine and the concentrations before and after the addition were determined. On the evidence of these concentrations, the percent recovery for each studied compound was calculated (Table **2**). The majority of the compounds had reasonably high recovery (> 90%) except cineole (< 50%). Compounds such as eugenol and isoeugenol showed intermediate levels of recovery (79-84%). The recovery of some of the smoke taint compounds was better than 100% with relative standard deviation (RSD) < 10% (Table **2**). This could be due to hydrolysis of soluble precursors at higher injector block temperatures as has also been reported previously [5, 30]. Another reason for this discrepancy could be matrix enhancement of the GC response; usually from the active sites in the liner and column being shielded by compounds in the matrix resulting in a larger response for the target compounds. Nevertheless, these results are similar to spiked recoveries observed in previous studies, suggesting that wine components may affect the extraction of volatile phenols [31].

The intra-day (repeatability) and inter-day (reproducibility) precision of the method were calculated by means of eight samples extracted in triplicate at the same time and another eight extractions performed on different days. No significant differences were observed between the sets of data produced either intra- or inter-day. As can be seen in (Table **2**), the precision were broadly comparable for the intra- (2.2-12.2%) and inter-day (1.9-14.2%) runs indicating the robustness of the method.

The detection limits (LOD) determined for most of the chemicals analysed was < 5 μg/L (Table **2**). These values were close to the lowest concentration level of the working range. It was verified that these analytes presented rates of recovery and levels of detection compatible with their thresholds of perception and the concentrations expected in non-smoked fruit and wine [32] (Table **3**). In summary, taking into account recovery, repeatability, reproducibility, LOD and LOQ, the method developed here provides an acceptable level of accuracy for the determination of volatile phenols which may contribute to smoke taint in wines prepared from smoke affected fruit.

Determination of Volatile Compounds in Wines

Previous research has established a strong link between smoke exposure and development of smoke aroma in winegrapes and the wine product. We used the GC-MS based analytical method developed here to examine the levels of

Table 1. Characteristics of the Phenols Determined in the Analysis, their Ions, Retention Time an Calibration Curves Generated by Using GC-MS Based Analytical Method to Examine Smoke Taint Related Phenols

Compound	Quantifying Ions (*m/z*)	Retention Time (min)	Studied Range (µg/L)	r^2	Mean Slope	RSD (%)
phenol	94, 66, 65, 39	4.284	2.5-1000	0.9983	0.2106 ± 0.010	3.2
cineole	154, 139, 108, 111	4.856	2.5-1000	0.9975	0.2561 ± 0.008	3.2
***o*-cresol**	108, 107, 79, 77	5.022	2.5-1000	0.9994	0.1772 ± 0.010	2.9
***p*-cresol**	107, 108, 79, 77	5.198	2.5-1000	0.9996	0.3914 ± 0.010	2.9
***m*-cresol**	108, 107, 79, 77	5.23	2.5-1000	0.9991	0.3473 ± 0.010	3.1
guaiacol	109, 124, 81	5.409	1-1000	0.9998	0.1715 ± 0.003	1.9
2,4-dimethylphenol	122, 107,121, 77	5.962	1-1000	0.9996	0.3126 ± 0.010	2.8
4-ethylphenol	107, 122, 77	6.122	1-1000	0.9993	0.3846 ± 0.010	2.9
4-methylguaiacol	138, 123, 95	6.439	1-1000	0.9984	0.1465 ± 0.003	1.8
4*n*-propylphenol	107, 136, 77	7.009	1-1000	0.9981	0.3428 ± 0.010	3.2
4-ethylguaiacol	137, 152, 122	7.263	2.5-1000	0.9964	0.2823 ± 0.010	2.1
4-vinyl guaiacol	150, 134, 107	7.588	1-1000	0.9962	0.0891 ± 0.002	2.0
syringol	139, 154, 111	7.909	2.5-1000	0.991	0.0473 ± 0.003	7.3
eugenol	164, 149, 131, 103	7.985	2-1000	0.9983	0.1092 ± 0.007	6.7
4*n*-propylguaiacol	137, 166, 122, 94	8.027	1-1000	0.9914	0.3790 ± 0.004	1.1
vanillin	151, 152, 109, 123	8.371	5-1000	0.9977	0.0311 ± 0.003	9.7
4-methylsyringol	168, 153, 125, 151	8.746	5-1000	0.9873	0.0416 ± 0.004	9.1
isoeugenol	164, 77, 149, 91	8.795	2.5-1000	0.9957	0.0981 ± 0.007	7.1
acetovallinone	151, 166, 123	9.118	10-1000	0.9918	0.0088 ± 0.001	8.5
allysyringol	194, 179, 167	10.025	5-1000	0.9973	0.0298 ± 0.002	7.9
syringaldehyde	182, 181, 96, 111	10.488	5-1000	0.9981	0.0218 ± 0.001	4.0
acetosyringone	181, 196, 153	11.039	5-1000	0.997	0.0309 ± 0.002	7.1

free and glyco-conjugates of phenols in wine samples prepared from smoke exposed and unexposed grapes. Fig. (**1B**) shows the total ion chromatogram of one of the smoke affected wine samples showing the presence of various smoke related phenols.

The analytical method was used successfully to show that bushfire smoke affected wines, compared to unaffected control wines, contained markedly elevated levels of a range of smoke taint compounds for all the varieties and hence different wine matrices examined (Table **3**). Cineole, eugenol, and isoeugenol were not detected in the smoked or unsmoked control wines. This may be due to degradation of these analytes during analysis as suggested previously [1, 12] or these analytes were present at levels lower than the detection limits of the method described above.

Vanillin, the main phenolic aldehyde, and its derivatives contribute to vanilla aromas [33]. Vanillin was detected at slightly elevated levels in wines prepared from smoke affected grapes compared to unsmoked control wines (Table **3**). Free acetovallinone content was significantly higher in smoke affected wine for all varieties, but the levels in Cabernet Sauvignon, Merlot and Sangiovese wines were approximately twice those in Chardonnay and Shiraz wines. Syringol and acetosyringone were the most dominant sinapyl volatile phenols. This is consistent with a previous report where enhanced levels of syringol have been observed in smoke affected grapes [1]. Other related compounds such as syringaldehyde which contribute to the enhancement of aged wine's flavour were detected in free form in only two of the varieties: Cabernet Sauvignon (122 µg/L) and Merlot (98 µg/L) (Table **3**). The reason for this differential result is not clear but may indicate a varietal difference in accumulation.

Wines produced from grapes not exposed to smoke had low levels of some of the volatile phenols studied here in both the free as well as bound forms as evident from only slightly elevated levels after acid hydrolysis (Table **3**). These results suggest that small amounts of the phenolic glycosides are naturally present in grapes and are released during yeast fermentation or aging of bottled wines. Previous studies have

Table 2. Performance Characteristics of the Analytical Method Developed to Detect and Measure Various Phenols Potentially Associated with Smoke Taint in Wines

Compound	Detection Limit (LOD, µg/L)	Quantitation Limit (LOQ, µg/L)	Recovery (%)	Repeatability (RSD, %)	Reproducibility (RSD, %)
phenol	1.1	3.2	96.4	4.1	5.8
cineole	1.3	4.0	46.4	-	-
***o*-cresol**	0.4	1.3	117.5	4.0	3.2
***p*-cresol**	0.3	1.0	110.6	3.7	4.6
***m*-cresol**	0.3	0.9	110.7	4.4	5.8
guaiacol	0.5	1.4	121.9	5.9	5.3
2,4-dimethylphenol	1.1	3.4	-	-	-
4-ethylphenol	0.5	1.3	121.5	3.0	1.9
4-methylguaiacol	0.4	1.1	108.5	4.7	6.6
4*n*-propylphenol	0.4	1.0	106.0	9.6	4.2
4-ethylguaiacol	0.4	1.3	114.4	3.9	6.3
4-vinyl guaiacol	0.9	2.6	-	-	-
syringol	2.4	7.1	95.3	9.6	8.3
eugenol	2.1	6.1	79.0	2.5	-
4*n*-propylguaiacol	0.5	1.5	104.3	4.9	4.5
vanillin	2.1	6.4	98.6	12.2	14.2
4-methylsyringol	1.5	4.6	94.2	5.2	6.8
isoeugenol	2.1	6.4	83.7	12.8	6.4
acetovallinone	2.8	8.5	134.2	9.6	6.9
4-allysyringol	1.8	5.4	145.8	12.2	10.4
syringaldehyde	2.8	8.4	100.3	2.4	7.2
acetosyringone	1.2	3.7	107.3	2.2	4.3

reported glycosides of guaiacol in the berries of Tempranillo, Grenache [34], Shiraz [5, 13], Merlot [12] and vanillin as glycoside in grapes, cherry and strawberry [35]. Recently glycosides of phenol, cresols, methylsyringol and syringol have been detected in unsmoked Chardonnay berry juice [1, 17]. It is also possible that some of these phenols derive at least partially, from degradation of certain lignified zones of the fruit for example, the seed [36]. Therefore, it will be interesting to process grapes and yeast fermentation samples with or without seeds to shed further light on the provenances of these chemicals.

Concentrations of hydrolytically released *p*-coumaryl alcohols from their bound forms ranged from 52 µg/L (unsmoked Chardonnay, control wine) to 1260 µg/L (smoked Merlot wine). The glycoside-bound *p*-coumaryl pool of compounds was generally dominated by cresols (Table **3**). Among all the wines prepared from smoke affected fruit, the highest concentrations of hydrolytically released *p*-coumaryl alcohols were observed in Chardonnay, Cabernet Sauvignon and Merlot, while the lowest concentrations were observed in wines from Sangiovese and Shiraz grapes. Concentrations of the hydrolytically released phenols from the coniferyl alcohol group ranged from 141 µg/L in smoke unaffected Shiraz wines to 1698 µg/L in smoke tainted Cabernet Sauvignon wines (Table **3**). Concentrations of the hydrolysed sinapyl alcohol group of compounds showed a two order of magnitude range (69 µg/L in control Chardonnay wines to 6487 µg/L in smoke tainted Cabernet Sauvignon wines) (Table **3**). Generally, the highest concentrations of hydrolytically released compounds were observed in red wines, while the lowest concentrations were observed in wines from Chardonnay grapes. This observation was consistent with previous reports that wines made from white grapes tended to have lower levels of guaiacol and 4-methylguaiacol and that this was due to the absence of skin contact during winemaking with the wines being made from free-run juice [14]. This suggests that smoke taint compounds accumulate

Table 3. Concentrations (μg/L) of Free and Bound forms (Determined After Acid Hydrolysis) of Volatile Phenols in Wines Made from Smoke Affected and Unaffected (Control) Grapes. Wines were analysed by the Method Described Here and Values Represent Mean ± s.e. of Analytical Replicates (n=3)

		Location							
		Mildura (34°42' South 142°28' East)	**Mildura (34°42' South 142°28' East)**	**Mildura (34°42' South 142°28' East)**	**Whitfield (36°45' South 146°24' East)**	**Cheshunt Sth (36°55' South 146°23' East)**	**Whitfield (36°45' South 146°24' East)**	**Edi Upper (36°41' South 146°29' East)**	**Cheshunt (36°47' South 146°25' East)**
		Variety							
Free	Volatile phenols	Chardonnay (Control)	Shiraz (Control)	Cabernet sauvignon (Control)	Chardonnay	Shiraz	Sangiovese	Cabernet sauvignon	Merlot
p-coumaryl alcohol	phenol	21.32±1.4	9.3±1.1	12.5±0.8	60.9±0.7	107.1±5.6	125.3±3.2	249.8±13.9	59.0±3.4
	o-cresol	ND	ND	ND	15.8±0.4	60.6±3.7	106.1±2.3	108.4±5.5	25.4±1.3
	p-cresol	2.9±0.3	10.7±1.2	6.5±0.3	28.7±0.4	35.1±2.3	62.6±1.5	77.6±4.7	23.6±0.8
	m-cresol	3.7±0.4	9.6±0.9	3.6±0.3	30.3±0.3	37.9±2.6	69.2±2.1	83.7±5.2	25.5±1.3
	4-ethylphenol	ND	ND	ND	ND	ND	ND	ND	ND
coniferyl alcohol	guaiacol	1.7±0.2	21.8±1.4	8.4±0.6	86.5±1.5	283.6±14.5	487.0±9.0	306.8±15.7	100.7±4.3
	4-methylguaiacol	ND	ND	ND	61.3±1.7	68.4±3.6	123.8±2.5	180.5±10.9	45.6±2.3
	4-ethylguaiacol	ND	ND	ND	42.4±1.3	21.5±1.7	44.7±0.8	80.8±4.6	25.7±0.6
	eugenol	ND	ND	ND	ND	ND	ND	ND	ND
	4*n*-propylguaiacol	ND	1.7±0.2	ND	6.3±0.1	ND	4.1±0.1	9.0±0.4	2.6±0.1
	vanillin	27.7±2.8	32.7±0.4	35.6±1.8	48.0±1.0	49.3±1.5	46.3±1.3	47.5±1.3	29.8±0.8
	acetovallinone	40.4±2.1	73.4±1.0	165.3±1.9	303.5±8.5	305.5±11.9	779.0±27.0	707.8±27.8	617.2±23.0
sinapyl alcohol	syringol	20.3±1.5	ND	474.3±28.3	370.6±5.4	570.0±104.9	421.6±19.4	1649.8±114.1	831.4±21.5
	4-methylsyringol	ND	23.9±3.2	10.9±0.5	160.5±2.4	197.2±8.6	154.2±5.6	686.7±45.0	232.7±8.2
	allysyringol	ND	61.8±2.3	ND	90.3±3.6	118.3±9.0	41.0±0.8	127.0±6.4	49.8±3.6
	syringaldehyde	ND	ND	ND	ND	ND	ND	122.1±2.9	98.5±4.2
	acetosyringone	32.0±2.0	299.3±12.7	164.0±12.1	286.9±8.7	1054.3±17.5	966.3±28.0	1253.0±50.9	1755.5±32.1
Bound									
p-coumaryl alcohol	phenol	10.6±0.4	6.3±0.5	10.2±0.5	149.6±3.1	70.0±2.3	110.8±7.7	178.2±2.2	224.8±5.6
	o-cresol	ND	ND	ND	328.2±1.24	23.4±.8	19.6±3.3	49.9±.8.2	461.8±13.5
	p-cresol	4.2±0.6	6.7±0.2	22.5±3.5	105.9±3.7	68.9±3.3	62.7±2.6	246.0±8.6	245.4±12.1
	m-cresol	6.2±0.4	9.2±0.4	ND	138.8±9.6	52.4±2.6	72.7±9.4	314.7±15.0	328.7±19.0
	4-ethylphenol	32.1±1.0	ND	ND	31.6±0.6	ND	ND	ND	ND

(Table 3) Contd….

	Location								
		Mildura (34°42' South 142°28' East)	**Mildura (34°42' South 142°28' East)**	**Mildura (34°42' South 142°28' East)**	**Whitfield (36°45' South 146°24' East)**	**Cheshunt Sth (36°55' South 146°23' East)**	**Whitfield (36°45' South 146°24' East)**	**Edi Upper (36°41' South 146°29' East)**	**Cheshunt (36°47' South 146°25' East)**
coniferyl alcohol	guaiacol	3.5±0.2	17.7±0.3	7.3±0.4	130.0±3.5	209.9±7.1	253.6±9.9	235.6±2.5	377.3±11.4
	4-methylguaiacol	ND	ND	ND	63.4±2.4	57.8±3.8	114.6±9.3	132.1±3.7	210.3±7.2
	4-ethylguaiacol	8.9±0.3	ND	2.4±0.3	16.9±0.7	9.4±0.5	27.0±2.8	37.6±0.8	60.9±2.1
	eugenol	ND	ND	ND	ND	ND	ND	ND	ND
	4*n*-propylguaiacol	ND	ND	ND	1.1±0.1	ND	1.96±0.3	4.4±0.7	10.2±0.6
	vanillin	135.7±5.3	113.5±2.4	178.7±4.8	736.2±64.5	395.9±21.7	809.3±16.3	740.4±30.4	299.0±16.0
	acetovallinone	76.8±3.6	9.9±0.8	92.7±4.4	376.2±26.5	197.2±23.6	398.8±18.3	548.3±69.8	22.5±2.2
sinapyl alcohol	syringol	18.2±0.9	18.6±0.6	71.0±3.4	506.2±30.5	566.8±45.1	1433.8±162.7	2985.4±186.5	3201.2±167.5
	4-methylsyringol	ND	ND	ND	ND	7.2±0.5	9.0±1.5	49.2±1.5	11.7±1.1
	allysyringol	ND	ND	ND	ND	ND	ND	ND	ND
	syringaldehyde	ND	134.8±16.0	ND	ND	504.6±25.4	906.5±25.3	3040.7±208.5	1223.8±62.6
	acetosyringone	51.5±3.7	265.7±9.7	283.2±11.5	229.2±14.8	330.8±13.4	384.6±42.4	411.8±51.4	525.4±51.6

differentially in different tissues of grapes. It would therefore be interesting to analyse these compounds in each of the berry tissues (skin, seeds and flesh) separately to localise their distribution in the berry.

Guaiacol and 4-methylguaiacol concentrations in wines, made from bushfire smoke exposed fruit, were considerably higher than those reported in wines prepared from fruit exposed to smoke under experimental conditions [2, 4, 14, 17]. This is likely to be a function of the density and duration of smoke exposure, which has been shown to influence guaiacol levels in smoke tainted wine [37]. However, for the samples analysed here, the bushfire smoke density and duration of exposure were not known, although these are likely to be denser and longer than the experimental smoke exposure conditions based on anecdotal reports. It is also possible that there are differences in varietal sensitivity and accumulation of smoke taint compounds and it is worth verifying whether this indeed is the case. The results from this work collectively demonstrate that smoke unaffected wines contain high totals of background (constitutive) levels of lignin derived compounds ranging from 498 µg/L in Chardonnay to 1549 µg/L in Cabernet Sauvignon (Table **3**). The primary effect of smoke exposure is less a matter of generating new smoke taint compounds than of elevating the levels of lignin-derived compounds that are naturally found in grapes and wines. Thus, in this respect, in wines made from smoke exposed grapes, the background levels were increased by 5 -10 fold (Fig. (**2**)).

CONCLUSIONS

We have optimised the conditions for the analysis of smoke derived volatile phenols that may possess smoky aromas in winegrapes and finished wines by GC-MS. Under the optimised conditions developed in this study, SPE can be considered an appropriate technique for the extraction of bound forms of smoke taint compounds from complex matrices such as wines. The detection and quantitation limits, and the accuracy obtained are adequate for the quantification of the studied phenols.

Several of these volatile phenols were detected in wines prepared from fruit exposed to smoke from 2006-07 bushfire events in the north eastern Victoria. In view of the results obtained here and the method's capability for analysing a wider range of smoke taint compounds than has been hitherto reported, this method will be a valuable tool in furthering smoke taint research. Furthermore, evaluation of additional smoke taint associated compounds with this method provides opportunities to explore the impact on predictive assays and additive or cumulative effects on sensory analyses.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

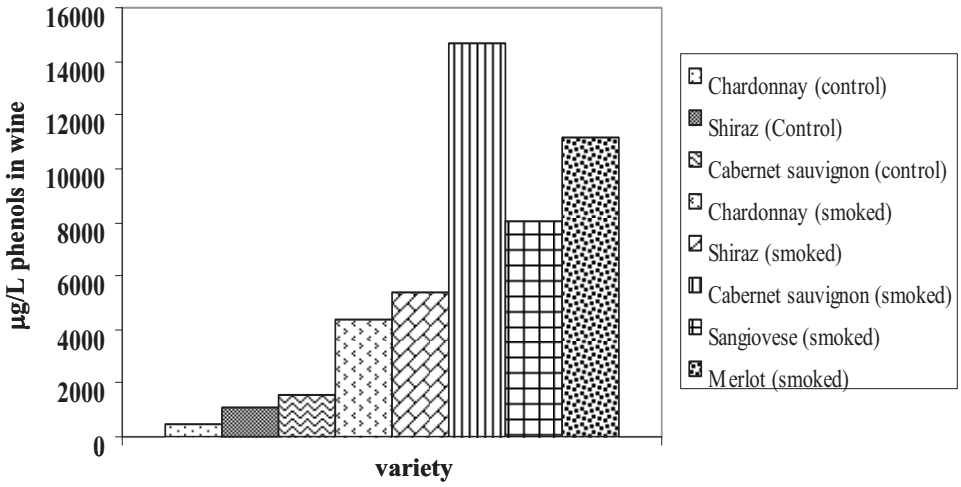

Fig. (2). Concentration of total phenols in wines prepared from grapes exposed to smoke as a result of bushfires in the 2006-07 season in north eastern Victoria Values represent mean of analytical replicates (n=3). Total represents the sum of free and bound forms of *p*-coumaryl, coniferyl and sinapyl alcohols investigated in this study.

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Exposure of grapes to smoke of vegetation with varying lignin composition and accretion of lignin derived putative smoke taint compounds in wine

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ABSTRACT

Smoke taint in wines from bushfire smoke exposure has become a concern for wine producers. Smoke taint compounds are primarily derived from pyrolysis of the lignin component of fuels. This work examined the influence of the lignin composition of pyrolysed vegetation on the types of putative smoke taint compounds that accrue in wines. At veraison, Merlot vines were exposed to smoke generated from five vegetation types with differing lignin composition. Smoke was generated under pyrolysis conditions that simulated bushfire temperature profiles. Lignin and smoke composition of each fuel type along with putative smoke taint compounds in wines were determined. The results showed that, regardless of fuel type, the commonly reported guaiacyl lignin derived smoke taint compounds, guaiacol and 4-methylguaiacol, represented about 20% of the total phenols in wines. Quantitatively, syringyl lignin derived compounds dominated the total phenol pools in both free and bound forms. The contributions of *p*-hydroxyphenyls were generally similar to the guaiacyl sources. A further unexpected outcome of the study was that pine smoke affected wines had significantly elevated levels of syringols compared to the controls although pine fuel and its smoke emission lacked syringyl products.

1. Introduction

Much of the global wine grape crop is produced in Mediterranean-type environments (Jones, White, Cooper, & Storchmann, 2005) where fire is a frequent occurrence (Keeley, Pausas, Rundel, Bond, & Bradstock, 2011). While fire and the resultant smoke play an influential role in shaping natural ecosystems in these environments (Keeley et al., 2011), smoke from such fires can also potentially taint grapes and consequently wines if smoke drifts through vineyards in the landscape while vines are bearing fruit (Whiting & Krstic, 2007). Several recent investigations of smoke exposure from either experimental fires using model vegetation fuels (Kennison, Gibberd, Pollnitz, & Wilkinson, 2008; Sheppard, Dhesi, & Eggers, 2009) or wild/prescribed fires (Hayasaka, Baldock, Parker et al., 2010; Singh et al., 2011, in press) have established that grapes exposed to smoke from pyrolysis of vegetation fuels produce wines with elevated levels of substituted phenols that impart undesirable organoleptic properties.

Smoke-borne compounds that are considered responsible for smoke taint in grapes or wines are thought to originate primarily from pyrolysis of the lignin component of vegetation fuels (Hayasaka, Baldock, Parker et al., 2010; Singh et al., in press) analogously to that which occurs in smoke used for curing/flavouring of food (Gilbert & Knowles, 1975; Tóth & Potthast, 1984; Wittkowski, Ruther, Drinda, & Rafiei-Taghanaki, 1992). Lignin is derived mainly from polymerisation of three monolignol precursors: *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (Fahmi, Bridgwater, Thain, & Donnison, 2007; Pettersen, 1984; Weng & Chapple, 2010), which respectively constitute the *p*-hydroxyphenyl, guaiacyl and syringyl units of lignin. When pyrolysed, these lignin units release respectively phenol, guaiacol and syringol along with their substituted forms such as methyl, ethyl, propyl, vinyl, allyl, propenyl (Gilbert & Knowles, 1975). The lignin makeup broadly varies with vegetation type. Lignin of grasses contains all three precursors (Buranov & Mazza, 2008; Fahmi et al., 2007; Ralph & Hatfield, 1991); in lignin of angiosperm hardwoods, syringyl units dominate while most of the balance is from guaiacyl units with a minor contribution of *p*-hydroxyphenyl units (Pettersen, 1984; Rencoret et al., 2011); whereas lignin of gymnosperms, such as pines,

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predominantly contains guaiacyl units with some *p*-hydroxyphenyls but lacks syringyl units (Lebo, Gargulak, & McNally, 2001; Pettersen, 1984; Weng & Chapple, 2010).

Several variables can affect the composition of lignin pyrolysis products in smoke and subsequently the types of lignin-derived putative smoke taint compounds that accrue in wines made from smoke exposed grapes. These include firstly, the monolignol composition of pyrolysed vegetation. Even for a given vegetation, its lignin makeup may vary as a function of its age (Rencoret et al., 2011), the proportion of various plant components (Yokoi et al., 1999), and state of decay of the fuel which can alter the monolignol composition due to preferential demethoxylation of the dimethoxy- or methoxy-substituted phenylpropanoid units, i.e., degradation of syringyl and/or guaiacyl units, by rot-fungi (Faix, Bremer, Schmidit, & Stevanovic, 1991; Higuchi, 1990; Schmidt, 2006; Vane, Drage, Snape, Stephenson, & Foster, 2005 and references therein). Secondly, pyrolysis conditions, particularly temperature and oxygen availability (Guillén & Ibargoitia, 1996; Simon, de la Calle, Palme, Meier, & Anklam, 2005; Toth & Potthast, 1984) can affect lignin pyrolysis products. While lignin may start to pyrolyse at temperatures as low as 280–290 °C (Browne, 1958; Butt, 2006), the composition of lignin pyrolysis products varies as temperature increases, with yields of the organoleptically important phenolic compounds in smoke reportedly peaking in the region of 559 °C (Guillén & Ibargoitia, 1996) and 650 °C (Toth & Potthast, 1984). The above variables underscore the need to define vegetation type, fuel and pyrolysis conditions and smoke composition in order to compare results across studies as well as to explore the links between these factors and the types of putative taint compounds that accrue in grapes and wines. However, such definitions are rather rare in most of the smoke taint studies in grapes and wines to date.

The earlier work on smoke taint in grapes and wines focussed on two guaiacyl lignin derived phenols: guaiacol and 4-methyl guaiacol (Kennison, Wilkinson, Williams, Smith, & Gibberd, 2007; Kennison et al., 2008; Sheppard et al., 2009). However, it has been pointed out that smoke affected wines have sensory properties additional to those expected from these two phenols. This suggests other phenols in smoke are contributing to the extra undesirable sensory effects. Recently, Hayasaka, Baldock, Parker et al. (2010) and Singh et al. (in press) have shown that there are indeed several lignin pyrolysis products in bushfire smoke affected wines at elevated levels. Although these findings broaden our understanding of the range of putative taint compounds that can accrue in wine as a result of smoke exposure, these do not allow for exploring the link, if any, between vegetation type of the smoke source and the types of taint compounds that accrue in grapes or wines.

In the work reported here, a controlled and replicated smoke generation experiment was carried out using vegetation fuel sources that differed in their lignin makeup. The aim was to expose fruit-bearing mature Merlot vines to the resultant smoke and to examine the accretion of putative smoke taint compounds in wines in relation to the lignin makeup of the fuels as well as the smoke emissions. For this purpose, five distinct and major vegetation types with varying lignin composition were used. Each fuel type was (1) reconstituted in proportion to biomass components that burn in a decadal fire event and (2) pyrolysed under conditions that reproduce wildfire temperature profiles (Gould et al., 2007). Recent work has shown that, at least in young wines, the putative smoke taint compounds are predominantly sequestered as glycoconjugates and only a small proportion is present as volatile phenols (Hayasaka, Baldock, Parker et al., 2010; Kennison et al., 2008; Singh et al., 2011, in press; Wilkinson et al., 2011). Thus, in this study both volatile and glycoside-bound phenols were considered.

2. Materials and methods

2.1. Fuel types and fuel compilation

The experiment was carried out in the Margaret River wine region (33°57′S, 115°01′E), in the south west of Western Australia. In this viticultural region, vineyards typically adjoin forested and/or grassed landscapes. The vegetation of these landscapes contains mainly hardwood species such as jarrah (*Eucalyptus marginata* Donn ex Sm.), karri (*Eucalyptus diversicolor* F. Muell.) and marri (*Corymbia calophylla* Lindl.); plantations of the softwood species radiata pine (*Pinus radiata* D. Don.); and pasture grasses such as wild oats (*Avena fatua* L.). Thus, for this study, five biomass fuels representing each of these main vegetation types were used as fuel sources. The fuels were stored in thin layers for several weeks to equilibrate their moisture contents. After drying, each of the tree fuels for smoke generation was compiled from foliage, duff, twigs ($Ø < 6$ mm) and round wood ($Ø \geqslant 6$ mm) in proportion to the respective components that occur in a 10 years old fuel accumulation (Burrows, 1994; O'Connell & Menage, 1982) (Supplementary Table S1). For wild oats, all of its above ground biomass was considered a single component (100% fuel source) since all of it combusts during a fire event.

2.2. Grapevine smoke exposure experiment

The smoke exposure experiment was set up as a completely randomised block design in a commercial vineyard containing 10 years old *Vitis vinifera* L. cv. Merlot vines. To minimise variability in experimental units within a block, each block was carefully selected for vines of uniform canopy size and crop load. The treatments, for smoke generation and exposure, consisted of the five vegetation fuel types described above plus a control (i.e., vines not exposed to smoke). Within each block, the treatments plus the control were randomly allocated to experimental units. Each experimental unit consisted of a panel of five vines. Experimental units were separated by at least two panels of vines to avoid smoke cross contamination. Each block containing the full treatment structure was replicated five times, thus there were a total of 30 experimental units.

Smoke exposure of the experimental vines was carried out, 14 d post-veraison, in a purpose built tent as described by Kennison et al. (2008). For smoke generation, 1 kg of fuel sample was combusted inside a custom built pyrolysis chamber that allowed a controlled replication of the wild fire temperature versus time profiles reported in Gould et al. (2007). The resulting smoke was delivered, *via* a flexible steel tube, to a 63 m^3 tent enclosing each replicate vine panel. Each smoke exposure event lasted 30 min. The smoke density – defined as an obscuration by particulate matter larger than 2.5 μm ($PM_{2.5}$) – was recorded for the entire duration of each smoke exposure event using a Laser Focus VLF-250 nephelometer (Xtralis, Mawson Lakes, South Australia). In each case, obscuration exceeded the instrument's maximum reading of 32%. Control vines were similarly enclosed in an identical tent for the same duration to minimise differences in environmental conditions between smoke treated and control vines.

2.3. Harvest and vine size assessments

The fruits from each replicate panel were harvested separately at commercial maturity, total soluble solids of ~23 °Brix, 6 weeks after smoking treatments. The mass of fruit from each replicate, as well as the number of bunches and the mass of 200 randomly selected berries were determined. Leaf area per panel was estimated from the product of average leaf area per cane and the total

number of canes per panel. Details on harvest and leaf area are given in Supplementary Table S2.

2.4. Wine making

The fruit from each replicate was kept separate and wines made individually for each of the 30 samples. Each lot was crushed and de-stemmed with the addition of 100 mg/l potassium metabisulphite. The total acids were adjusted to 7.0 g/l with the addition of tartaric acid. The must was inoculated with 300 mg/l *Saccharomyces cerevisiae* EC1118 (Lallemand Inc., Montreal, Canada) and 100 mg/l diammonium phosphate added as a nitrogen supplement. Each replicate was fermented on skins in open neck 25 l glass demijohns and hand plunged regularly. The specific gravity and temperature were recorded every 12 h. At three °Brix the must was pressed off the skins and fermentation continued to dryness (<1 g/l residual sugars) in capped 15 l demijohns. Each replicate was racked from gross lees and inoculated with *Oenococcus oeni* (Viniflora CH 16, CHR Hansen, Denmark) at 10 mg/l to initiate malolactic conversion. The ferments were kept at 23 °C until malic acid levels stabilised (<0.1 g/l malic acid). Upon completion of malolactic fermentation, the wines received a further 60 mg/l potassium metabisulphite and cold stabilised at −4 °C for 21 d, filtered through a 0.2 μm pore size cartridge (Sartorius Sartopure 2 Maxicap, Sartorius, Gottingen, Germany) and bottled under food grade nitrogen with stelvin closures.

2.5. Smoke sampling during the vine smoke exposure experiment and prescribed burns

Samples of smoke generated during the vineyard smoke exposure experiments and prescribed burning were collected using a Markes Unity 2 thermal desorption (TD) unit (Markes International Ltd., Llantrisant, UK) as described by Vitzthum von Eckstaedt, Grice, Ioppolo-Armanios, Chidlow, and Jones (2011). The TD sampling tubes were packed manually with approximately 280 mg Tenax-TA adsorbent (60–80 mesh) with minimal compression. All tubes were thermally conditioned for 4 h at 330 °C prior to their first use and for 30 min at 310 °C prior to every sampling event. In the vineyard trials, smoke was drawn through at a rate of 200 ml/min with a miniport diaphragm pump (KNF Neuberger GmbH, Breisgau, Germany) for 30 min. Full details of the prescribed burn sampling procedure are given in Supplementary Fig. S1. The sample tubes were stored at 4 °C until analysed.

2.6. Chemical analyses

2.6.1. Lignocellulose analysis

For each fuel type, 1 kg samples were reconstituted in proportion to the components listed in Table S1. Subsamples were then analysed for cellulose, hemicellulose and lignin at the Western Australian Chemistry Centre (Perth) according to the method of van Soest and Wine (1967). Each analyte was determined in triplicate.

2.6.2. Lignin composition of fuels via pyrolysis gas chromatography-mass spectrometry (Py GC–MS)

For lignin composition analysis, subsamples of fuels reconstituted as described above were pulverized to <0.5 μm particle size. Approximately 0.1 mg was then weighed into quartz tubes and flash pyrolysed for 20 s at 550 °C using a CDS Analytical 5250 Automated Pyroprobe (Oxford, Pennsylvania, USA). The transfer line from the pyroprobe to the GC–MS system was operated at 300 °C and the GC–MS analyses were performed on an HP6890A Gas Chromatograph (Hewlett Packard, Santa Clara, California, USA) interfaced to an HP5973A Mass Selective Detector. A 60 m × 0.25 mm × 0.25 μm DB-5MS capillary column (Agilent J&W, Santa Clara, USA) was used for the analyses with a helium carrier in constant flow mode at 1.2 ml/min with a 40:1 inlet split. The GC oven was cooled to −20 °C, held for 1 min and then heated at 8 °C/min to 40 °C. The oven temperature was then ramped to 320 °C at 4 °C/min and held at 320 °C for 25 min. The mass selective detector was scanned between m/z 20 and 620, at 2.48 scans per second with an electron energy of 70 eV. All the analyses were carried out in triplicate. The lignin derived compounds were identified by comparing their mass spectra with the NIST and/or Wiley spectral libraries or by comparison to reported spectra in the literature (Ralph & Hatfield, 1991). Individual lignin pyrolysates were quantified by their relative percentage area of the total ion chromatograms of all the lignin pyrolysates.

2.6.3. Smoke composition

The smoke samples were analysed by thermal desorption gas chromatography mass spectrometry (TD GC–MS) on an HP6890A GC interfaced to an HP5973A Mass Selective Detector and Unity 2 single tube, two stage desorption unit as described in Bates et al. (2008) and Vitzthum von Eckstaedt et al. (2011). Briefly, the smoke sample containing tubes were thermally desorbed at 300 °C for 5 min (first stage desorption); the desorbed samples were transferred in a helium gas stream (>25 ml/min) to a cold trap (10 °C) to refocus the compounds. The refocusing cold trap was subsequently heated at 100 °C/s to 300 °C and held isothermally for 1 min (second stage desorption). The compounds desorbed from the refocusing trap were transferred, in a helium stream at 1 ml/min, to the GC column via a deactivated fused silica capillary transfer line that was maintained at 120 °C. Samples were analysed on a 60 m × 0.25 mm × 0.25 μm Agilent DB5-MS column with a helium carrier gas in constant flow mode at 1.1 ml/min. The GC was set at constant pressure and run in splitless mode. All sample splits were carried out at the desorption stage. The DB-5MS column was run with an initial temperature of 40 °C ramped at 4 °C/min to 300 °C and held isothermally for 10 min.

2.6.4. Free and glycoside-bound putative smoke taint compounds in wines

Lignin-derived putative smoke taint (volatiles and glycoside-bound forms) compounds in wines were extracted and analysed using GC–MS as described by Singh et al. (2011, in press). In the present study, the glycoside-bound lignin pyrolysis products in wines refer to values determined after strong acid hydrolysis as detailed in Singh et al. (in press).

2.7. Data analyses

Data were analysed according to a completely randomised block design model (with one factor and five replicates). Reported treatment effects discussed herein, unless indicated otherwise, were significant at $p < 0.05$. Analyses were carried out using SPSS 20 (IBM® SPSS® Statistics) and GenStat 13th Edition (VSN International Ltd., UK).

3. Results

3.1. Lignocellulose composition of fuels

The cellulose contents of all fuels varied over a relatively narrow range (24–29%) except in oat for which cellulose made up about half of the fuel mass (Table 1). The hemicellulose contents of the tree fuels (hardwood or softwood) were also similar averaging at 8.6%, which was substantially lower than the 28% for oats fuels. In terms of lignin, the five vegetation fuel types fell into three

Table 1
Lignocellulosic compositions of the fuels used for smoke generation.

Fuel	Cellulose	Hemicellulose	Lignin
Karri	28.4 ± 0.1	8.1 ± 0.2	29.3 ± 0.3
Jarrah	24.2 ± 0.4	6.1 ± 0.2	23.5 ± 0.1
Marri	29.4 ± 0.7	9.0 ± 0.2	24.9 ± 0.2
Pine	23.7 ± 0.4	9.4 ± 0.2	44.5 ± 0.4
Wild oats	48.9 ± 0.1	28.0 ± 0.3	7.8 ± 0.2

Data are mean ± 1 s.e. (n = 3).

distinct groupings. The oats fuel at 7.8% had the lowest lignin concentration, the three hardwoods with an average of 26% were intermediate and the softwood fuel, with a lignin concentration nearly six times that of oats, had the highest level (44.5%).

3.2. Fuel lignin composition

The lignin pyrolysis products of fuels from the Py GC–MS analysis are shown in Table 2. All the angiosperm fuels (the three hardwoods and wild oats) contained lignin pyrolysates from all three lignin units (Table 2). As expected, the pine fuel contained no syringyl products. For the pine and oat fuels, 70–80% of the total lignin products pyrogram peak area was contributed by guaiacyl derivatives. For the hardwood fuels, ⩾80% of the lignin pyrolysis products were syringyl and guaiacyl phenols.

Of the *p*-hydroxyphenyl derivatives, phenol and the three isomers of cresol were the dominant pyrolysis products (accounting for 75–87% of peak areas) irrespective of fuel type (Table 2). The balance was mainly made up by dimethylphenols and 4-ethylphenol. With respect to the guaiacyl lignin products, 4-vinylguaiacol was the single most abundant pryolysate in all the five fuel types, particularly in the wild oats and pine fuels. Other pyrolysates with high relative abundance included guaiacol, 4-methylguaiacol and 4-ethylguaiacol, vanillin, *cis*-isoeugenol and acetovanillone (Table 2). For the angiosperm fuels, the relative abundances of the syringyl-derived pyrolysates were similar among the hardwood trees; however, in the wild oats, the relative abundances of the syringyl-derived products, except syringol, were consistently less than those of the hardwoods.

3.3. Volatile phenols in smoke emissions

Smoke emissions from pyrolysis of the five fuels during the vine smoke exposure experiment contained a range of lignin pyrolysates (Table 3). All five fuels had relatively high levels of the following *p*-hydroxyphenyl derivatives: phenol, *o*-, *p*- and *m*-cresols, 4-ethylphenol and 2,4-dimethylphenol. Of the guaiacyl derivatives, the dominant compounds in the smoke emissions from each fuel were guaiacol, 4-methylguaiacol, 4-ethylguaiacol, eugenol and vanillin. By contrast, only syringol was the significant pyrolysate from the syringyl unit of lignin from each fuel type except the pine fuel which had no syringyl products in its smoke emissions. The smoke emissions from prescribed burns of marri-, karri-dominated forests as well as a wild oats pasture had similar lignin pryolysate profiles (Table 3) as those of the respective fuels during the vineyard experiment.

3.4. Putative smoke taint compounds in wines

3.4.1. Total (free and bound) lignin-derived putative smoke taint compounds in wines

Wines from unsmoked, control, grapes contained relatively high background levels (311 μg/l) of total (free and bound) lignin

Table 2
Lignin composition of fuels based on Py GC–MS analysis.

Lignin unit	Lignin pyrolysates	Relative abundance (% of total lignin derived pyrolysates) by fuel type				
		Jarrah	Karri	Marri	Oats	Pine
p-Hydroxy-phenyls	Phenol	5.9	8.7	3.4	4.6	6.8
	o-Cresol	2.4	1.7	0.8	1.1	3.2
	m- and *p*-Cresol	8.1	3.1	2.4	1.8	6.9
	2,4-Dimethylphenol	1.2	0.8	0.5	0.3	1.8
	4-Ethylphenol	2.4	1.2	1.0	1.1	2.3
	4-Hydroxybenzaldehyde	nq	nq	nq	0.8	nq
	4-Allylphenol	nq	nq	nq	0.5	0.8
	Subtotal	20.0 ± 0.2	15.5 ± 0.6	8.1 ± 0.5	10.2 ± 0.2	21.8 ± 0.1
Guaiacyls	Guaiacol	3.0	3.7	9.4	9.4	6.4
	4-Methylguaiacol	3.6	4.9	4.2	3.1	11.9
	4-Ethylguaiacol	4.1	1.4	0.9	3.1	3.1
	4-Vinylguaiacol	14.0	10.2	12.7	41.3	26.9
	Eugenol	1.0	0.8	1.0	1.3	2.6
	4-Propylguaiacol	nq	nq	nq	nq	0.8
	Vanillin	2.4	2.0	2.8	3.9	4.6
	trans-Isoeugenol	1.2	1.3	1.2	0.4	1.9
	cis-Isoeugenol	5.5	2.4	7.8	5.5	12.7
	Homovanillyl alcohol	nq	nq	nq	0.8	nq
	Acetovanillone	2.2	3.0	1.7	1.2	1.5
	Homovanillic acid	2.0	3.1	nq	nq	5.8
	Subtotal	39.0 ± 0.7	32.8 ± 0.6	41.7 ± 2.0	70.0 ± 0.6	78.2 ± 0.1
Syringyls	Syringol	8.0	6.6	5.0	6.7	nd
	4-Vinylsyringol	11.6	15.2	18.6	4.7	nd
	4-(2-Propenyl) syringol	1.8	2.4	3.0	0.8	nd
	Z-4-(1-propenyl)-syringol	1.8	1.7	1.7	0.5	nd
	Syringaldehyde	4.0	5.2	4.1	1.2	nd
	E-4-(1-propenyl)-syringol	10.4	14.3	15.1	3.1	nd
	Acetosyringone	3.4	6.3	2.7	2.4	nd
	3,5-Dimethoxy-4-hydroxycinnamaldehyde	nq	nq	nq	0.4	nd
	Subtotal	41.0 ± 0.9	51.7 ± 0.9	50.2 ± 0.9	19.8 ± 0.6	-
	Total	100.0	100.0	100.0	100.0	100.0

Fuel samples were analysed in triplicates; nq, not quantified.

Table 3
Volatile phenols in smoke emissions based on TD GC–MS analysis.

Lignin units	Compounds in smoke	Relative abundance of lignin pyrolysates in smoke emissions from							
		Vineyard smoke exposure experiment					Prescribed burns		
		Jarrah	Karri	Marri	Oats	Pine	Karri	Marri	Oats
p-Hydroxy-phenyls	Phenol	54.53	48.69	45.82	56.73	33.44	35.14	14.80	34.23
	o-Cresol	9.96	8.99	9.00	11.79	7.51	6.73	3.06	5.37
	m and *p*-Cresol	17.44	23.45	9.16	13.90	14.38	11.93	13.27	7.38
	2,4-Dimethyl phenol	1.59	1.42	1.33	1.56	2.89	4.39	1.02	1.34
	3,5-Dimethyl phenol	nq	nq	1.23	0.44	1.28	nd	nq	0.67
	2-Ethylphenol	nq	0.52	0.75	nq	1.47	nd	nq	nq
	4-Ethylphenol	3.68	1.80	2.40	2.67	4.76	2.93	3.06	4.70
	4-Vinylphenol	nd	nd	nd	nd	8.66	nd	nd	9.40
	4-Allyl phenol	nd	nd	nd	nd	0.78	nd	nd	nd
	Subtotal	87.19	84.87	69.69	87.10	75.17	61.13	35.20	63.09
Guaiacyls	Guaiacol	6.40	7.34	12.31	6.23	8.43	22.55	11.73	8.72
	4-Methylguaiacol	2.54	3.15	5.81	3.11	4.81	7.98	9.69	4.70
	4-Ethylguaiacol	1.78	2.55	4.90	1.45	2.43	5.42	6.63	2.01
	4-Vinylguaiacol	0.25	0.45	2.40	0.67	3.99	1.54	13.27	8.72
	Eugenol	0.19	0.22	1.23	0.11	1.60	nd	1.02	0.67
	Vanillin	0.63	0.37	0.21	0.33	0.69	nd	nd	4.03
	4-Propylguaiacol	0.19	0.15	0.75	nd	1.19	nd	nd	nd
	cis- and *trans*-Isoeugenol	nq	nd	nd	0.22	1.47	nd	nd	1.34
	Acetovanillone	nd	nd	nd	nd	0.23	nd	nd	nd
	Subtotal	11.98	14.23	27.60	12.12	24.83	37.48	42.35	30.20
Syringyls	Syringol	0.82	0.90	2.72	0.78	nd	1.39	22.45	6.04
	4-Methylsyringol	nd	nd	nd	nd	nd	nd	nd	0.67
	Subtotal	0.82	0.90	2.72	0.78	nd	1.39	22.45	6.71

Data are based on triplicate sample analyses; nd, not detected, nq, not quantified.

degradation products (Table 4). Smoke exposure of vines 14 d after the onset of grape ripening significantly increased the total concentrations of lignin-derived compounds in wines. This increase was fuel type dependent and ranged from 74% in the karri smoke treatment to 146% in the wild oats smoke treatment (Table 4). Significant increases also occurred across all three lignin unit derivatives; however, the average magnitude of increases differed in the following order: guaiacyl derivatives (362%) > *p*-hydroxyphenyl derivatives (221%) > syringyl derivatives (72%) (Table 4). Irrespective of fuel type or smoke exposure, syringyl derivatives dominated the total pool of lignin-derived compounds, accounting for between 54% and 78% (Table 4). The remainder was approximately equally apportioned between the *p*-hydroxyphenyl and guaiacyl groups of compounds in oat and pine smoke-affected wines, while in the control and hardwood smoke affected wines, the *p*-hydroxyphenyl derived compounds accounted for a consistently higher proportion than the guaiacyl derivatives.

The smoke-derived compounds in wines showed no correspondence with the lignin composition of the pyrolysed fuels. Thus, wines from grapes exposed to smoke of the pine fuel, the lignin of which contains no syringyl moieties or detectable syringyl derivatives in its smoke, unexpectedly contained high levels of

Table 4
Effects of smoke exposure and fuel type on levels (μg /l) of total phenols (sum of free and glycoside-bound) in wines.

Lignin unit	Putative taint	Treatments					
		Control	Jarrah	Karri	Marri	Wild oats	Pine
p-Hydroxy-phenyls	Phenol	5.2 ± 0.2	29.1 ± 1.0	20.0 ± 1.0	40.1 ± 2.4	46.1 ± 1.4	35.6 ± 2.1
	o-Cresol	8.9 ± 0.3	21.8 ± 0.4	16.2 ± 0.9	32.3 ± 1.0	34.4 ± 1.2	26.9 ± 1.7
	m-Cresol	6.3 ± 0.3	21.4 ± 0.6	18.6 ± 0.8	32.6 ± 1.5	31.2 ± 0.7	30.2 ± 1.5
	p-Cresol	9.3 ± 0.3	22.4 ± 0.5	19.2 ± 0.7	34.1 ± 1.5	32.4 ± 0.5	31.3 ± 1.7
	4-Ethylphenol	12.4 ± 0.6	20.7 ± 1.1	17.7 ± 0.7	30.5 ± 1.3	29.4 ± 0.8	22.8 ± 0.7
	Subtotal	42.1 ± 1.8	115.4 ± 1.3	91.7 ± 2.7	169.6 ± 7.3	173.5 ± 3.4	146.8 ± 7.4
Guaiacyls	Guaiacol	19.2 ± 0.5	54.7 ± 2.9	44.5 ± 1.4	69.6 ± 2.9	125.1 ± 1.4	84.4 ± 6.8
	4-Methylguaiacol	8.1 ± 0.9	29.4 ± 2.4	18.4 ± 1.0	35.2 ± 2.6	33.9 ± 1.9	58.3 ± 7.4
	4-Ethylguaiacol	nq	1.9 ± 0.1	2.0 ± 0.1	5.9 ± 0.2	6.2 ± 0.2	4.9 ± 0.5
	4-Propylguaiacol	nq	1.2 ± 0.1	5.1 ± 0.7	7.6 ± 0.2	5.8 ± 0.5	4.3 ± 0.2
	4-Vinylguaiacol	nq	6.3 ± 0.4	6.7 ± 0.4	8.1 ± 0.5	6.9 ± 0.9	5.9 ± 0.3
	Vanillin	252.2 ± 19.4	221.8 ± 33.6	270.6 ± 18.8	209.0 ± 14.2	177.6 ± 13.4	166.2 ± 9.6
	Acetovanillone	276.0 ± 8.6	328.2 ± 18.4	339.2 ± 9.2	302.0 ± 4.0	329.3 ± 12.5	322.7 ± 13.3
	Subtotal[1]	27.3 ± 0.8	93.5 ± 4.2	76.7 ± 1.3	126.4 ± 4.5	177.9 ± 2.0	157.8 ± 14.3
Syringyls	Syringol	82.2 ± 1.3	168.0 ± 18.1	133.7 ± 9.8	153.1 ± 15.2	154.4 ± 8.6	129.9 ± 5.9
	4-Methylsyringol	nq	8.4 ± 0.6	8.8 ± 0.5	13.1 ± 0.8	7.9 ± 0.4	6.8 ± 0.2
	Syringaldehyde	48.8 ± 2.0	67.3 ± 8.5	86.2 ± 2.3	54.5 ± 2.9	47.9 ± 2.4	52.3 ± 2.7
	Acetosyringone	110.5 ± 9.1	205.4 ± 15.6	143.2 ± 15.0	213.4 ± 19.7	203.0 ± 19.4	217.3 ± 11.0
	subtotal	241.5 ± 11.0	449.1 ± 42.7	371.9 ± 27.4	434.1 ± 36.0	413.2 ± 23.1	406.3 ± 10.2
	Total lignin-derivatives[1]	311.2 ± 11.1	658.0 ± 44.2	540.1 ± 29.6	731.3 ± 45.0	764.6 ± 23.7	711.0 ± 27.5

Data shown are mean ± 1 standard error (n = 5).
[1] Excludes vanillin and acetovanillone.

Table 5
Effects of fuel type and smoke exposure on levels (μg/l) of glycoconjugated phenols in Merlot wines.

Lignin unit	Bound phenols	Treatments					
		Control	Jarrah	Karri	Marri	Wild oats	Pine
p-Hydroxy-phenyls	Phenol	3.6 ± 0.2	21.7 ± 0.4	15.4 ± 0.4	29.8 ± 0.6	30.4 ± 0.6	26.3 ± 0.7
	o-Cresol	6.9 ± 0.2	17.1 ± 0.3	13.7 ± 0.4	26.4 ± 0.6	26.2 ± 0.5	21.5 ± 0.7
	m-Cresol	5.5 ± 0.2	17.5 ± 0.4	16.1 ± 0.4	27.2 ± 0.6	25.1 ± 0.4	25.1 ± 0.5
	p-Cresol	8.2 ± 0.2	18.3 ± 0.4	16.5 ± 0.4	27.7 ± 0.8	25.8 ± 0.3	26.1 ± 0.6
	4-Ethylphenol	12.4 ± 0.6	17.7 ± 0.7	16.0 ± 0.5	26.7 ± 0.6	24.1 ± 0.5	19.2 ± 0.3
	Subtotal	36.6 ± 2.0	92.3 ± 1.8	77.7 ± 1.4	137.8 ± 4.6	131.6 ± 2.2	118.2 ± 4.0
Guaiacyls	Guaiacol	15.1 ± 0.4	38.9 ± 1.2	37.4 ± 0.7	54.3 ± 0.7	87.8 ± 0.8	64.3 ± 2.8
	4-Methylguaiacol	7.6 ± 0.5	22.7 ± 1.0	14.9 ± 0.4	26.0 ± 0.9	24.9 ± 1.0	46.6 ± 2.9
	4-Ethylguaiacol	nd	1.9 ± 0.1	2.0 ± 0.1	3.1 ± 0.1	3.5 ± 0.1	3.3 ± 0.2
	4-Propylguaiacol	nd	nd	4.0 ± 0.4	5.1 ± 0.2	4.3 ± 0.3	4.3 ± 0.2
	4-Vinylguaiacol	nd	6.3 ± 0.4	6.7 ± 0.4	8.1 ± 0.5	6.9 ± 0.9	5.9 ± 0.3
	Subtotal	22.7 ± 0.7	69.8 ± 3.2	65.0 ± 1.4	96.6 ± 1.8	127.4 ± 1.6	124.4 ± 10.5
Syringyls	Syringol	9.8 ± 0.6	64.1 ± 8.1	34.7 ± 2.6	52.0 ± 4.9	52.7 ± 2.6	26.5 ± 2.5
	4-Methylsyringol	nd	nd	nd	nd	nd	nd
	Syringaldehyde	48.8 ± 2.0	67.3 ± 8.5	86.2 ± 2.3	54.5 ± 2.9	47.9 ± 2.4	52.3 ± 2.7
	Acetosyringone	13.7 ± 0.7	30.6 ± 4.5	24.0 ± 1.6	25.0 ± 1.5	10.9 ± 0.5	9.6 ± 0.8
	Subtotal	72.3 ± 5.9	162.0 ± 38.3	144.9 ± 10.2	131.5 ± 13.1	111.5 ± 4.2	88.4 ± 9.4
	Total	131.6 ± 5.8	324.1 ± 25.9	287.6 ± 10.6	365.9 ± 15.0	370.5 ± 10.9	331 ± 15.1

Data are mean ± 1 standard error (n = 5, each observation is a mean of three analytical determinations); nd, not detected.

syringyl products (Table 4). Indeed, smoke of gymnosperm and angiosperm fuels elicited equivalent levels of total syringyl derivatives in wines.

Of the *p*-hydroxyphenyl lignin derivatives, phenol, *o*-, *p*-, *m*-cresol and 4-ethylphenol were consistently present in both smoke affected and unaffected wines. However, in smoke affected wines all these compounds were significantly elevated (Table 4). While phenol was generally the single largest component of the *p*-hydroxyphenyl derivatives, the combined cresols (free and glycoside-bound *o*-, *p*-, and *m*-cresol) dominated (67–75%) the total *p*- hydroxyphenyl pool (Table 4).

Of the guaiacyl group, vanillin and acetovanillone were present at considerably higher total (glycoside-bound plus free) concentrations than any others. Nonetheless, the levels of these two compounds were independent of smoke exposure (Table 4). The guaiacyl derivatives that increased due to smoke exposure were guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-propylguaiacol and 4-vinylguaiacol (Table 6). Of the total pool of five guaiacyl derivatives that responded to smoke exposure, guaiacol was by far the largest (54–70%) component with 4-methylguaiacol a distant second at 19–37% contribution (Table 4).

Control wines contained moderately high, 241 μg/l, total (volatile plus glycoconjugates) concentrations of the syringyl-derived compounds syringol, syringaldehyde and acetosyringone (Table 4). In smoke-affected wines, total concentrations of all these syringyl compounds including 4-methylsyringol were significantly elevated with the exception of syringaldehyde in pine or wild oats smoke affected wines (Table 4). Unexpectedly, wines made from grapes exposed to pine fuel smoke contained syringol and acetosyringone which, respectively, were 1.6 and 2.0 times the levels found in control wines (Table 4).

Some of the lignin pyrolysis products that were present in Py GC–MS (fuels) and/or TD GC–MS (smoke emissions) were either not detected or present below their detection limits in wines (~5 μg/l) (*cf.* Tables 2–4).

3.4.2. Effect of smoke exposure on glycoside-bound lignin derivatives in wine

Averaged across fuel types, the total pool of glycoconjugated lignin derivatives was more than 2.8 times the levels found in control wines (Table 5). Once again, the largest response to smoke exposure occurred in the total concentration of the guaiacyl derivatives (4.3-fold) followed by *p*-hydroxyphenyl (3-fold) with the least responsive being the total of syringyl products (1.8-fold).

Control wines contained measurable levels (3.6–12.4 μg/l) of glycoconjugates of phenol, *o*-cresol, *m*-cresol, *p*-cresol and 4-ethylphenol (Table 5). While no additional non-volatiles of *p*-hydroxyphenyl origin were detected in smoke affected wines, smoke exposure increased the concentrations of these five compounds by up to 8-fold (Table 5). The effects of fuel type and/or the smoke exposure treatments on the levels of the glycoside-bound *p*-hydroxyphenyl derivatives closely tracked the effects observed on the total (free- and glycoside-forms) *p*-hydroxyphenyl compounds (*cf.* Tables 4 and 5). Thus, karri smoke affected wines, while significantly different from the control wines, contained lower concentrations of each of the glycoside-bound *p*-hydroxyphenyl compounds than wines from grapes exposed to smoke from any of the other four fuels (Table 5). Similarly, regardless of fuel type or smoke exposure, the cresol isomers as a group dominated (56–60%) the glycoside-bound pool of *p*-hydroxyphenyl derivatives (Table 5).

The total concentrations of glycoconjugated guaiacyl derivatives (guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-propylguaiacol and 4-vinylguaiacol) in smoke affected wines were 3–5.5 times the level in unsmoked, control wines (Table 5). Of the fuel types, the pine and oat fuels yielded significantly higher total concentrations of glycoside-bound guaiacyl derivatives in wines than any of the hardwood fuels.

Generally, smoke exposure also significantly elevated individual glycoside-bound compounds of guaiacyl lignin origin. Quantitatively, however, guaiacol and 4-methylguaiacol were the dominant components, respectively accounting for 56–69 % and 20–38% of the total glycoside-bound guaiacyl derived compounds in smoke affected wines. Interestingly, the bound 4-methylguaiacol levels of wines from pine smoke exposed grapes were 1.9 times those from the wines affected by the other fuel types. In control wines, glycoside-conjugates of 4-ethylguaiacol, 4-propylguaiacol and 4-vinylguaiacol were below quantitation limits as was 4-propylguaiacol from the jarrah treatment.

Control wines contained substantial background levels (72 μg/l) of total glycoconjugates of syringyl compounds (Table 5). Smoke exposure further raised the already high background concentrations, depending on vegetation fuel type, by 22–124% (Table 5). However, syringol was the only compound significantly elevated

Table 6
Effects of fuel type and smoke exposure on volatile phenols levels (μg/l) in Merlot wines.

Lignin unit	Volatile phenols	Treatments					
		Control	Jarrah	Karri	Marri	Wild Oats	Pine
p-Hydroxyl-phenyls	Phenol	1.6 ± 0.1	7.4 ± 0.2	4.6 ± 0.2	10.3 ± 0.8	15.7 ± 0.3	9.3 ± 0.6
	o-Cresol	2.0 ± 0.1	4.7 ± 0.1	2.5 ± 0.2	5.9 ± 0.2	8.2 ± 0.2	5.4 ± 0.3
	m-Cresol	0.8 ± 0.1	3.9 ± 0.2	2.5 ± 0.2	5.4 ± 0.4	6.1 ± 0.2	5.1 ± 0.4
	p-Cresol	1.1 ± 0.1	4.1 ± 0.1	2.7 ± 0.1	6.4 ± 0.3	6.6 ± 0.2	5.2 ± 0.4
	4-Ethylphenol	nq	3.0 ± 0.1	1.7 ± 0.1	3.8 ± 0.4	5.3 ± 0.1	3.6 ± 0.3
	Subtotal	5.5 ± 0.3	23.1 ± 1.3	14.0 ± 1.4	31.8 ± 3.2	41.9 ± 1.5	28.6 ± 3.7
Guaiacyls	Guaiacol	4.1 ± 0.2	15.8 ± 0.5	7.1 ± 0.2	15.3 ± 1.2	37.3 ± 0.4	20.1 ± 0.9
	4-Methylguaiacol	0.5 ± 0.1	6.7 ± 0.3	3.5 ± 0.3	9.2 ± 0.6	9.0 ± 0.2	11.7 ± 1.0
	4-Ethylguaiacol	nd	nd	nd	2.8 ± 0.1	2.7 ± 0.1	1.6 ± 0.1
	4-Propylguaiacol	nd	1.2 ± 0.1	1.1 ± 0.1	2.5 ± 0.1	1.5 ± 0.1	nd
	4-Vinylguaiacol	nd	nd	nd	nd	nd	nd
	Subtotal	4.6 ± 0.3	23.7 ± 1.2	11.7 ± 0.5	29.8 ± 2.9	50.5 ± 0.7	33.4 ± 3.8
Syringyls	Syringol	72.4 ± 1.0	103.9 ± 2.3	99.0 ± 3.2	101.1 ± 3.7	101.7 ± 2.5	103.4 ± 1.5
	4-Methylsyringol	nd	8.4 ± 0.6	8.8 ± 0.5	13.1 ± 0.8	7.9 ± 0.4	6.8 ± 0.2
	Syringaldehyde	nd	nd	nd	nd	nd	nd
	Acetosyringone	96.8 ± 5.7	174.8 ± 6.9	119.2 ± 7.5	188.4 ± 10.9	192.1 ± 10.6	207.7 ± 8.6
	Subtotal	169.2 ± 10.4	287.1 ± 18.7	227.0 ± 18.5	302.6 ± 26.1	301.7 ± 23.3	317.9 ± 10.4
	Total	179.3 ± 7.1	333.9 ± 11.5	252.7 ± 12.5	364.2 ± 19.6	394.1 ± 15.2	379.9 ± 14.3

Data are mean ± 1 standard error (n = 5, each observation is a mean of three analytical determinations); nd, not detected.

across all fuel types including the pine fuel. Whilst exposure of grapes to smoke of the gymnosperm fuel, compared to the control, significantly increased the syringol concentration in wines (26.5 vs. 9.8 μg/l), the effect of the gymnosperm fuel smoke was less than the effect of smoke from the angiosperm fuels, i.e., hardwoods and wild oats (Table 5). Interestingly, no glycoside-bound 4-methylsyringol was detected (<1.5 μg/l) in any of the wines.

Syringaldehyde and acetosyringone were the other glycoside-bound syringyl-derivatives affected by smoke. In particular, while wines from the control, pine or wild oats treatments contained comparable levels of these compounds, smoke from the hardwood fuels significantly elevated concentrations of glycoside-bound syringaldehyde and acetosyringone in wines (Table 5).

3.4.3. Effect of smoke exposure on free phenols in wine

The total level of volatile lignin derivatives in smoke affected wines was, on average, nearly double the levels found in control wines (Table 6). Smoke exposure also significantly increased the total concentrations of volatiles in each of the three lignin types. However, the relative responses were different. Relative to the respective levels in control wines, the volatile guaiacyl derivatives were the most responsive to smoke exposure (~6.5 times), followed by the total of *p*-hydroxyphenyl products (~5-fold) while the syringyl derivatives were the least responsive (1.7 times) reflecting the high background level of syringyl derivatives in wines. However, irrespective of smoke exposure, the syringyl derivatives were the dominant (77–94%) components of the total pool of volatile phenols in the wines (Table 6). The remainder of the total volatile phenols pool was approximately equally contributed by the *p*-hydroxyphenyl and guaiacyl derivatives.

Of the volatiles of the *p*-hydroxyphenyl derivatives, while phenol was the single largest (32–38%) component in wines from smoke exposed grapes, regardless of smoke exposure, the cresols as a group once again dominated (50–70%) the total of free *p*-hydroxyphenyl derivatives (Table 6). Of the free guaiacyl derivatives, only guaiacol and 4-methylguaiacol were consistently found in quantifiable levels regardless of smoke exposure. As expected, however, the concentrations of these analytes were significantly higher in wines from smoke exposed grapes than from control grapes (Table 6). The other guaiacyl lignin derivatives (4-ethylguaiacol, 4-propylguaiacol and 4-vinylguaiacol) were present at trace levels. Thus, free guaiacol and 4-methylguaiacol were the dominant volatile components, jointly accounting for 82–100% of the total pool of guaiacyl derivatives, as was the case with the glycoside-bound forms (*cf.* Table 5 and Table 6).

Control wines contained substantial levels of volatile syringol and acetosyringone (72 and 97 μg/l, respectively). Smoke exposure, regardless of fuel type, significantly increased the levels of these compounds (Table 6). 4-Methylsyringol was not detected in control wines but was present at low levels in all wines made from grapes exposed to smoke. Although all wines, irrespective of smoke exposure, had moderate levels (⩾48 μg/l) of glycoside-bound syringaldehyde, 19 months after bottling, no free syringaldehyde was detected (<3 μg/l) in any of the wines.

Smoke exposure also significantly increased (depending on fuel type by 37–88%) the total concentrations of the free syringyl derivatives. Regardless of smoke exposure, >96% of the total free pool of syringyl derivatives in wines was contributed by acetosyringone (53–65%) and syringol (33–46%). Surprisingly, the wines from vines exposed to pine fuel smoke, showed the highest concentration of total free syringyl derivatives (318 μg/l).

3.4.4. Distribution of the lignin-derived compounds between the free and glycoside-bound pools

In all wines, after 19 months of bottle storage, both the *p*-hydroxyphenyl and guaiacyl derivatives were predominantly present as glycoconjugates (Fig. 1), with the free volatile components of each group accounting for less than 30% of the total pools. By contrast, most (61–78%) of the syringyl derivatives were present in free forms (Fig. 1). Nonetheless, even after 19 months, some syringyl derivatives such as syringaldehyde were detected only in the bound form.

3.4.5. Influence of canopy size on accretion of lignin pyrolysis products in wine

The concentrations of several of the lignin pyrolysis products in wines from smoke exposed grapes showed consistent negative correlations with vine leaf area as well as with leaf area per bunch (Fig. 2).

4. Discussion

4.1. Fuel and smoke composition

In this study, taxonomically distinct groups of vegetation fuels with different lignin composition were used to generate smoke,

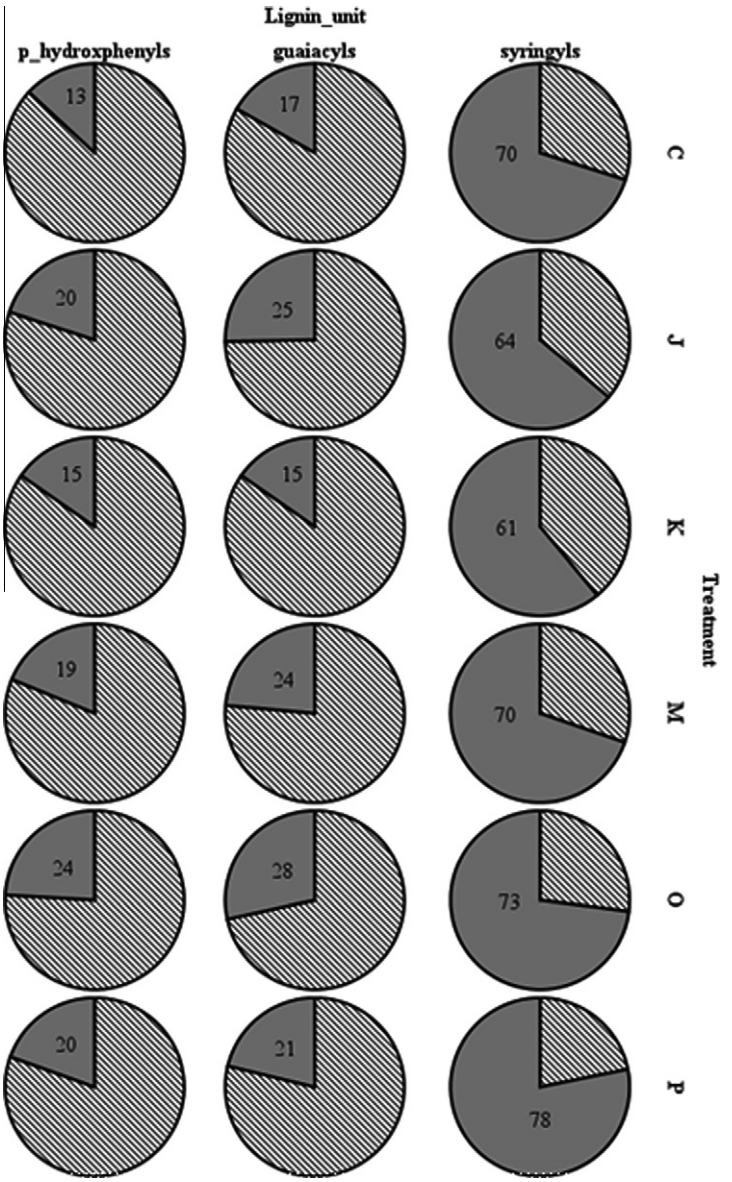

Fig. 1. Distributions (%) of the total putative smoke taint compounds pool between the free (solid slices) and glycoconjugate (hashed slices) components shown by lignin unit source and treatment. Treatment fuels are: C, unsmoked control; J, jarrah fuel; K, karri fuel; M, marri fuel; O, oat fuel; and P, pine fuel.

under conditions that reproduce bushfire pyrolysis temperature profiles, for fumigating grapevines at the start of grape ripening. The primary aim was to examine influence of the lignin makeup of vegetation on lignin pyrolysis products (potential smoke taint compounds) that accrue in wine as a result of exposure of grapes to smoke so generated. In a bushfire event, all components of vegetation, including partially decomposed litter from the soil surface, contribute to the resulting smoke. Each of the fuels used here was thus compiled in proportion to components of the respective vegetation fuel types that are pyrolysed during a decadal bushfire event – the typical forest fire management burn cycle in the studied wine region. In fuels such as these that comprise variously decomposed components, it is pertinent that the fuels retain compositional variation characteristic of the vegetation types used. In this respect, the lignocellulose analyses results were broadly consistent with the expected composition of these vegetation types (Lebo et al., 2001). Nonetheless, the radiata pine fuel had a higher lignin concentration than was expected. This was due to the high (90%) contribution of needles, which have lignin concentration in the range of 37% (in freshly senesced needles) to >50% (in old, partially decomposed needles) (Girisha, Condron, Clinton, & Davis, 2003).

A further detailed analysis of the lignin composition of the five fuel types indicated that while the angiosperms (hardwood trees and wild oats fuels) contained lignin pyrolysis products from all three lignin units, the dominant components were the guaiacyl derivatives in wild oats, and the syringyl followed by guaiacyl compounds in hardwoods. Guaiacol and substituted guaiacols were the principal components of pine fuel lignin; however, it contained no syringyl derivatives as expected for a gymnosperm specimen (Greenwood, van Heemst, Guthrie, & Atcher, 2002; Kjallstrand, Ramnas, & Petersson, 2000; Nolte, Schauer, Cass, & Simoneit, 2001; Pettersen, 1984) although trace levels are sometimes reported (Kristensen, Coulson, & Gordon, 2009) in spite of the general absence of the requisite syringyl lignin biosynthetic enzymes in gymnosperms (Weng & Chapple, 2010). On the whole, despite the inclusion of partially degraded components in the fuel samples, the relative proportions of the lignin derivatives were still distinct

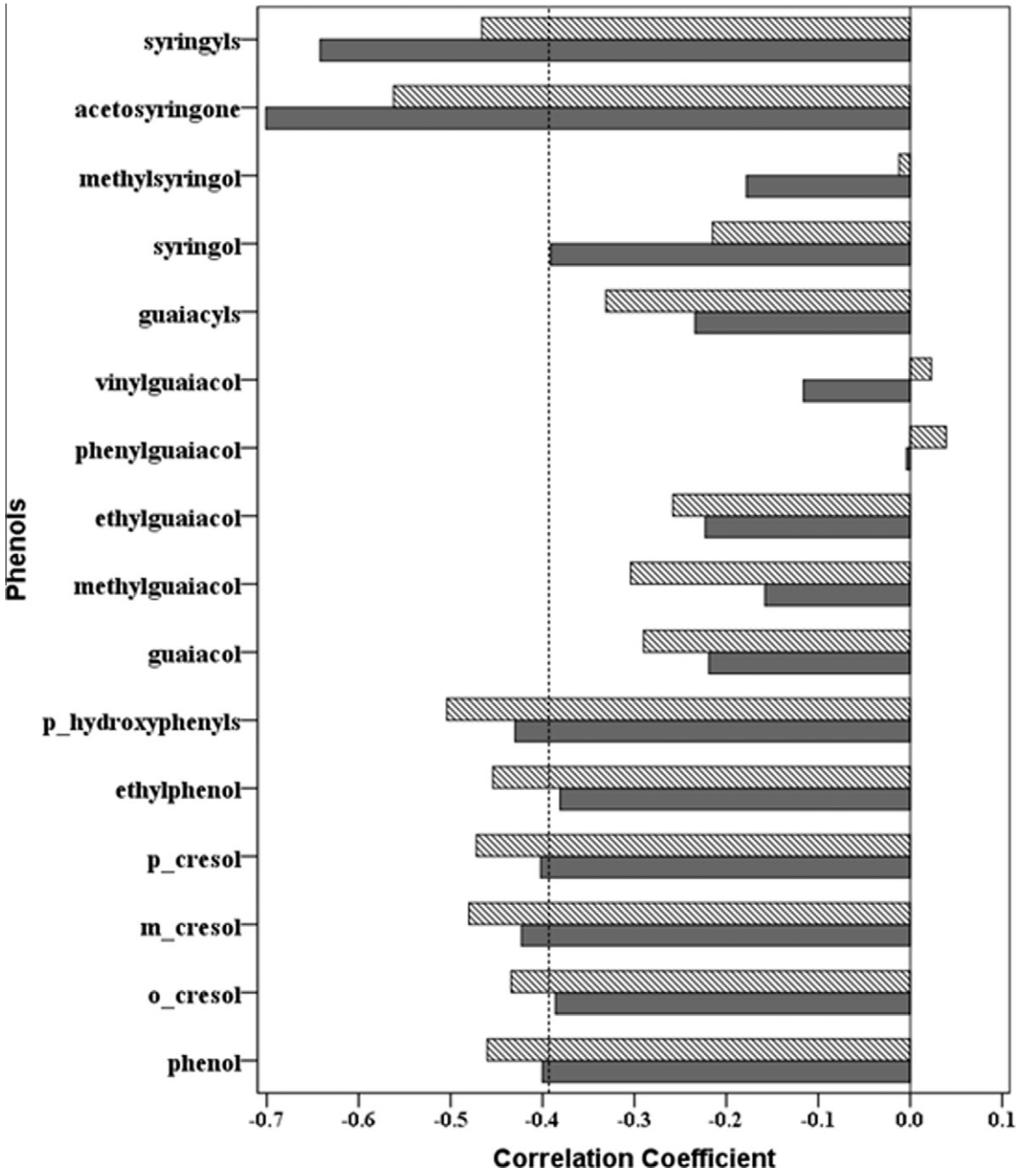

Fig. 2. Correlations of totals of free and bound phenols with leaf area per vine (solid bar) and the leaf area per bunch (hashed bar). Data shown are for individual phenols as well as sums by lignin units. The dashed horizontal line denotes the critical value (−0.394) at $p = 0.05$, thus coefficients below −0.394 or above 0.394 are significant at $p < 0.05$.

enough to characterise the fuels into the three lignin types (grass, hardwood and softwood) (Buranov & Mazza, 2008; Higuchi, 1990; Pettersen, 1984) (Table 2 and Supplementary Fig. S2a,b).

Smoke emissions from the vineyard smoke exposure experiment and the prescribed burns of three of the vegetation types contained phenol, guaiacol and several of their substituted forms as well as syringol and trace levels of 4-methylsyringol. Emissions from the radiata pine fuel contained no syringyl derivatives consistent with the Py GC–MS results presented here and with earlier reports that members of the gymnosperm taxa lack syringyl lignin (Bari, Baumbach, Kuch, & Scheffknecht, 2009; Kjallstrand et al., 2000; Nolte et al., 2001; Schauer, Kleeman, Cass, & Simoneit, 2001) although some exceptions have been noted (e.g., Fine, Cass, & Simoneit 2001). While these results confirm that most of the volatile lignin degradation products that accrue in wines are present in smoke emissions, the results from the pine fuel, as discussed below, exemplify an exception to this generalisation. Volatile phenols emission profiles from the prescribed burns and from smoke generated during the vineyard experiment were broadly similar, indicating that (1) the pyrolysis conditions during the vineyard smoke exposure successfully simulated bushfire/prescribed burn conditions, and (2) as a result, the vines and grapes were exposed to the types of volatile phenols that would occur in bushfire and/or prescribed burn smoke. Although the emissions profiles of volatile phenols from the prescribed burns and vineyard experiment were similar in this study (*cf.*, Tables 2 and 3), the relative abundances,

especially of syringyl and guaiacyl derivatives, were lower than those reported from prescribed burn emissions in the Adelaide Hills, South Australia (Hayasaka, Baldock, Parker et al., 2010). However, in the latter case, sampling was carried out in winter when conditions are likely to favour smouldering, which increases the yield of these methoxyphenols (Kjallstrand et al., 2000).

4.2. Putative smoke taint compounds in wine

Much of the earlier work into smoke taint in wines focussed on accretion of guaiacyl lignin derivatives (Kennison et al., 2007, 2008; Sheppard et al., 2009; Singh et al., 2011). The results from this study demonstrate that wines made from smoke exposed grapes, compared to controls, can accrue significantly elevated levels of other phenols (Tables 4–6) that are emitted in smoke during pyrolysis of lignin (Table 3). This effect was generally invariant of the vegetation source of smoke. More recently, Hayasaka, Baldock, Parker et al. (2010) also reported increased accumulation of phenol, syringol and their substituted forms in bushfire affected wines. However, in this controlled smoke exposure experiment, as in our study of wines made from grapes exposed to wildfire generated smoke (Singh et al., in press), a broader range of lignin pyrolysis products are identified than has been reported hitherto. This is indicative of the possibility that more compounds may contribute to smoke taint aroma of smoke affected wines than the few volatile phenols implicated to date. This is consistent with the suggestion that sensory descriptors of smoke affected wines are varied and more complex than those imparted by the commonly used smoke taint marker compounds, guaiacol and 4-methylguaiacol (Hayasaka, Baldock, Parker et al., 2010; Kennison et al., 2007).

This study also revealed that while the levels of the commonly reported smoke taint markers, guaiacol and 4-methyl guaiacol, were certainly the dominant components of the guaiacyl lignin derived compounds, these represented only about 20% of the total pool of lignin derived putative smoke taint compounds in wines. Instead, the quantitatively dominant contributors (>50%) were pyrolysis products of syringyl lignin, while phenol and substituted phenols were of broadly comparable abundance as guaiacol and substituted guaiacols (Table 4). Comparable proportions have been found in wines of several varieties exposed to wildfire smoke (Singh et al., in press). However, some perspective is warranted on relative contributions of different phenol groups to the total pool. Firstly, the quantitative relative abundance of putative taint compounds does not necessarily indicate the respective compound's taint impact owing to differences in aroma and/or taste perception threshold concentrations. Thus, for example, while syringol was present at a far higher level than guaiacol (101 vs. 19 μg/l), its perception threshold concentration is also high (for example, in red wine 570 vs. 75 μg/l, cited in Petruzzi et al., 2010). Yet, in grapes exposed to high density bushfire smoke for an extended period, the total pool of syringol and substituted syringols can reach 25 times (~10 ppm) the levels found in this limited duration smoke exposure experiment (Singh et al., in press), and hence can far exceed their apparent high perception threshold . Secondly, the comparison above, of guaiacols vs. syringols, may be slightly skewed towards the latter. Here, the total of each of these compounds was estimated from the sum of the volatiles released during fermentation and those released from acid hydrolysis of the glycoside-bound phenols. According to Hayasaka, Baldock, Parker et al. (2010), however, yields of phenol, guaiacol and their substituted forms from acid hydrolysis of the respective glycoconjugates are considerably less (<10%) than that of glycoside-bound syringol and 4-methylsyringol (33%).

Wines made from smoke unexposed grapes (control treatment) contained a high total (241 μg/l) of endogenous volatile and glycoside-bound syringyl products. Given that grapevine lignin contains syringyl derivatives archetypal of a woody angiosperm (Guillén & Ibargoitia,1996) and that grape juice was fermented on skins (thus facilitating hydrolytic release of lignin units into wine (Loscos, Hernandez-Orte, Cacho, & Ferreira, 2009)), the presence of syringyl derivatives, albeit at high levels, is plausible (see also Singh et al., in press). The observed significant increases in the concentrations of total syringols and substituted syringols in wines impacted by smoke of the angiosperm fuels (the three hardwoods and wild oat) may be attributable to exogenous uptake due to the presence of syringyl derivatives in fuels (Table 2) and more significantly in their smoke emissions (Table 3). The provenance for the elevated levels of syringols in wines from pine smoke exposed grapes (Tables 4–6) however cannot be similarly attributed since syringyl derivatives were expectedly lacking in this fuel and its smoke emissions. During smoke exposure of vines/grapes due care was taken to avoid smoke cross contamination from smoke of the angiosperm fuels. At any rate, if contamination was a mechanism, the control wines would have comparable levels to the pine smoke impacted wines. A further experiment is underway to understand the mechanism for the observed increase in syringyl products in wines from pine smoke exposed grapes.

4.3. Relative abundances of free and bound phenols in wine

Nineteen months after bottling, the distribution of putative smoke taint compounds in wines between glycoside-bound and volatile pools differed according to lignin class. Regardless of the applied smoke treatments, pyrolysates of *p*-hydroxyphenyl and guaiacyl origin were predominantly (72–87%) present as glycoconjugates, with free forms making up the balance. By contrast, syringol and substituted syringols released after acid hydrolysis made up <40% of the total pool of syringyl derivatives in wines (Fig. 1). The predominance of glycoside-bound phenol, guaiacol and their substituted forms over the volatile forms is broadly consistent with previous findings (Hayasaka, Baldock, Parker et al., 2010; Hayasaka, Dungey, Baldock, Kennison, & Wilkinson, 2010; Singh et al., 2011; Wilkinson et al., 2011). The results for the syringyl derivatives, however, contrasts with those reported in Hayasaka, Baldock, Parker et al. (2010) who found that levels of volatile syringol and 4-methylsyringol in both red and white wines were <4% of the total pool (i.e., total of volatiles after acid hydrolysis) for each of these compounds. The reason for this disparity is not clear. However, the wines in this study, unlike those of Hayasaka, Baldock, Parker et al. (2010), were analysed 19 months after bottling, the typical red wine maturation duration. The relatively long storage under the mildly acidic environment of wines coupled with syringols possibly having a weak glycoside bond (Hayasaka, Baldock, Parker et al., 2010) may have facilitated slow release of syringol and substituted syringols from their glycosides thus tilting the balance in favour of the free forms. Regardless of the mechanism, however, the observation that 19 months after bottling more than 70% of the phenol, guaiacol and their substituted forms exist as glycoconjugates presents a significant practical problem in smoke taint management. It also presents a challenge for efforts to remove volatile smoke taint compounds from wines through reverse osmosis filtration (Fudge, Ristic, Wollan, & Wilkinson, 2011) since the major "taint" reservoir (glycoconjugates) still remains in wines.

4.4. Influences of vine canopy size and type of lignin pyrolysed on concentrations of putative smoke taint in wine

In nearly all of the lignin pyrolysis products, the concentrations of phenols in wines were negatively correlated to vine canopy leaf area as well as the leaf area per bunch (Fig. 2). The mechanism of accretion of smoke borne lignin pyrolysates in berries is not well understood. Some of the possibilities include direct uptake of

lignin products in smoke emissions by berries and/or foliar uptake and subsequent translocation and sequestration in berries. Tracer studies have shown leaves and/or berries take up phenols (Beattie & Seibel, 2007; Hayasaka, Baldock, Pardon, Jeffery, & Herderich, 2010), although only trace quantities are subsequently translocated to berries (Hayasaka, Baldock, Pardon, Jeffery et al., 2010). If the mechanism of uptake in berries was primarily through the leaves, then a positive relationship between canopy size (absorptive surface area) and taint concentration would be expected instead of the inverse relationship found in the current study. This suggests that direct uptake by berries may be a significant contributor to accumulation of phenols in berries. Phenol, guaiacol, syringol and their substituted forms from lignin pyrolysis are emitted in gas phase and particle phase, and particularly for some of the syringyl derivatives such as acetosyringone (Schauer et al., 2001). While canopy area may have minimal effect on uptake of emissions from gas phase, a denser canopy can intercept particulate phase emissions, and thus reduce contact with the surface of berries. The negative relationship we observed between leaf area per bunch and concentration of phenols (i.e., the more the grapes are sheltered by foliage the less the "taint" concentration) is consistent with this explanation. Furthermore, the correlation was stronger for acetosyringone whose relative emission in particle phase is high compared to other lignin pyrolysis emissions (Schauer et al., 2001).

The vegetation fuels used in this study were chosen to investigate links between the pyrolysis of different lignin sources and the appearance of putative smoke taint compounds in wines. The results, particularly of wines from the pine smoke treatment, have revealed that the lignin pyrolysis products that accrue in wines do not necessarily discern the lignin composition of the pyrolysed fuel. Further work is needed to address marker compound(s) based apportionment or attribution of vegetation-type source of smoke taint.

5. Conclusions

In this study, vegetation fuels with varying lignin makeup were used to generate smoke and fumigate vines at the start of berry ripening. The aim was to examine whether putative smoke taint compounds that accumulate in wines following exposure of grapes to a bushfire event reflect the lignin composition of vegetation that is pyrolysed. The results showed a broader range of lignin pyrolysis products in wines than have been reported to date, suggesting more compounds are likely to contribute to the perceived smoke taint than have been implicated. The effect of vegetation fuel types was less about changes in the identity of compounds than their quantities. Thus, and more significantly, fuel lignin makeup does not appear to be a good indicator of the types of lignin pyrolysis products that become elevated in wines. This is predicated on the finding that radiata pine fuel which did not contain syringyl units in its lignin nor pyrolysis products of syringyl units in its smoke emission gave rise to significantly elevated levels of syringols and substituted syringols in wines made from grapes exposed to pine smoke. The mechanism for this observation is not clear, but further work is underway to better understand this response.

This work also demonstrated that several phenols other than guaiacyl products (i.e., guaiacol and 4-methylguaiacol, the routinely used smoke "taint" measures) are present in wines at similar or higher concentrations than the guaiacyl phenols. Significant among these are the *p*-hydroxyphenyl lignin degradation products (phenol, *o*-, *m*-, and *p*-cresol) and syringol. The cumulative levels of these "other" phenols are considerably higher than those of the guaiacols. Since smoke tainted wines have sensory characteristics beyond those that could be attributed to guaiacols, the *p*-hydroxyphenyl products and syringols, which become elevated following smoke exposure, are possible candidates to account for the additional smoke taint characteristics in wines.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2012.05.036.

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3 Fig. S1.

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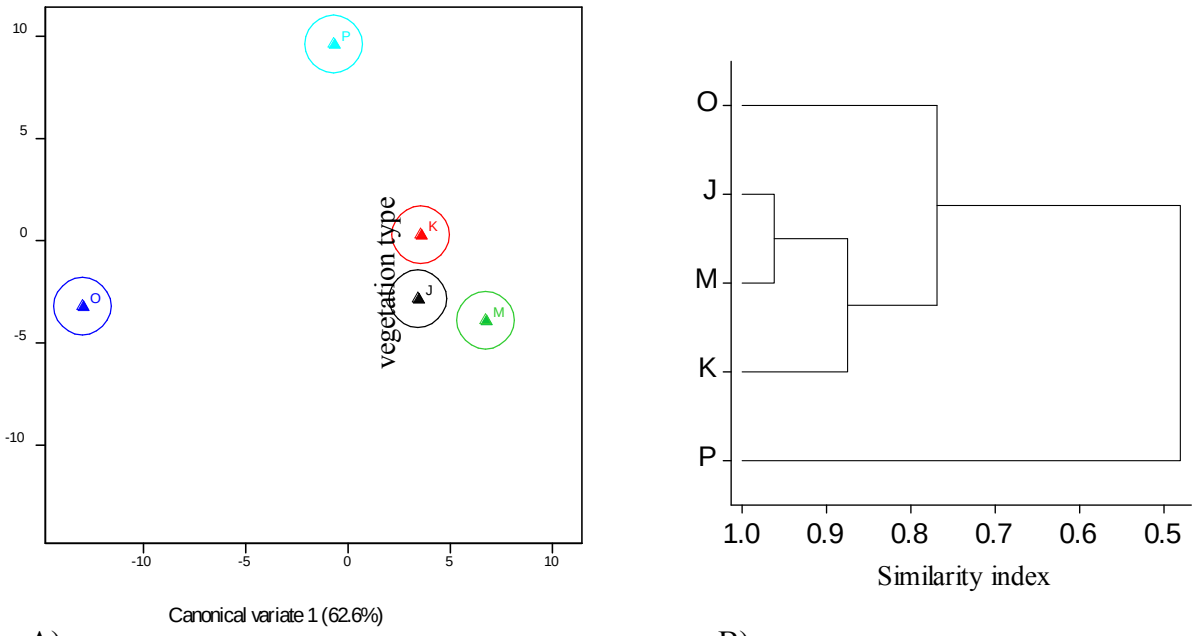

5 A) B)

6 Fig. S2A, B.

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Table S1. Percentage of "biomass" component used for reconstituting fuels used for smoke generation as well as lignocellulose and lignin composition analyses.

Fuel	Biomass fuel component					Total
	Leaves	Duff	Bark	Twigs ∅ < 6 mm	wood ∅ > 6 mm	
Karri	50.1	26.7	3.8	14.3	5.1	100
jarrah	16.9	53.4	3.9	25.8	0.0	100
marri	16.9	53.4	3.9	25.8	0.0	100
	Needles	Twigs ∅ < 5 mm	wood ∅ 5-20 mm	wood ∅ > 20 mm		
pine	90.0	4.0	5.0	1.0	-	100
	Straw, blade and panicle					
wild oats	100					100

For the tree fuels, the percentages given in Table S1 are considered to be representative of 10 year old fuel accumulations for the respective vegetation types. For the jarrah fuel, the percentage biomass components were derived from data of Burrows (1994). No such data were found for marri, and its fuel components were approximated using the jarrah ratios since the two species usually co-occur in the southwest forests. Karri fuel components were interpolated to 10 year accumulations from the data published by O'Connell and Menage (1982). For the pine fuel, the components were combined from a comparison of unburnt and wildfire burnt pine plantations in the region. For wild oats, all of its aboveground biomass was considered a single component (100% fuel source) since all of it burns whenever there is a fire event.

Table S2. Estimates of vine canopy size, fruit yield and berry quality characteristics.

Treat	LA	Cane	Bunch	Fruit mass	mean berry wt.	Juice TSS	Juice pH	Juice TA	Malic acid
	m^2	#	#	kg	g	°Brix		g/l	g/l
Control	37± 5	88± 4	116±8	22 ± 1	2.22 ± 0.09	23.0 ± 0.2	3.58 ± 0.03	5.2 ± 0.3	1.60 ± 0.08
Jarrah	32± 4	90± 2	112± 5	19 ± 2	2.08 ± 0.09	22.7 ± 0.2	3.64 ± 0.02	4.8 ± 0.2	1.34 ± 0.07
Karri	36± 3	95± 4	121±10	22 ± 2	2.24 ± 0.06	22.7 ± 0.2	3.60 ± 0.01	5.2 ± 0.1	1.60 ± 0.02
Marri	30± 1	95± 3	116± 7	19 ± 2	2.16 ± 0.05	22.6 ± 0.1	3.62 ± 0.02	4.9 ± 0.1	1.50 ± 0.06
Oats	29± 3	100± 6	119± 7	19 ± 1	2.25 ± 0.09	22.4 ± 0.2	3.67 ± 0.01	4.9 ± 0.1	1.60 ± 0.09
Pine	31± 2	97± 4	125± 6	23 ± 4	2.08 ± 0.09	23.6 ± 0.9	3.61 ± 0.02	5.2 ± 0.1	1.50 ± 0.09

To estimate vine canopy size/leaf area, the total number of canes for all vines in each of the 30 panels were counted and recorded shortly after fruit harvest. Then samples of two canes with their subtending leaves were taken from two vines in each panel. The leaves of these sample canes were removed, counted and immediately weighed. Total leaf area for each of these canes was calculated from the average mass of 50 randomly selected 16 cm^2 leaf disk cuttings. The total leaf area per panel was then estimated from the product of average leaf area per cane and the total number of canes per panel. There were no significant treatment effects ($p > 0.05$) on vine canopy size measures (leaf area and cane numbers), yield and yield components (berry weight, berry and bunch numbers, and fruit yield), as well as harvest quality indicators (levels of total soluble solids, titratable acidity, malic acid and pH).

Appendix 3. Kelly, D., Zerihun, A., Hayasaka Y. and Gibberd, M. (2014). Wine making practice affects the extraction of smoke-borne phenols from grapes into wines. Australian Journal of Grape and Wine Research. 20(3), 386-393.

Winemaking practice affects the extraction of smoke-borne phenols from grapes into wines

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Abstract

Background and Aims: Exposure to smoke and uptake of taint imparting phenols in grapes and wines is a significant problem in bushfire-prone regions of Australia and other countries. The effects of smoke exposure on taint occurrence in wines, however, can be variable. This study assessed the influence of cultivar on uptake and accumulation of smoke-borne phenols in grapes and of subsequent processing and winemaking methods on extraction of phenols into wines.

Methods and Results: Smoke-exposure experiments were conducted in commercial vineyards of Chardonnay, Merlot and Sauvignon Blanc 14 days after the onset of veraison. At maturity, grapes were harvested for winemaking, which included malolactic fermentation (MLF) for Merlot. Volatile and glycoconjugated phenols were determined in grapes and the resultant wines. All cultivars had a similar concentration of smoke-derived total phenols in their grapes. The apparent extraction of total phenols from grapes into wines, however, differed markedly among the three traditional winemaking methods. Red winemaking (Merlot) with skin contact extracted 88% of total grape phenols, whereas white winemaking either by crushing before pressing (Sauvignon Blanc) or by whole-bunch pressing without crushing (Chardonnay), respectively, released 39 and 18% of total phenols. For Merlot wines, MLF did not affect the extraction of total smoke-derived phenols.

Conclusions: Under standardised exposure conditions (duration, intensity and phenology), the three cultivars studied accumulated a similar concentration of total phenols in grapes. The grape-processing and winemaking methods, however, can bring about a fourfold difference in the concentration of total phenols of wines. The smoke-derived phenols extracted from grapes into wine and the distribution of these phenols between the volatile and conjugated pools were not affected by MLF.

Significance of the Study: The key findings reported here have the potential to improve decision-making by grapegrowers and winemakers on the effect of cultivar and winemaking practice on potential smoke taint in wine.

Keywords: *Chardonnay, cresols, glycoconjugated phenols, guaiacol, Merlot, phenol, Sauvignon Blanc, smoke exposure, syringol,* Vitis vinifera *L., volatile phenols*

Introduction

Wine grapes exposed to smoke from wildfires and controlled burns produce wines with an elevated concentration of volatile and glycoconjugated phenols (Kennison et al. 2007, Hayasaka et al. 2010, 2013, Kelly et al. 2012, Singh et al. 2012). At such an elevated concentration, volatile phenols and glycoconjugated phenols impart unpleasant characters to wines, including burnt, smoky, medicinal and dirty aromas and flavour attributes (Kennison et al. 2009, Ristic et al. 2011, Parker et al. 2012). Such wines have low consumer acceptance, and thus smoke exposure can cause significant negative economic impact on grapegrowers and winemakers (Whiting and Krstic 2007). Consequently, when a vineyard is subject to smoke, grapegrowers and winemakers endeavour to understand the likely level of smoke taint in grapes and in wines from a smoke-exposed vineyard. Several factors, however, are likely to influence the level of accumulation of smoke-borne putative taint compounds in grapes and into wines. First, from the work reported to date, it is unclear whether different winegrape cultivars accumulate a similar concentration of smoke-borne phenols under comparable smoke-exposure conditions. Differences among cultivars in the concentration of phenols from wildfire smoke-exposed fruit have been reported (Hayasaka et al. 2010, Dungey et al. 2011, Singh et al. 2012), but these differences may be due to variation in the intensity and duration of smoke exposure as well as the timing of smoke exposure in relation to vine phenology (Kennison et al. 2009).

Second, the transformations and estimates of the expected concentration of phenols in finished wines under different winemaking practices are not well understood. Recently, there have been advances in the analysis of phenols in smoke-affected fruit and resultant wines (Hayasaka et al. 2010). However, estimates of the expected proportion of volatile phenols and glycoconjugated phenols extractable from grapes into wines (that can be used as industry guidelines) may not be fully inferred from these reports either due in part to the use of

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non-traditional winemaking (Hayasaka et al. 2010) or to the limited range of glycoconjugated phenols reported (Ristic et al. 2011). In smoke-affected grapes, a high proportion of glycoconjugated phenols is sequestered in the skin (Dungey et al. 2011), and skin maceration and contact during winemaking may affect the extraction of these compounds into wine. Thus, it is imperative to understand the likely extent of extraction of a comprehensive range of glycoconjugated phenols from smoke-impacted grapes under commonly used white and red winemaking practices.

Third, the sensory impact of smoke taint in wine is influenced by the distribution of volatile and glycoconjugated phenols (Parker et al. 2012). The production of red wines often includes malolactic fermentation (MLF) by inoculation with lactic acid bacteria (LAB). The metabolic activity of LAB can influence wine aroma complexity by hydrolysing wine aroma glycosides (Ugliano et al. 2003, D'Incecco et al. 2004). The effect of MLF, however, on the distribution of glycoconjugated phenols is yet to be reported.

The objectives of this study were threefold:

- To determine whether the cultivar influences the uptake of smoke-borne phenols and accumulation in grapes of the three cultivars Chardonnay, Sauvignon Blanc and Merlot. To minimise potential confounding factors, the smoke-exposure experiments were replicated both with respect to the panels of vines exposed and to the exposure conditions (i.e. fuel composition, mass, pyrolysis of fuel), and the vines were exposed to smoke at the same phenological stage, 14 days post-veraison, in commercial vineyards.
- To determine the likely proportion of glycoconjugated phenols that are extracted from grapes into wines under commercial red and white winemaking techniques, including when fruit is crushed and de-stemmed and when fruit is whole-bunch pressed. These results may provide guidelines for expected smoke-derived phenols in wines based on the concentration determined in affected grapes under different winemaking practices.
- To assess whether the glycosidase activity of LAB contributes significantly to hydrolysis of glycoconjugated phenols to volatile phenols, by comparing MLF and no MLF Merlot wines.

Materials and methods

Fuel types and fuel compilation

The experiments were conducted in 10-year-old commercial Sauvignon Blanc, Chardonnay and Merlot vineyards located in the Margaret River wine region (33°57'S, 115°01'E) in the southwest of Western Australia. The spacing and canopy management details of these vineyards are set out in Supporting Information Table S1. These vineyards are typically located in close proximity to forests and agricultural areas, and bushfire emissions that may contribute to the accumulation of smoke compounds in wine grapes can arise from remnant native forest, plantations and farmland vegetation. Two fuels, the softwood species radiata pine (*Pinus radiata* D. Don) and a pasture grass, wild oats (*Avena fatua* L.) were collected and prepared as described in Kelly et al. (2012) to compare uptake of smoke-borne phenols between cultivars.

Grapevine smoke exposure

The design and conduct of the smoke-exposure experiments followed that of Kelly et al. (2012). Briefly, the experiments were established as randomised block designs. To minimise variability within an experimental block, each block had vines with uniform canopy size and yield. The treatments and controls within each block were randomly allocated to experimental units where the smoke generation and exposure consisted of the fuels described above plus a control (not exposed to smoke). The smoke-exposure treatments and controls were replicated three times for Chardonnay and Sauvignon Blanc and five times for Merlot. Each experimental unit consisted of a panel of five vines separated by at least two panels of vines to avoid cross contamination. Smoke exposure of the experimental vines occurred 14 days post-veraison as per Kelly et al. (2012), with smoke events lasting 30 min. For Chardonnay, due to logistical constraints, the smoke-exposure treatment involved one fuel type only, which is wild oats.

Winemaking

Fruit was harvested at commercial maturity, ~23°Brix total soluble solids, approximately 6 weeks after smoke exposure. The fruit from each replicate panel was harvested, processed and fermented separately. The Chardonnay and Sauvignon Blanc wines were made by conventional white winemaking methods where there was minimal skin contact before commencement of fermentation. The Sauvignon Blanc replicates were separately de-stemmed, crushed and pressed while the Chardonnay replicates were whole-bunch pressed, each with addition of 100 mg/L potassium metabisulfite (PMS) (Chem Supply AR grade, Gillman, SA, Australia). For both cultivars the must was inoculated with *Saccharomyces cerevisiae* EC1118 (Lallemand Inc., Montreal, QC, Canada) at 300 mg/L and supplemented with 100 mg/L diammonium phosphate (Sigma-Aldrich, Sydney, NSW, Australia). Each replicate was fermented in 25-L glass demijohns to dryness (<1 g/L residual sugars), racked from gross lees with the addition of 60 mg/L PMS and cold stabilised at −4°C for 21 days. The wines were filtered through a 0.2-μm pore-size cartridge (Sartorius Sartopure 2 Maxicap, Sartorius, Gottingen, Germany) and bottled under food grade nitrogen with Stelvin closures.

The Merlot wines were made by traditional red winemaking methods as described in Kelly et al. (2012). After the ferments reached dryness (<1 g/L residual sugars), the wines were racked from gross lees, and divided into two equal portions by volume. The first half (no MLF wines) were cold stabilised at −4°C for 21 days with the addition of 60 mg/L PMS and the second half (MLF wines) was inoculated with *Oenococcus oeni* (Viniflora CH 16, CHR Hansen, Hørsholm, Denmark) at 10 mg/L to initiate malolactic conversion. The MLF replicates were kept at 23°C until the malic acid concentration dropped to <0.1 g/L (19–60 days) and subsequently cold stabilised at −4°C for 21 days with the addition of 60 mg/L PMS. Both the no MLF and MLF wines were filtered as described above.

Chemical analyses

Grape and wine samples were analysed for seven volatile phenols and 14 glycoconjugated phenols at The Australian Wine Research Institute's Commercial Services using the methods described by Hayasaka et al. (2013). Volatile and glycoconjugated phenols were analysed on five and three replicate samples, respectively. Wines were analysed at 7 months (Chardonnay), 30 months (Sauvignon Blanc) and at 40 months (Merlot), post-bottling.

Statistical analysis

Analysis of variance was carried out using the general linear model procedure using SPSS 20 (IBM SPSS Statistics, Chicago, IL, USA). Reported treatments effects are significant at $P < 0.05$.

Results and discussion

Accumulation of smoke-borne phenols in grapes

Volatile phenols. Volatile phenols are expected to occur, although at low concentration, constitutively in lignin-bearing plants or parts thereof. Accordingly, for all three cultivars, the concentration of volatile phenols in grapes from the unsmoked, control vines were either below the limit of detection of the analytical method used ($<2.5 \times 10^{-9}$ mol/kg) or present at trace concentration. Smoke exposure early in the grape-ripening phase significantly increased the concentration of many of the volatile phenols, particularly in Sauvignon Blanc grapes (Table 1). Nonetheless, the concentration of total volatile phenols in smoke-exposed grapes was still low, $\leq 331 \times 10^{-9}$ mol/kg. Volatile phenols are toxic and reactive (Whetten and Sederoff 1995) and the low overall concentration in grapes, therefore, indicates that following uptake of volatile phenols, they are converted to and stored as physiologically compatible complexes by binding with sugars (Hayasaka et al. 2010). In Chardonnay, only *o*-cresol was present at a measurable concentration. Generally, cultivar responses to smoke exposure in terms of the concentration of individual volatile phenols and/or their total pools in grapes were of the order: Sauvignon Blanc > > Merlot > Chardonnay. Where smoke exposure increased the total pool of volatile phenols, the major contributors were the cresol isomers, guaiacol and syringol. While smoke exposure affected the concentration of volatile phenols in grapes, there was no consistent effect of fuel type (smoke source) across cultivars or phenol types.

Glycoconjugated phenols. Depending on cultivar, grapes from the unsmoked vines contained up to 175×10^{-9} mol/kg endogenous total glycoconjugated phenols, including glucosylglucosides (GG), pentosylglucosides (PG) and rhamnosylglucosides (RG). Smoke exposure, averaged across fuel types and cultivars, increased the total pool of grape glycoconjugated phenols by >14-fold (96 vs 1392×10^{-9} mol/kg, Table 2). The source of smoke (fuel type), however, had no significant effect. Similarly, there was no significant effect of cultivar on the total glycoconjugated phenols of grapes at commercial harvest, nor was there a significant cultivar by fuel type interaction. Earlier work suggested cultivar sensitivity in the accumulation of smoke-borne phenols (Whiting and Krstic 2007), although it was not clear whether the cultivars were at similar stage of berry development when the smoke-exposure event occurred. This is an important consideration in determining the effect of the cultivar, since the uptake of smoke-borne phenols changes markedly throughout berry development (Kennison et al. 2009). Our results indicate that when smoke-exposure events occur at comparable stage of berry development (in this case, 14 days post-veraison), the cultivar has no effect on the accumulation of total glycoconjugated phenols. These observations underscore the importance of standardising smoke-exposure conditions in experiments, such as duration, intensity and timing in relation to grape development, and further suggest that the apparent variation in cultivar sensitivity of earlier reports may relate more to phenology at the time of exposure and exposure conditions than to cultivar differences. However, an exhaustive comparison of cultivar responses requires standardisation of other factors, such as canopy management and environmental conditions, which were not considered in this study.

Although cultivar and fuel type had little influence on the concentration of total glycoconjugated phenols in grapes, both treatments affected the composition of the phenols. For example, while the white cultivars accumulated an equivalent concentration of total phenols and total cresols, the concentration of these phenols in the red cultivar, Merlot, was significantly lower (i.e. SB = CH > M). This apparent red versus white cultivar dichotomy in phenol uptake does not apply to all cultivars because accumulation of total guaiacol was similar between Sauvignon Blanc and Merlot (~445×10^{-9} mol/kg), which was higher than the ~$\leq 166 \times 10^{-9}$ mol/kg observed in Chardonnay. Further quantitative differences between cultivars were also evident when composition was considered by the glycone moieties of glycoconjugated phenols. While a clear cultivar pattern was not apparent across all the glycoconjugate types and all phenols, for all the RG, the following cultivar ranking was evident: Sauvignon Blanc > Chardonnay = Merlot.

Table 1. Effect of fuel type and smoke exposure on the concentration of volatile phenols in the fruit of the grape cultivars Sauvignon Blanc, Chardonnay and Merlot.

Volatile phenols	Concentration of volatile phenols ($\times 10^{-9}$ mol/kg)							
	Sauvignon Blanc			Chardonnay		Merlot		
	Control	Pine†	Grass‡	Control	Grass‡	Control	Pine†	Grass‡
o-Cresol	nd	136.9 ± 15.9	175.7 ± 14.1	nd	20.0 ± 1.5	nd	25.9 ± 8.0	33.3 ± 6.3
m-Cresol	nd	24.0 ± 2.3	24.7 ± 3.1	nd	nd	nd	nd	nd
p-Cresol	nd	20.3 ± 1.8	nd	nd	nd	nd	nd	nd
Subtotal	nd	181.2 ± 19.1	200.4 ± 17.2	nd	20.0 ± 1.5	nd	25.9 ± 8.0	33.3 ± 6.3
Guaiacol	nd	59.6 ± 14.3	83.2 ± 11.7	nd	nd	nd	17.7 ± 5.3	27.4 ± 5.5
4-Methylguaiacol	nd	31.8 ± 5.4	14.5 ± 0.1	nd	nd	nd	4.3 ± 2.9	nd
Subtotal	nd	91.4 ± 19.7	97.7 ± 11.7	nd	nd	nd	22.0 ± 7.5	27.4 ± 5.5
Syringol	nd	40.2 ± 11.5	32.4 ± 23.4	nd	nd	25.9 ± 8.5	20.8 ± 2.4	16.9 ± 7.3
4-Methylsyringol	nd	nd	nd	nd	nd	nd	nd	nd
Subtotal	nd	40.2 ± 11.5	32.4 ± 23.4	nd	nd	25.9 ± 8.5	20.8 ± 2.4	16.9 ± 7.3
Total	nd	312.8 ± 36.2	330.5 ± 19.8	nd	20.0 ± 1.5	25.9 ± 8.5	68.7 ± 14.2	77.6 ± 14.9

Data are the mean ± 1 standard deviation (SD) ($n = 5$, except Sauvignon Blanc exposed to smoke from oat grass and Chardonnay where $n = 3$). †Smoke generated from the softwood species radiata pine (*Pinus radiata* D. Don) and ‡from a pasture grass, wild oats (*Avena fatua* L.). nd, not detected.

Table 2. Effect of fuel type and smoke exposure on the concentration of glycoconjugated phenols in the fruit of the grape cultivars Sauvignon Blanc, Chardonnay and Merlot.

Glycoconjugated phenols	Concentration of glycoconjugated phenols ($\times 10^{-9}$ mol/kg)							
	Sauvignon Blanc			Chardonnay		Merlot		
	Control	Pine†	Grass‡	Control	Grass‡	Control	Pine†	Grass‡
Phenol–PG	6.1 ± 0.3	178.2 ± 20.2	167.1 ± 29.0	25.3 ± 0.2	302.1 ± 20.2	3.3 ± 0.4	75.5 ± 10.7	84.8 ± 11.9
Phenol–RG	3.4 ± 0.5	135.8 ± 16.2	203.9 ± 44.4	1.7 ± 0.1	57.7 ± 3.7	0.9 ± 0.1	45.4 ± 7.3	59.2 ± 10.9
Cresol–PG	12.8 ± 0.2	273.4 ± 42.1	301.6 ± 52.9	53.7 ± 1.2	443.6 ± 25.2	21.2 ± 2.3	294.6 ± 63.3	287.4 ± 47.0
Cresol–RG	5.4 ± 0.3	183.9 ± 28.3	180.2 ± 19.9	6.1 ± 0.3	118.8 ± 8.5	1.9 ± 0.3	84.1 ± 15.3	79.7 ± 13.0
Guaiacol–GG	0.7 ± 0.01	55.6 ± 11.2	95.8 ± 8.7	2.7 ± 0.1	5.6 ± 0.3	1.8 ± 0.3	68.4 ± 13.7	101.0 ± 20.8
Guaiacol–PG	8.7 ± 0.5	211.7 ± 38.4	276.4 ± 31.9	39.1 ± 0.5	140.2 ± 7.8	12.6 ± 0.7	276.1 ± 64.2	350.0 ± 73.0
Guaiacol–RG	1.9 ± 0.1	100.5 ± 19.7	158.9 ± 6.7	1.7 ± 0.2	20.0 ± 1.9	0.9 ± 0.1	36.3 ± 7.3	50.1 ± 9.3
4-Methylguaiacol–GG	0.2 ± 0.01	33.3 ± 7.5	19.1 ± 2.3	1.4 ± 0. 1	1.3 ± 0.2	0.3 ± 0.01	28.2 ± 9.4	15.0 ± 4.2
4-Methylguaiacol–PG	1.5 ± 0.1	83.5 ± 15.1	34.4 ± 1.3	13.0 ± 0.2	39.3 ± 2.6	2.5 ± 0.3	79.4 ± 28.1	43.3 ± 9.6
4-Methylguaiacol–RG	2.0 ± 0.2	106.3 ± 19.4	52.9 ± 2.4	6.2 ± 0.2	22.9 ± 1.1	1.2 ± 0.1	73.6 ± 13.1	41.6 ± 6.0
Syringol–GG	4.3 ± 0.3	51.9 ± 14.7	168.6 ± 19.9	6.5 ± 0.1	55.5 ± 6.2	4.3 ± 0.5	71.0 ± 23.9	209.2 ± 59.5
Syringol–PG	5.1 ± 0.2	15.5 ± 3.0	35.9 ± 3.7	11.5 ± 0.2	31.0 ± 1.8	6.1 ± 0.7	15.2 ± 4.1	31.1 ± 7.8
4-Methylsyringol–GG	0.6 ± 0.01	7.6 ± 1.8	14.9 ± 3.0	2.8 ± 0.1	13.3 ± 1.3	0.7 ± 0.1	13.9 ± 6.2	22.7 ± 6.8
4-Methylsyringol–PG	1.3 ± 0.1	3.0 ± 0.5	4.3 ± 0.9	2.6 ± 0.1	6.7 ± 0.4	1.4 ± 0.1	4.2 ± 1.1	4.8 ± 0.9
Total	54.0 ± 2.2	1440.2 ± 206.0	1714.0 ± 174.5	174.3 ± 1.9	1258.0 ± 74.4	59.1 ± 4.0	1165.9 ± 258.1	1379.9 ± 260.7

Data are mean ± 1 standard error ($n = 5$, except Sauvignon Blanc exposed to smoke from oat grass and Chardonnay where $n = 3$). †Smoke generated from the softwood species radiata pine (*Pinus radiata* D. Don) and ‡from a pasture grass, wild oats (*Avena fatua* L.). GG, glucosylglucoside; nd, not detected; PG, pentosylglucoside; RG, rhamnosylglucoside.

Clearly, in glycosylated form, these glycoconjugated phenols are aroma and, perhaps, flavour inactive. Whether such a difference in the concentration of diglycosides, which require sugar-specific exoglycosidases for cleavage of the sugar–sugar bonds that makes the resultant phenolic monoglucoside amenable to attack by a glucosidase and release of sensorially potent volatile phenols (Sarry and Günata 2004), influences the extent to which smoke taint can develop is not known.

The glycoconjugated phenols that reflected the fuel source of smoke were methylguaiacol–PG, and –RG and syringol–GG and –PG, which in part reflected the lignin composition of the fuels. Thus, for example, grapes exposed to the smoke of the pine fuel, whose lignin contains a relatively high concentration of methylguaiacol compared with that of the oat fuel (Kelly et al. 2012), accumulated a concentration of methylguaiacol–PG and –RG significantly higher than that of grapes exposed to oat smoke. The reverse occurred for the syringol diglycosides. Grapes exposed to smoke of oat fuel, which has a high concentration of syringols in its lignin compared with that of pine fuel (Kelly et al. 2012), contained a concentration of syringol–GG and –PG significantly higher than that of grapes exposed to pine smoke (Table 2). Notwithstanding these observations, the detection of an elevated concentration (~seven times the background concentration, Table 2, also see Kelly et al. 2012) of syringol diglycosides in grapes exposed to pine smoke, which has negligible syringols in its lignin, also shows that the accumulation of phenols into grapes does not match the phenol composition of the smoke's fuel source. The source of syringol in grapes exposed to pine fuel smoke remains unclear; however, in planta transformation (methoxylation) of the xenobiotically acquired hydroxy- and methoxy-phenols can be ruled out (Mr David Kelly and Dr Ayalsew Zerihun, unpubl. data, 2013).

Across cultivars and fuel types, the dominant (70–85%) contributors to the total glycoconjugated phenol pool were the diglycosides of phenol, cresol and guaiacol (Table 2). Interestingly, glycosides of syringol and methylsyringol made up ≤20% of the total smoke-derived glycoconjugated phenol in grapes. These results contrast with those reported in Hayasaka et al. (2010, 2013) in which syringol–GG was the single most dominant contributor to the total glycoconjugated phenols in grapes of a range of cultivars exposed to bushfire smoke. The source of this variance for the relative contribution is not clear apart from methodological differences in smoke generation (experimental vs wildfire smoke) as well as the intensity and duration of exposure.

Influence of winemaking techniques on wine phenols

Volatile phenols. The winemaking practices varied for the three cultivars examined. Thus, cultivar effects on volatile and glycoconjugated phenols are necessarily subsumed in the effect of winemaking practices, which influenced the wine volatile phenol concentration (Table 3). For both control and smoke-exposure treatments, Chardonnay wines made from whole-bunch-pressed juice had no measurable concentration ($<2.5 \times 10^{-9}$ mol/kg) of volatile phenols, as was generally the case in the grapes (Table 3). Although smoke-exposed grapes generally contained no volatile phenols, the apparent absence of volatile phenols in the resultant wines was unexpected. This is indicative of a low overall extraction of phenols into whole-bunch-pressed juice and subsequent negligible hydrolysis of the diglycoside bound phenols during and/or post-fermentation. These findings contrast with the high concentration of volatile phenols observed in Chardonnay fruit exposed to bushfire smoke and fermented on skins (Hayasaka et al. 2010) or in wines made after crushing and pressing Chardonnay juice

Table 3. Effect of fuel type and smoke exposure on the concentration of volatile phenols in wine of the grape cultivars Sauvignon Blanc, Chardonnay and Merlot.

Volatile phenols	Concentration of volatile phenols ($\times 10^{-9}$ mol/kg)							
	Sauvignon Blanc			Chardonnay		Merlot		
	Control	Pine†	Grass‡	Control	Grass‡	Control	Pine†	Grass‡
o-Cresol	nd	11.1 ± 3.5	12.3 ± 8.2	nd	nd	nd	77.7 ± 18.2	88.8 ± 11.9
m-Cresol	nd	27.7 ± 9.2	27.7 ± 16.0	nd	nd	nd	33.3 ± 8.6	33.3 ± 4.7
p-Cresol	nd	9.2 ± 4.1	nd	nd	nd	nd	27.7 ± 7.2	20.3 ± 1.8
Subtotal	nd	48.0 ± 14.1	40.0 ± 22.2	nd	nd	nd	138.7 ± 33.7	142.4 ± 17.2
Guaiacol	nd	43.5 ± 9.7	64.4 ± 20.3	nd	nd	40.3 ± 2.5	178.8 ± 30.9	207.8 ± 25.1
4-Methylguaiacol	nd	13.0 ± 4.2	nd	nd	nd	nd	60.8 ± 15.6	34.7 ± 4.8
Subtotal	nd	56.5 ± 13.8	64.4 ± 20.3	nd	nd	40.3 ± 2.5	239.6 ± 46.2	242.5 ± 29.8
Syringol	nd	15.6 ± 4.4	45.4 ± 9.9	nd	nd	122.0 ± 8.0	131.0 ± 6.9	205.0 ± 25.2
4-Methylsyringol	nd	nd	nd	nd	nd	nd	nd	nd
Subtotal	nd	15.6 ± 4.4	45.4 ± 9.9	nd	nd	122.0 ± 8.0	131.0 ± 6.9	205.0 ± 25.2
Total	nd	120.1 ± 28.0	149.8 ± 51.7	nd	nd	162.3 ± 9.0	509.3 ± 81.4	589.9 ± 58.6

Data are mean ± 1 standard error ($n = 5$, except Sauvignon Blanc exposed to smoke from oat grass and Chardonnay where $n = 3$); †Smoke generated from the softwood species radiata pine (*Pinus radiata* D. Don) and ‡from a pasture grass, wild oats (*Avena fatua* L.). nd, not detected.

(Singh et al. 2012). These examples highlight the effect of processing and/or winemaking practice on extraction of phenols.

Wines from the control treatments of Sauvignon Blanc and Merlot grapes had no measurable (<2.5×10^{-9} mol/kg) volatile phenols, except guaiacol and syringol in the Merlot wines, which were fermented on skins (Table 3). In contrast to that of the Chardonnay wines from whole-bunch-pressed juice, Sauvignon Blanc and Merlot wines from the smoke-exposed, de-stemmed and crushed grapes had an elevated concentration of six volatile phenols (Table 3). Between these two latter groups, however, a significantly higher concentration of volatile phenols was present in Merlot wines that were fermented on skins than in Sauvignon Blanc wines made from de-stemmed, crushed and pressed juice (Table 3). Interestingly, the volatile phenol concentration was comparable in smoke-affected Sauvignon Blanc grapes and the resultant wines, whereas in Merlot the concentration in wine was higher than that in grapes (Tables 1,3) suggesting the extended skin contact may have facilitated hydrolysis of glycoside-bound phenols as observed for example by Kennison et al. (2008).

Glycoconjugated phenols. The concentration of total glycoconjugated phenols varied significantly between Chardonnay (whole-bunch pressed without crushing), Sauvignon Blanc (de-stemmed, crushed and pressed) and Merlot (de-stemmed, crushed and fermented on skins) wines in the ratio of approximately 2:5:10, respectively (Table 4). Because the concentration of the total glycoconjugated phenols in grapes was comparable across cultivars and fuel types, the difference between wines primarily reflected the effect of fruit-processing and/or winemaking practices that are applied to these cultivars. These results highlight that extraction of glycoconjugated phenols not only differs between the red wine (made with skin contact) and white wine (made without skin contact) practices, but also between white grape-processing and/or winemaking practices, with grape crushing before pressing releasing considerably more (~2.5-fold) glycoconjugated phenols than whole-bunch pressing without crushing.

Fruit-processing and winemaking methods also had significant influence on the concentration of all 14 glycoconjugated phenols (Table 4). With the exception of syringol–PG and methylsyringol–PG, the ranking of the concentration of the remaining 12 glycoconjugated phenols among the wines of the three cultivars was the same as that for the total glycoconjugated phenols, that is Merlot > Sauvignon Blanc > Chardonnay. Of the total pool, the diglycosides of cresol and phenol contributed the largest proportion (ranging from 44% in Merlot wines to 70% in Chardonnay). The second largest class of phenols was the diglycosides of guaiacol accounting for between 20 (Chardonnay wines) and 33% (Merlot wines). Collectively, the phenol, cresol and guaiacol diglycosides made up 78% (Merlot) and 89% (Chardonnay and Sauvignon Blanc) of the total glycoconjugated phenols in these wines. The contribution of the syringol and methylsyringol glycosides to the total pool was only about 10% or less. The relative abundance of the different phenol classes in wines is broadly comparable with the respective proportion observed in grapes (data not shown but compare Tables 2,4). The low contribution of syringol glycosides (<10% of total) observed in this study, while similar to the results from Kelly et al. (2012), contrasts to results for wines from bushfire smoke-affected fruit in which syringol glycosides were the single largest component (Hayasaka et al. 2010, 2013, Singh et al. 2012). While the exact reason for this variance is unclear, given the similarity of the relative proportion in fruit and wine in the current study, the low values here suggest differences are probably related to conditions, such as exposure intensity and duration, during accumulation in grapes.

Averaged across cultivars, wines from smoke-exposed grapes had more than 16 times the concentration of total glycoconjugated phenols than the control wines (Table 4). The fuel source of smoke had no influence on the total glycoconjugated phenols. Of the individual glycoconjugated phenols, however, exposure to pine smoke generally tended to produce a higher concentration of phenols of the *p*-hydroxyphenyl– and guaiacyl–lignin origin, although the fuel effects were significant only for methylguaiacol–PG and –RG. In contrast, wines made from fruit exposed to grass smoke had a

Table 4. Effect of fuel type and smoke exposure on the concentration of glycoconjugated phenols in wine of the grape cultivars Sauvignon Blanc, Chardonnay and Merlot.

Glycoconjugated phenols	Concentration of glycoconjugated phenols ($\times 10^{-9}$ mol/kg)							
	Sauvignon Blanc			Chardonnay		Merlot		
	Control	Pine†	Grass‡	Control	Grass‡	Control	Pine†	Grass‡
Phenol–PG	6.0 ± 0.6	141.1 ± 21.5	114.5 ± 24.4	10.8 ± 0.4	41.3 ± 1.3	4.9 ± 0.5	164.6 ± 24.6	147.1 ± 15.7
Phenol–RG	1.4 ± 0.2	59.7 ± 3.6	60.8 ± 16.7	0.6 ± 0.2	19.0 ± 1.2	1.1 ± 0.1	74.9 ± 7.3	90.1 ± 8.7
Cresol–PG	9.7 ± 0.6	170.8 ± 28.4	148.3 ± 24.4	16.6 ± 1.1	94.5 ± 5.3	22.3 ± 2.3	265.4 ± 36.1	266.0 ± 23.5
Cresol–RG	3.1 ± 0.6	76.8 ± 9.3	55.1 ± 7.0	0.8 ± 0.1	19.8 ± 1.3	2.7 ± 0.3	105.7 ± 9.9	121.0 ± 12.5
Guaiacoli–GG	0.3 ± 0.01	0.6 ± 0.2	1.6 ± 0.7	0.2 ± 0.01	0.2 ± 0.01	2.0 ± 0.5	7.0 ± 0.8	8.2 ± 1.4
Guaiacol–PG	6.2 ± 0.5	113.0 ± 17.3	128.5 ± 8.7	11.8 ± 0.7	42.8 ± 2.2	14.2 ± 1.2	319.2 ± 52.7	387.8 ± 42.8
Guaiacol–RG	1.0 ± 0.1	35.3 ± 8.3	38.0 ± 2.9	0.4 ± 0.01	5.7 ± 0.2	1.1 ± 0.1	46.9 ± 5.7	76.1 ± 9.4
4-Methylguaiacol–GG	nd	0.2 ± 0.01	0.1 ± 0.01	nd	nd	0.2 ± 0.01	2.7 ± 0.8	2.2 ± 0.3
4-Methylguaiacol–PG	0.9 ± 0.1	52.2 ± 8.2	21.1 ± 1.1	2.6 ± 0.2	7.1 ± 0.6	3.2 ± 0.5	108.5 ± 19.4	66.6 ± 9.9
4-Methylguaiacol–RG	0.8 ± 0.1	25.4 ± 3.2	11.0 ± 0.8	0.6 ± 0.1	3.8 ± 0.5	1.6 ± 0.2	70.5 ± 6.0	54.1 ± 5.9
Syringol–GG	1.1 ± 0.1	6.0 ± 1.4	19.9 ± 3.7	1.8 ± 0.3	9.4 ± 0.6	2.7 ± 0.4	49.3 ± 13.6	129.6 ± 25.9
Syringol–PG	1.8 ± 0.2	4.2 ± 0.8	6.8 ± 0.6	2.7 ± 0.1	5.3 ± 0.4	8.6 ± 0.6	20.0 ± 2.9	38.8 ± 6.0
4-Methylsyringol–GG	0.1 ± 0.01	0.6 ± 0.1	1.2 ± 0.2	0.1 ± 0.01	0.6 ± 0.01	0.2 ± 0.01	4.8 ± 1.4	10.3 ± 2.6
4-Methylsyringol–PG	0.3 ± 0.01	0.5 ± 0.1	0.6 ± 0.1	0.3 ± 0.01	0.7 ± 0.01	10.4 ± 0.6	16.6 ± 2.1	15.9 ± 0.9
Total	32.7 ± 2.2	686.4 ± 76.6	607.5 ± 85.5	49.3 ± 0.8	250.2 ± 10.9	75.2 ± 3.9	1256.1 ± 173.9	1413.8 ± 139.1

Data are mean ± 1 standard error ($n = 5$, except Sauvignon Blanc exposed to smoke from oat grass and Chardonnay where $n = 3$). †Smoke generated from the softwood species radiata pine (*Pinus radiata* D. Don) and ‡from a pasture grass, wild oats (*Avena fatua* L.). GG, glucosylglucoside; nd, not detected; PG, pentosylglucoside; RG, rhamnosylglucoside.

concentration of phenols of the syringyl-lignin provenance (particularly, syringol–GG and –PG) significantly higher than that of the wines from the pine smoke treatment (Table 4). Such differences mirror broadly the concentration of these glycoconjugated phenols in fruit.

Effect of winemaking practice on extraction of grape glycoconjugated phenols into wines

The extraction of total glycoconjugated phenols from grapes into wines varied significantly among the wines of the three cultivars (Figure 1). Merlot wines, which were made according to the standard red winemaking practice of fermenting on skins until dryness, extracted about 85% of the grape glycoconjugated phenols, which is comparable with results for skin-fermented Cabernet Sauvignon and Chardonnay (Hayasaka et al. 2010). The extraction rate for the white wines, which did not involve skin contact, was considerably lower, averaging about 25% of the grape total glycoconjugated phenols. This average, however, masks the effect of different fruit-processing and -handling practices that are customarily used in white winemaking. Sauvignon Blanc wines, made following crushing of fruit prior to pressing, extracted 39% of fruit total glycoconjugated phenols, approximately twice the extraction rate of wines from whole-bunch pressed must without crushing, that is, Chardonnay, ~18% (Figure 1). It appears thus that white and red grape cultivars exposed to an identical bushfire smoke will have a markedly different concentration of putative smoke-taint compounds in wines under typical winemaking conditions. Whether these differences, however, translate into sensory differences (i.e. less negative impact in white wines than in red wines) is not clear, since sensory impacts may be modulated by the red versus white wine matrix effects (Boidron et al. 1988).

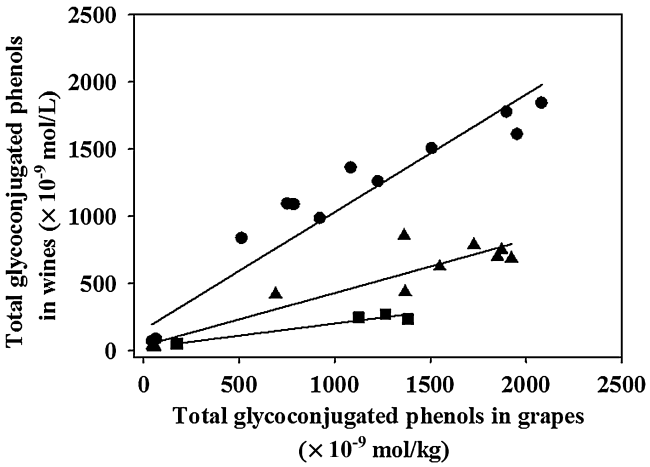

Figure 1. Apparent rate of extraction of total glycoconjugated phenols from grapes into wine as a result of whole-bunch pressing [Chardonnay (■), $y = 20.0 + 0.18\times$, R^2_adj = 0.95], crushing, de-stemming and pressing [Sauvignon Blanc (▲) $y = 35.3 + 0.39\times$, R^2_adj = 0.95] and fermentation on skins [Merlot (●) $y = 154.8 + 0.88\times$, R^2_adj = 0.93].

The extraction rate of the grape glycoconjugated phenols into wines varied between the different phenol classes and wines. In whole-bunch-pressed Chardonnay, the extraction of glycosides of phenols of the *p*-hydroxyphenyl, guaiacyl and syringyl classes was 14, 19 and 11%. In Sauvignon Blanc, the corresponding extraction rate was 39, 27 and 11%, and by comparison 90, 75 and 74% for Merlot wines. Such differences in extraction rate between winemaking practices reflect the localisation of a high proportion of the total grape glycoconjugated phenols in skins (Dungey et al. 2011).

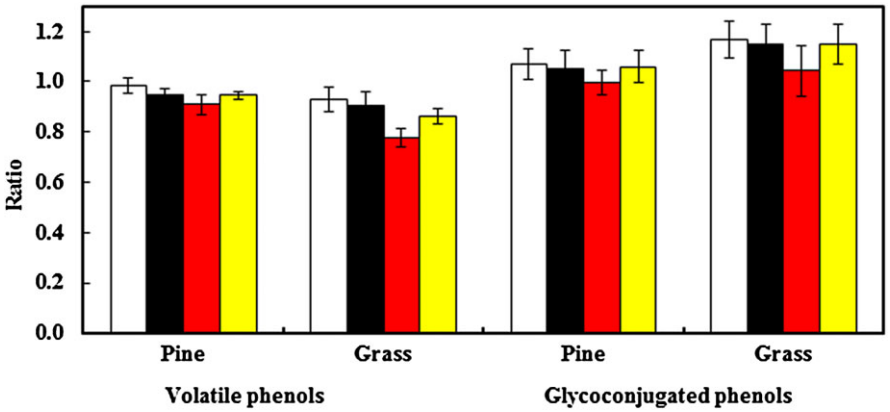

Figure 2. The effect of no malolactic fermentation (MLF) and MLF on the ratio of volatile phenols and glycoconjugated phenols in Merlot wines made from grapes exposed to smoke from pine and oat grass. Cresols and phenols (phenol not quantified in volatile form) (□), guaiacols (■), syringols (■) and totals (□). Data are means ± 1 standard error. For a reference, the data for the MLF wines are shown in Tables 3 and 4.

Differential extraction rates also occurred between glycoside type and conjugated phenol type. For example, guaiacol glucosylglucoside had a low extraction rate (≤7%) regardless of winemaking practice (cultivar) as also reported in Ristic et al. (2011) for Shiraz and Grenache wines. Similarly, low extraction (~10%) was observed for syringol glucosylglucoside in both Sauvignon Blanc and Chardonnay wines. These low apparent extraction rates, however, are not generalisable for all glucosylglucoside phenols, since high extraction of syringol glucosylglucoside (Merlot, 63%, this study; as well as >70% in Cabernet Sauvignon and Chardonnay wines fermented on skins, Hayasaka et al. 2010) can also occur. While the low apparent extraction rate for the white cultivars can be attributed to winemaking practice, the reason for the low extraction rate of guaiacol–GG compared with that of syringol–GG in wines fermented on skins is unclear.

Effect of MLF on extraction and hydrolysis of glycoconjugated phenols in Merlot wines

Red wines normally undergo MLF by inoculation with LAB, often after the completion of alcoholic fermentation. Although MLF is primarily used for de-carboxylating malate to lactate, the metabolic activity of LAB can also modify wine aroma complexity by transforming many compounds, including hydrolysis of glycosides (Ugliano et al. 2003, D'Incecco et al. 2004) and thus potentially releasing volatile phenols. The odour and flavour sensory profile of smoke-affected wines is largely dependent on the concentration of volatile phenols in wines, although some deconjugation of glycoconjugated phenols (at least of monoglucosides) can occur in the mouth (Parker et al. 2012). Therefore, it can be expected, that LAB-mediated hydrolysis of glycosides that alters the distribution of volatile and glycoconjugates of phenols, can also alter the sensory profile of smoke-affected wines. It is not clear whether LAB are capable of significant hydrolysis of glycoconjugated phenols. Our results showed no significant change in the concentration of total volatile phenols, of the total glycoconjugated phenols or of the phenol components between no MLF and MLF Merlot wines (Figure 2). The glycoconjugated phenols in smoke-affected wines were mostly present as diglycosides. It is unclear whether the nature/form of the glycosides present in wines contributed to the apparent lack of hydrolysis of glycoconjugates in the LAB-inoculated wines. The hydrolysis and release of aglycones from diglycosides can occur either at once by actions of diglycosidases or sequentially by cleavage of the sugar–sugar link by sugar-specific exoglycosidases followed by release of volatile phenols by glucosidases (Sarry and Günata 2004). While LAB contain the complementary suite of enzymes (exoglycosidase and glucosidase) that may make sequential hydrolyses possible (Boido et al. 2002, D'Incecco et al. 2004), the presence of a diglycosidase in LAB is yet to be shown (Sarry and Günata 2004). If LAB are capable of releasing aglycone moieties from their diglycoside conjugates though sequential hydroylases, then the lack of response here may be a strain-specific response (Ugliano et al. 2003), and further evaluation of other LAB strains is warranted to gain a more complete picture of LAB capacity on release of sensorially potent aglycones from their glycoconjugated phenols.

Conclusion

This study investigated three issues: (i) cultivar sensitivity to uptake and accumulation in grapes of smoke-borne phenols; (ii) influence of fruit-processing/winemaking practices on release of grape phenols into wines; and (iii) hydrolysis of glycoconjugated phenols and release of volatile phenols during MLF of red wines. For the three cultivars evaluated, when exposure to smoke occurred at the same stage of berry development (early in the berry ripening phase), no significant cultivar sensitivity was observed in the accumulation of total phenols in grapes, although the phenol composition varied. This finding has practical implications. For example, for a grapegrower with a property that adjoins bushland, the criteria for choosing planting material, for expansion or redevelopment, may not need to factor in cultivar sensitivity to smoke phenol uptake.

Fruit-processing and winemaking practices markedly influence the amount and/or proportion of grape phenols that are released into wines. While red winemaking practices that involve skin contact release a proportion of grape phenols considerably higher (≥80%) than that for white winemaking practices (no skin contact, average 25%), there is also significant difference in phenol extraction between different grape-processing methods for white winemaking: crushing before pressing releases ~40% of grape phenols compared with ~18% for whole-bunch pressing without crushing. Understanding how extraction of phenols from grapes changes as a function of fruit-processing and winemaking practices may aid in mitigating and managing smoke taint in smoke-exposed grapes. These results provide practical guidelines on the likely proportion of grape phenols to be expected in the wines for the three traditional winemaking methods studied.

The results from this work found no evidence that MLF in red winemaking increases extraction and hydrolysis of glycoconjugated phenols. Thus, at least for *Oenococcus oeni* (Viniflora CH 16), in wines containing an elevated concentration of glycoconjugated phenols, MLF should not significantly alter the concentration or distribution of volatile and glycoconjugated phenols of the resultant wines.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site: http://onlinelibrary.wiley.com/doi/10.1111/ajgw.12089/abstract

Table S1. Vine spacing and canopy management details for the three study vineyards.

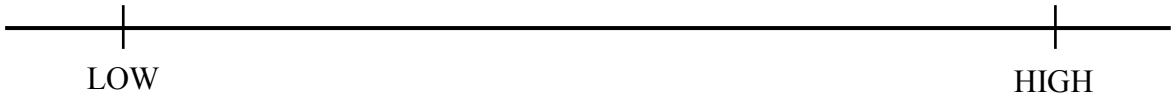
Appendix 4. Respondent sensory assessment results sheet.

Aroma

Rate each wine on the scale below for each of the descriptives by AROMA only.

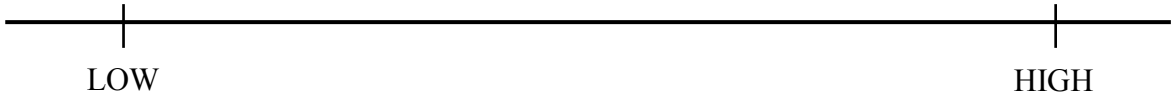
1). Overall fruit aroma

Intensity of overall fruit aroma: (red fruit, red berry, dark berry, strawberry and raspberry).



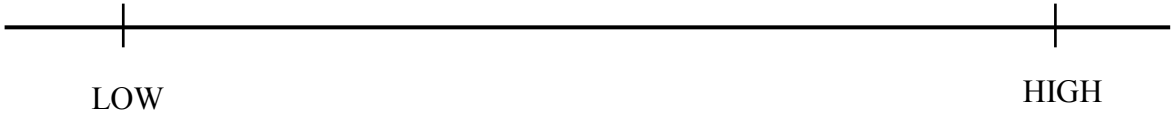
2). Ash

Burnt aroma associated with ashes: (ashtray, metallic and tarry).



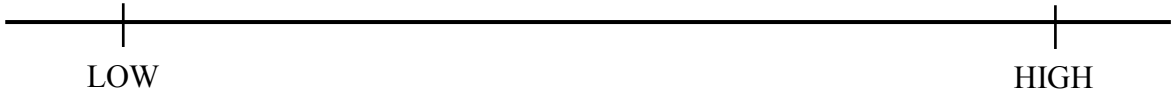
Aroma

3). Smoke — Perception of any type of smoke aroma: (smoky, charry, smoked meat and bacon).

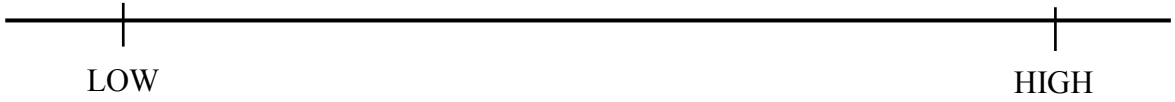


4). Medicinal

Aromatic characteristic of bandages or disinfectant: (disinfectant, phenols and Band-Aid).



5). Solvent — Volatile aroma associated with solvents: (varnish and shoe polish).

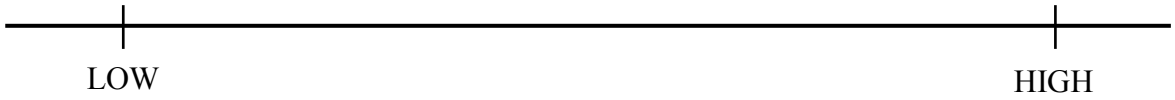


Taste

Rate each wine on the scale below for each of the descriptives by TASTE.

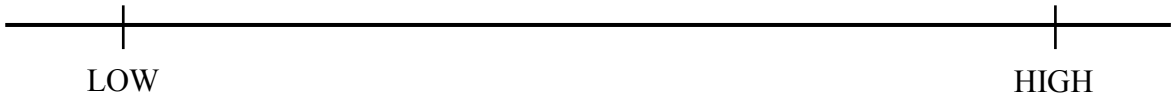
1). Overall fruit taste

Intensity of the overall fruit taste: (red fruit, red berry, dark berry, strawberry and raspberry).



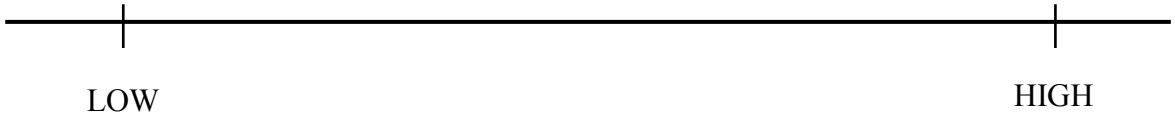
2). Smoky Flavour.

Smoke Flavour: (smoky, charry, smoked meat and bacon).



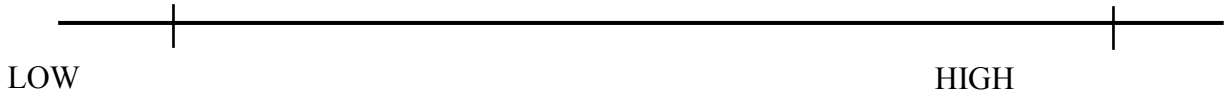
3). Sourness

Sour, acidic taste.

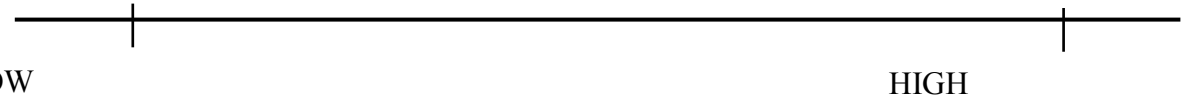


Taste

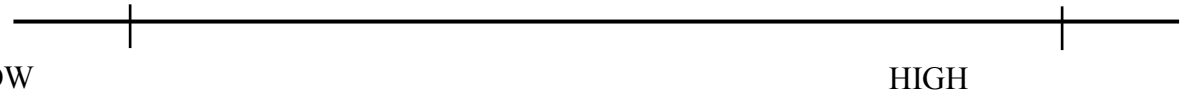
4). Metallic — 'Tinny', canned flavour associated with metals.



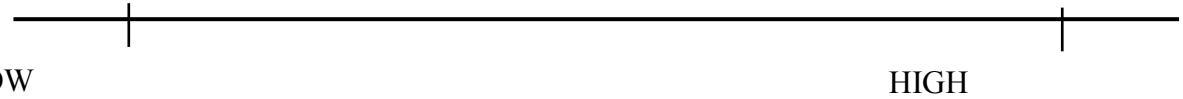
5). Bitter — Bitter taste or aftertaste.



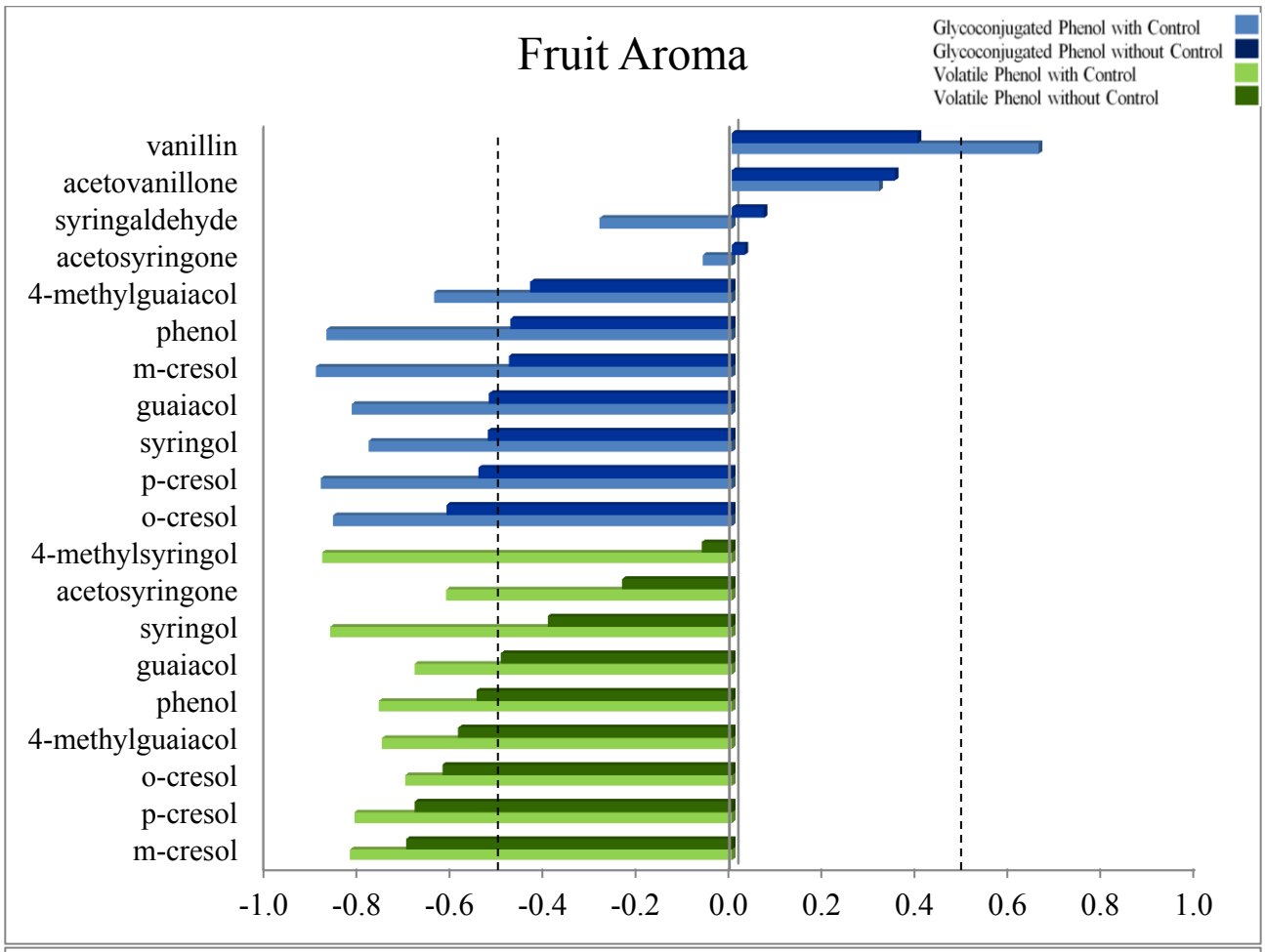
6). Ashy Aftertaste — Length of flavour associated with the residue of an ashtray flavour after spitting.

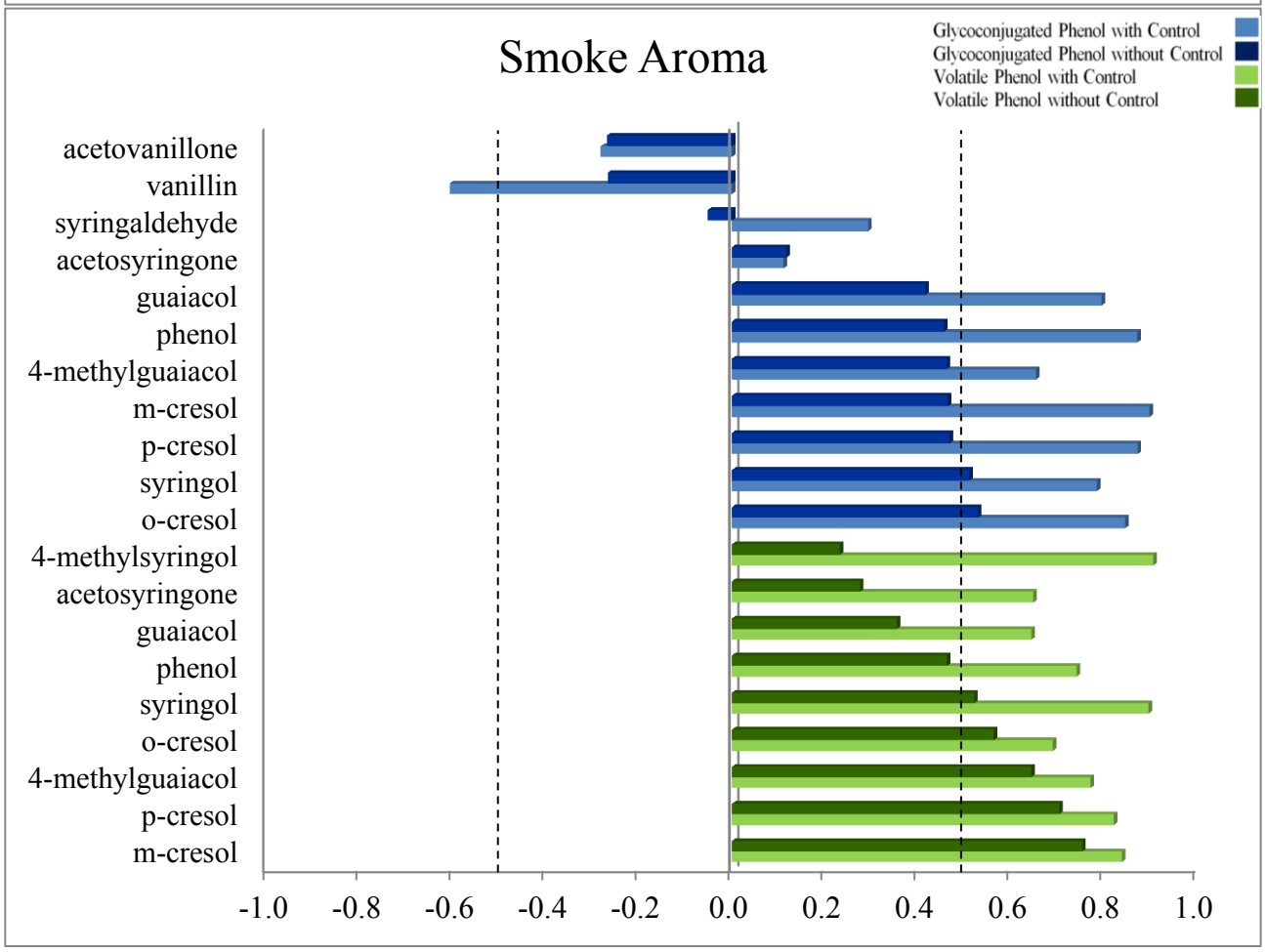


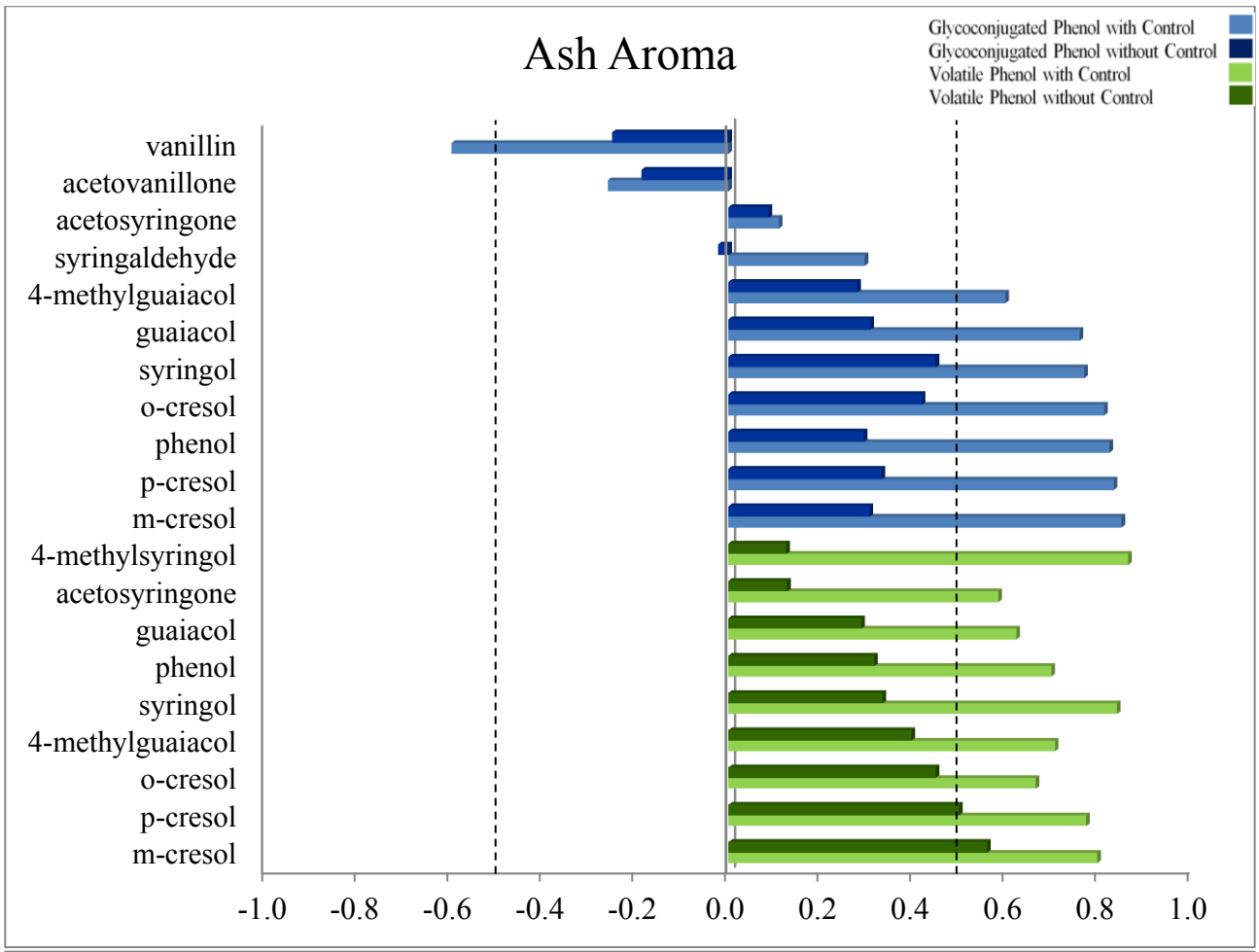
7). Drying — Drying, puckering mouth feel after spitting.

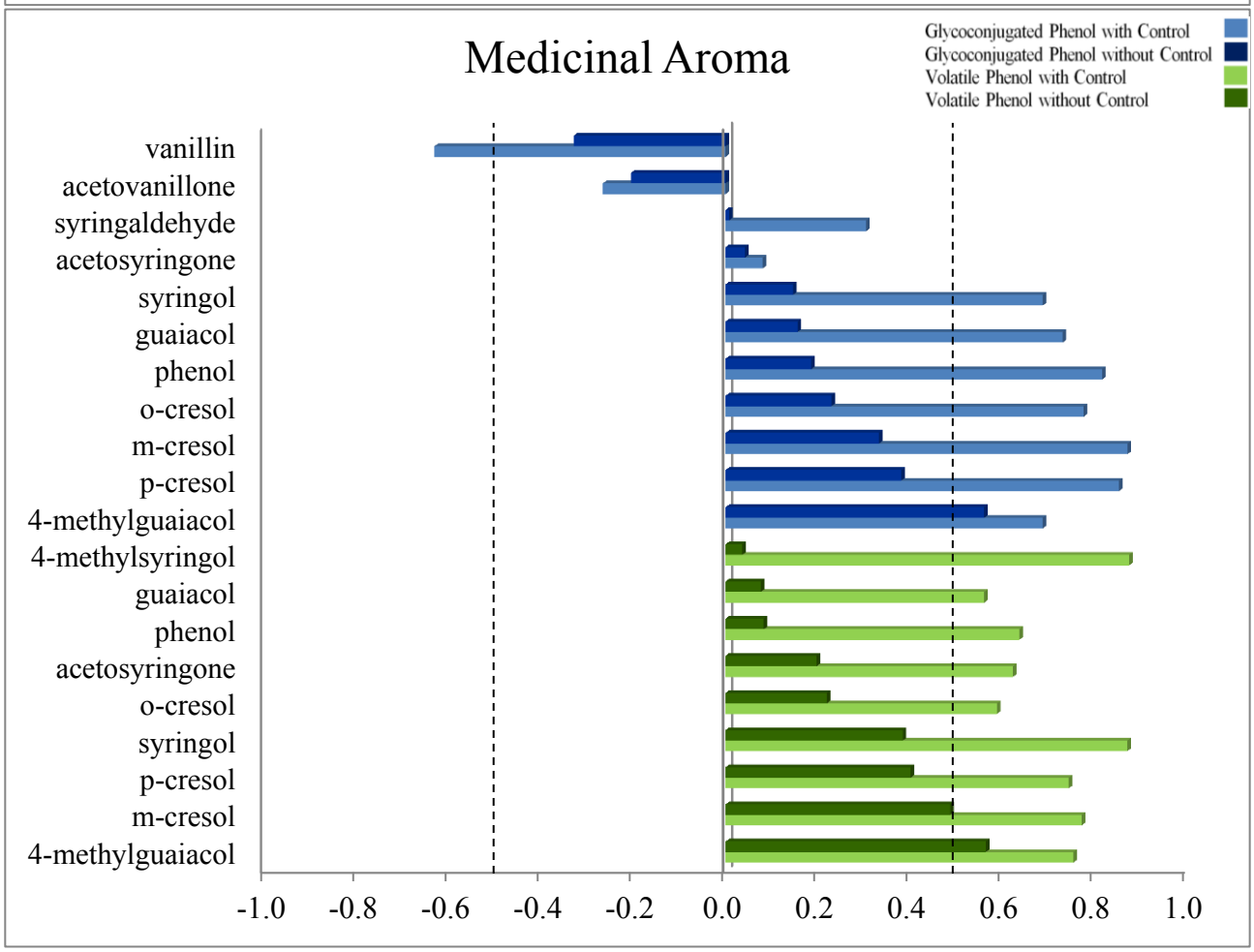


Appendix 5. Correlations of volatile and glycosidically bound phenols to aroma and taste descriptors of Merlot wines. The dashed vertical lines donate the critical value (0.497) at p=0.05, thus coefficients below -0.497 or above 0.497 are significant at p<0.05.






Ash Aroma
Glycoconjugated Phenol with Control
Glycoconjugated Phenol without Control
Volatile Phenol with Control
Volatile Phenol without Control
vanillin
acetovanillone
acetosyringone
syringaldehyde
4-methylguaiacol
guaiacol
syringol
o-cresol
phenol
p-cresol
m-cresol
4-methylsyringol
acetosyringone
guaiacol
phenol
syringol
4-methylguaiacol
o-cresol
p-cresol
m-cresol
-1.0
-0.8
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
0.8
1.0


Medicinal Aroma
Glycoconjugated Phenol with Control
Glycoconjugated Phenol without Control
Volatile Phenol with Control
Volatile Phenol without Control
vanillin
acetovanillone
syringaldehyde
acetosyringone
syringol
guaiacol
phenol
o-cresol
m-cresol
p-cresol
4-methylguaiacol
4-methylsyringol
guaiacol
phenol
acetosyringone
o-cresol
syringol
p-cresol
m-cresol
4-methylguaiacol
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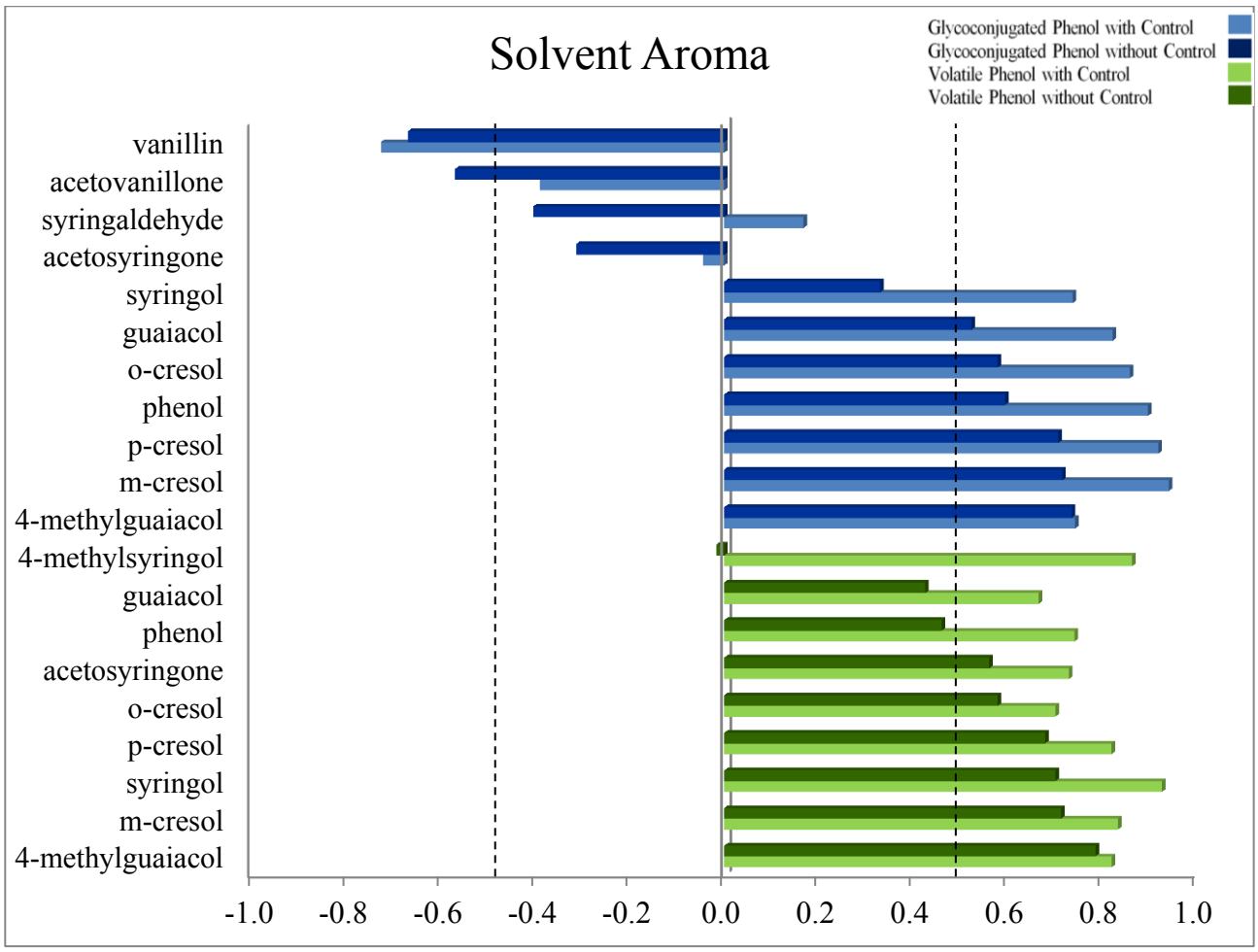
Solvent Aroma
Glycoconjugated Phenol with Control
Glycoconjugated Phenol without Control
Volatile Phenol with Control
Volatile Phenol without Control
vanillin
acetovanillone
syringaldehyde
acetosyringone
syringol
guaiacol
o-cresol
phenol
p-cresol
m-cresol
4-methylguaiacol
4-methylsyringol
guaiacol
phenol
acetosyringone
o-cresol
p-cresol
syringol
m-cresol
4-methylguaiacol
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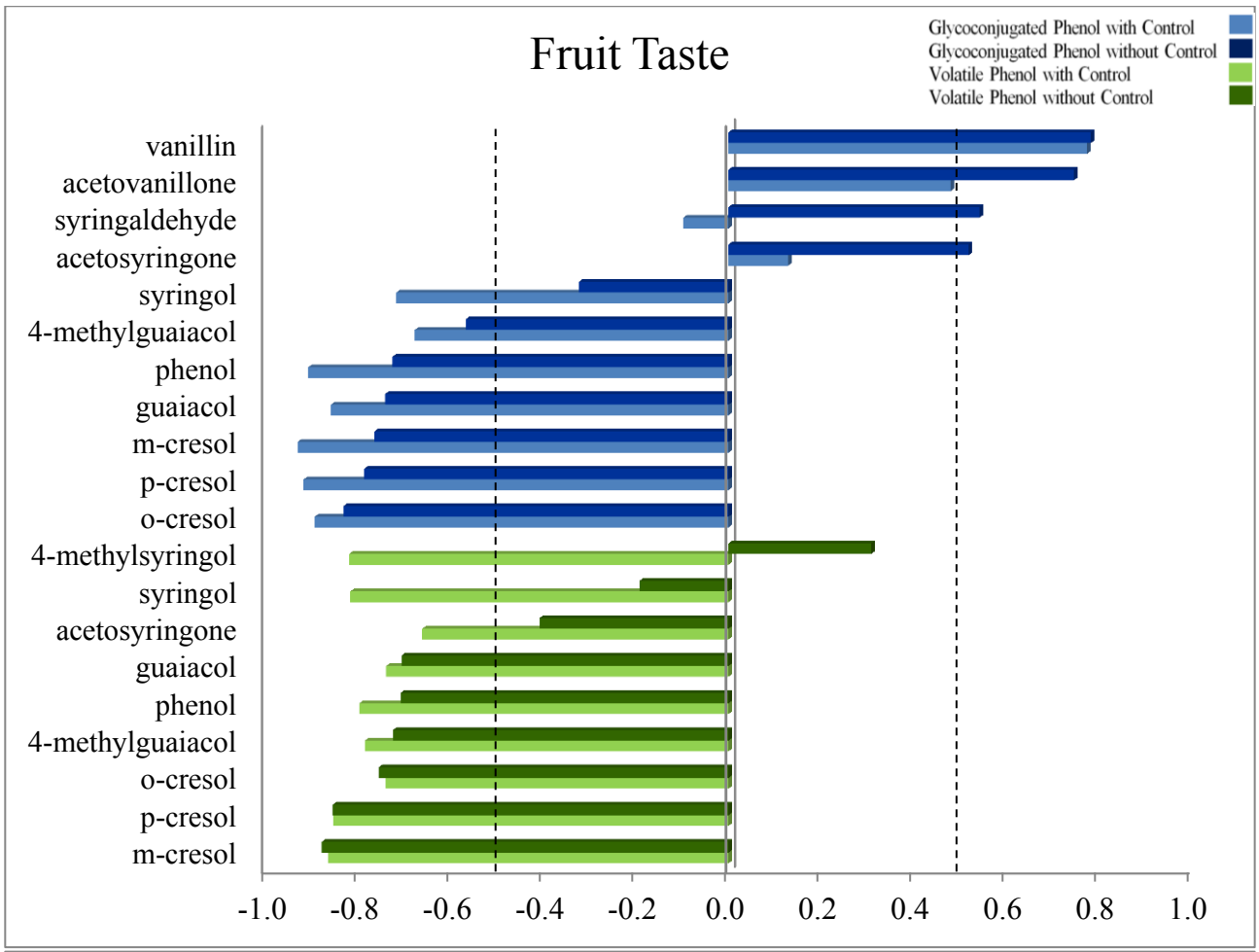
Fruit Taste
Glycoconjugated Phenol with Control
Glycoconjugated Phenol without Control
Volatile Phenol with Control
Volatile Phenol without Control
vanillin
acetovanillone
syringaldehyde
acetosyringone
syringol
4-methylguaiacol
phenol
guaiacol
m-cresol
p-cresol
o-cresol
4-methylsyringol
syringol
acetosyringone
guaiacol
phenol
4-methylguaiacol
o-cresol
p-cresol
m-cresol
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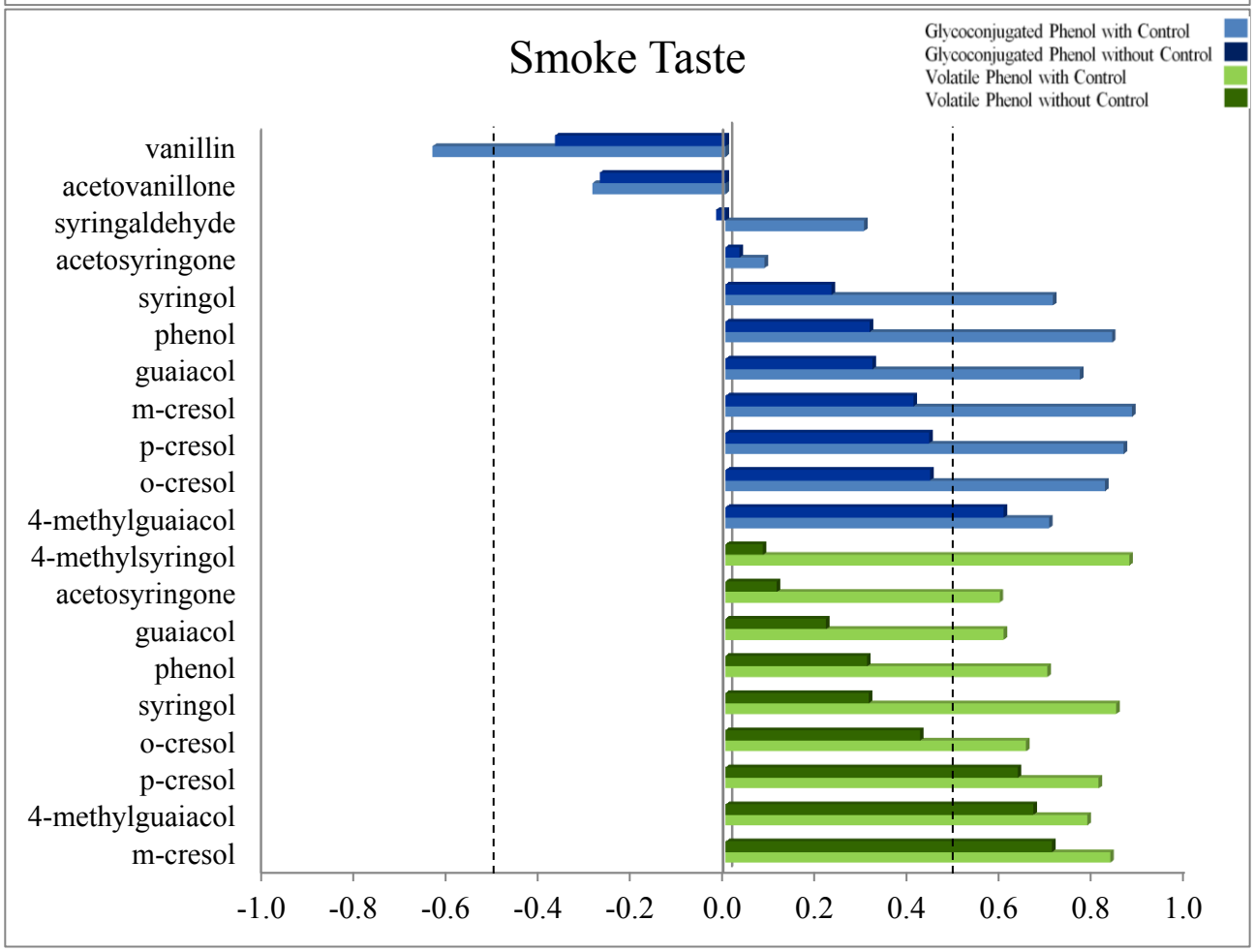
Smoke Taste
Glycoconjugated Phenol with Control
Glycoconjugated Phenol without Control
Volatile Phenol with Control
Volatile Phenol without Control
vanillin
acetovanillone
syringaldehyde
acetosyringone
syringol
phenol
guaiacol
m-cresol
p-cresol
o-cresol
4-methylguaiacol
4-methylsyringol
acetosyringone
guaiacol
phenol
syringol
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m-cresol
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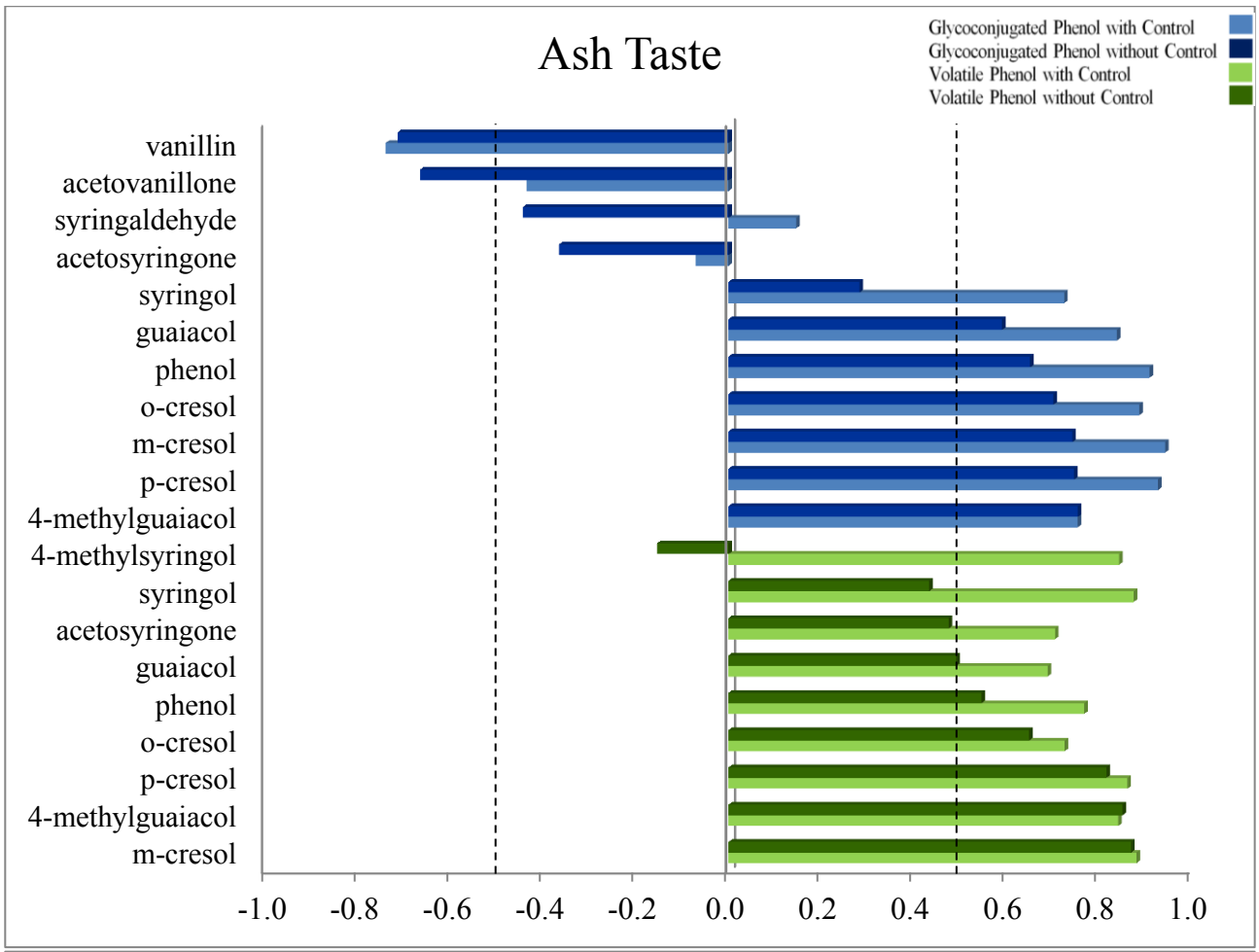
Ash Taste
Glycoconjugated Phenol with Control
Glycoconjugated Phenol without Control
Volatile Phenol with Control
Volatile Phenol without Control
vanillin
acetovanillone
syringaldehyde
acetosyringone
syringol
guaiacol
phenol
o-cresol
m-cresol
p-cresol
4-methylguaiacol
4-methylsyringol
syringol
acetosyringone
guaiacol
phenol
o-cresol
p-cresol
4-methylguaiacol
m-cresol
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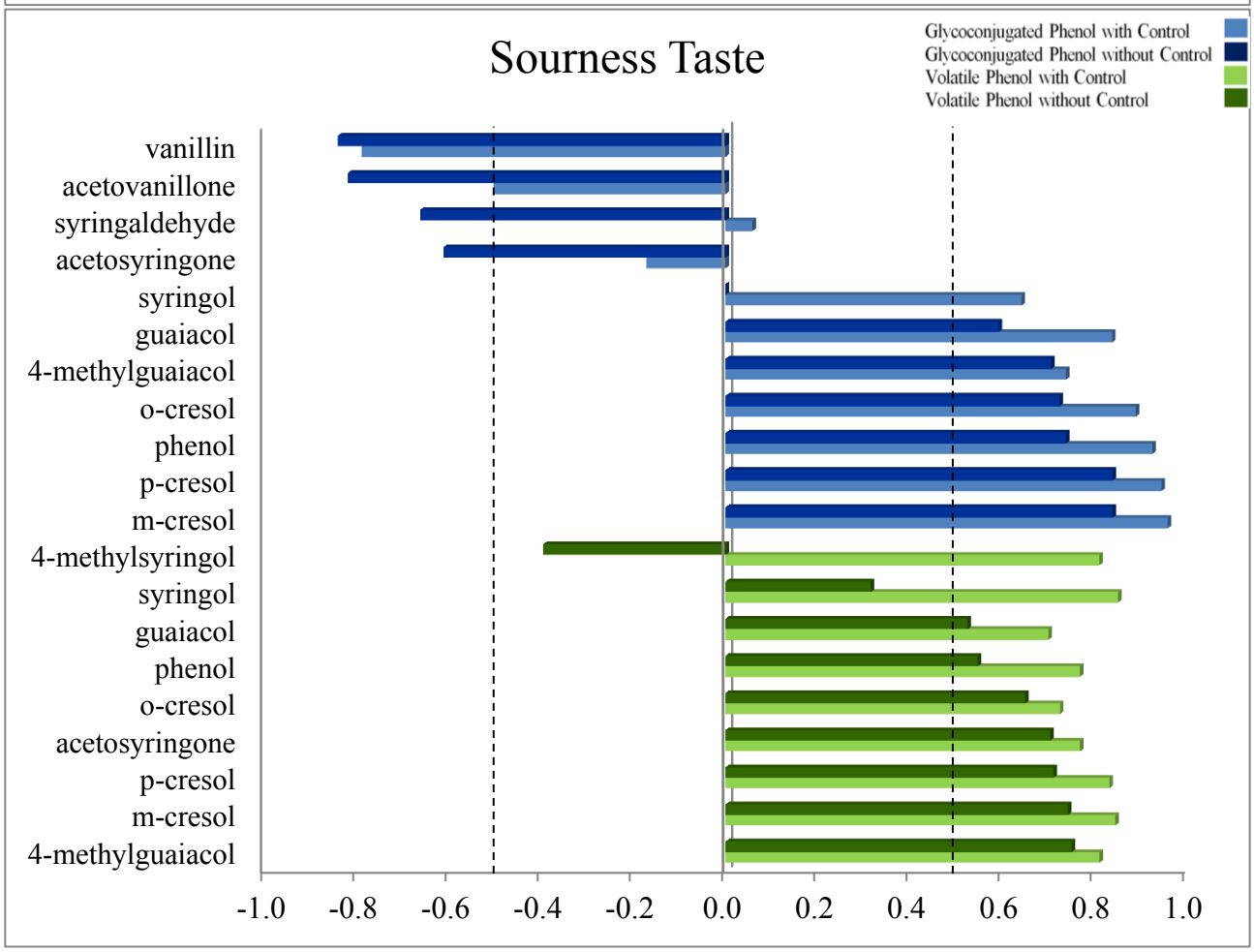
1.0


Sourness Taste
Glycoconjugated Phenol with Control
Glycoconjugated Phenol without Control
Volatile Phenol with Control
Volatile Phenol without Control
vanillin
acetovanillone
syringaldehyde
acetosyringone
syringol
guaiacol
4-methylguaiacol
o-cresol
phenol
p-cresol
m-cresol
4-methylsyringol
syringol
guaiacol
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acetosyringone
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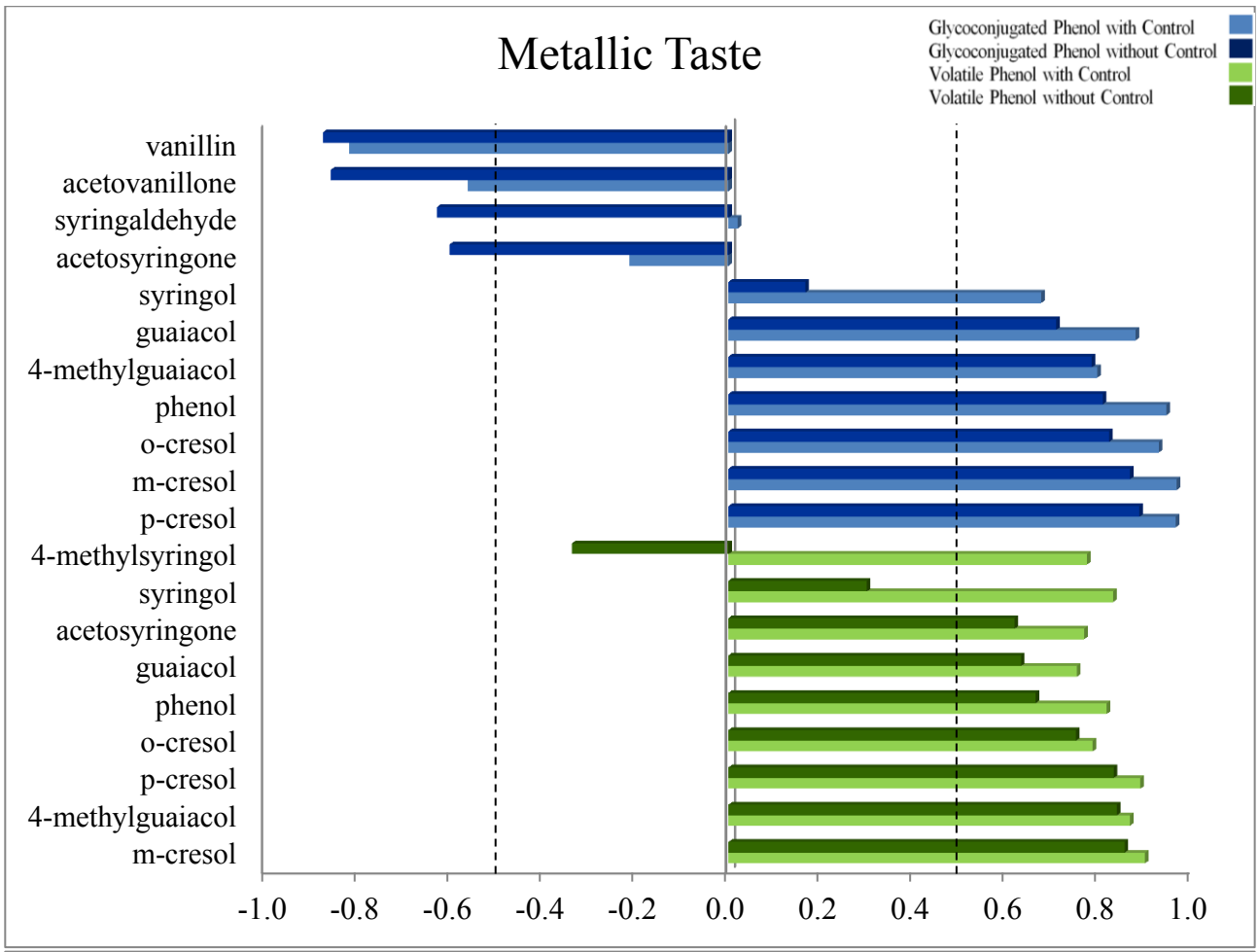
Metallic Taste
Glycoconjugated Phenol with Control
Glycoconjugated Phenol without Control
Volatile Phenol with Control
Volatile Phenol without Control
vanillin
acetovanillone
syringaldehyde
acetosyringone
syringol
guaiacol
4-methylguaiacol
phenol
o-cresol
m-cresol
p-cresol
4-methylsyringol
syringol
acetosyringone
guaiacol
phenol
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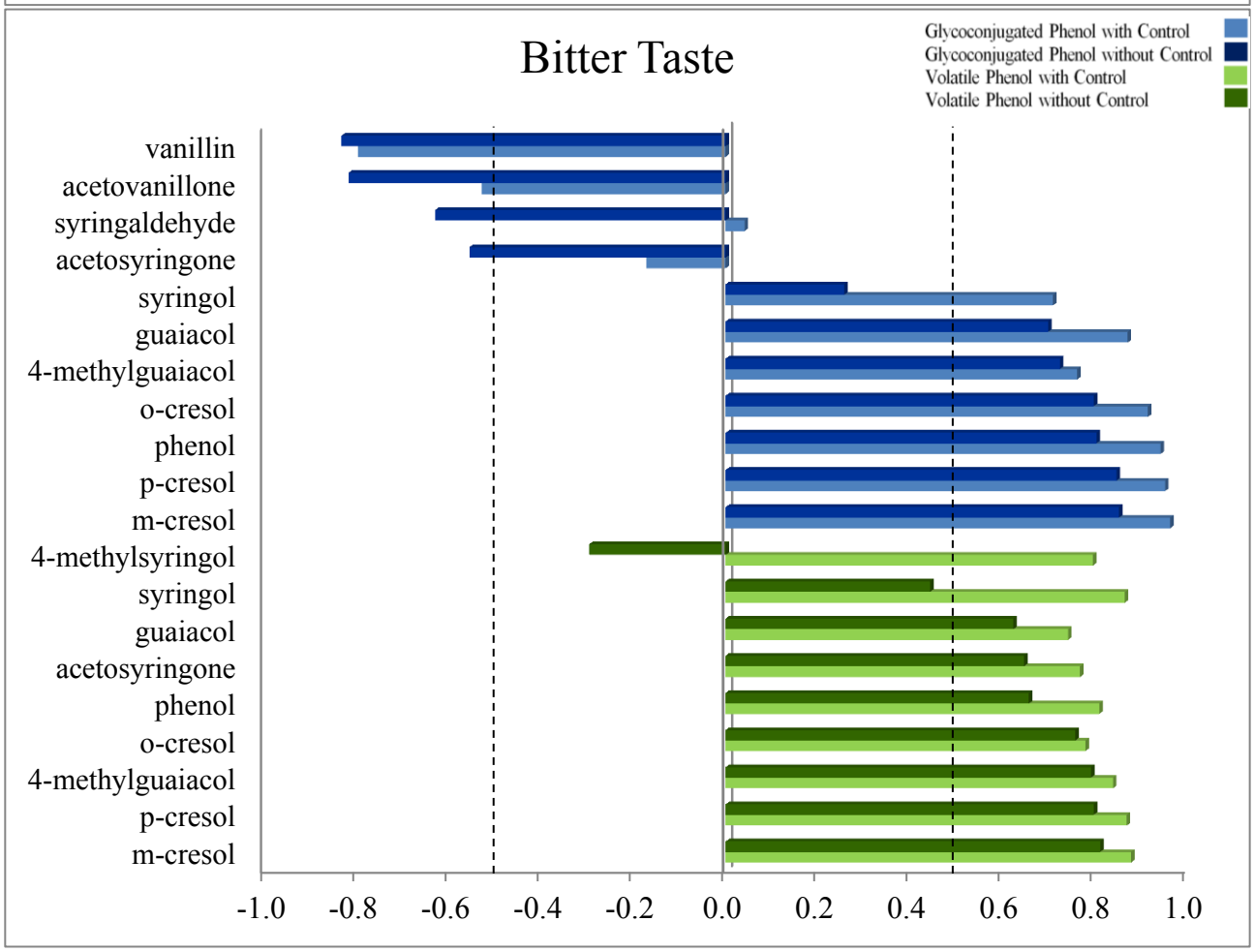
Bitter Taste
Glycoconjugated Phenol with Control
Glycoconjugated Phenol without Control
Volatile Phenol with Control
Volatile Phenol without Control
vanillin
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syringaldehyde
acetosyringone
syringol
guaiacol
4-methylguaiacol
o-cresol
phenol
p-cresol
m-cresol
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syringol
guaiacol
acetosyringone
phenol
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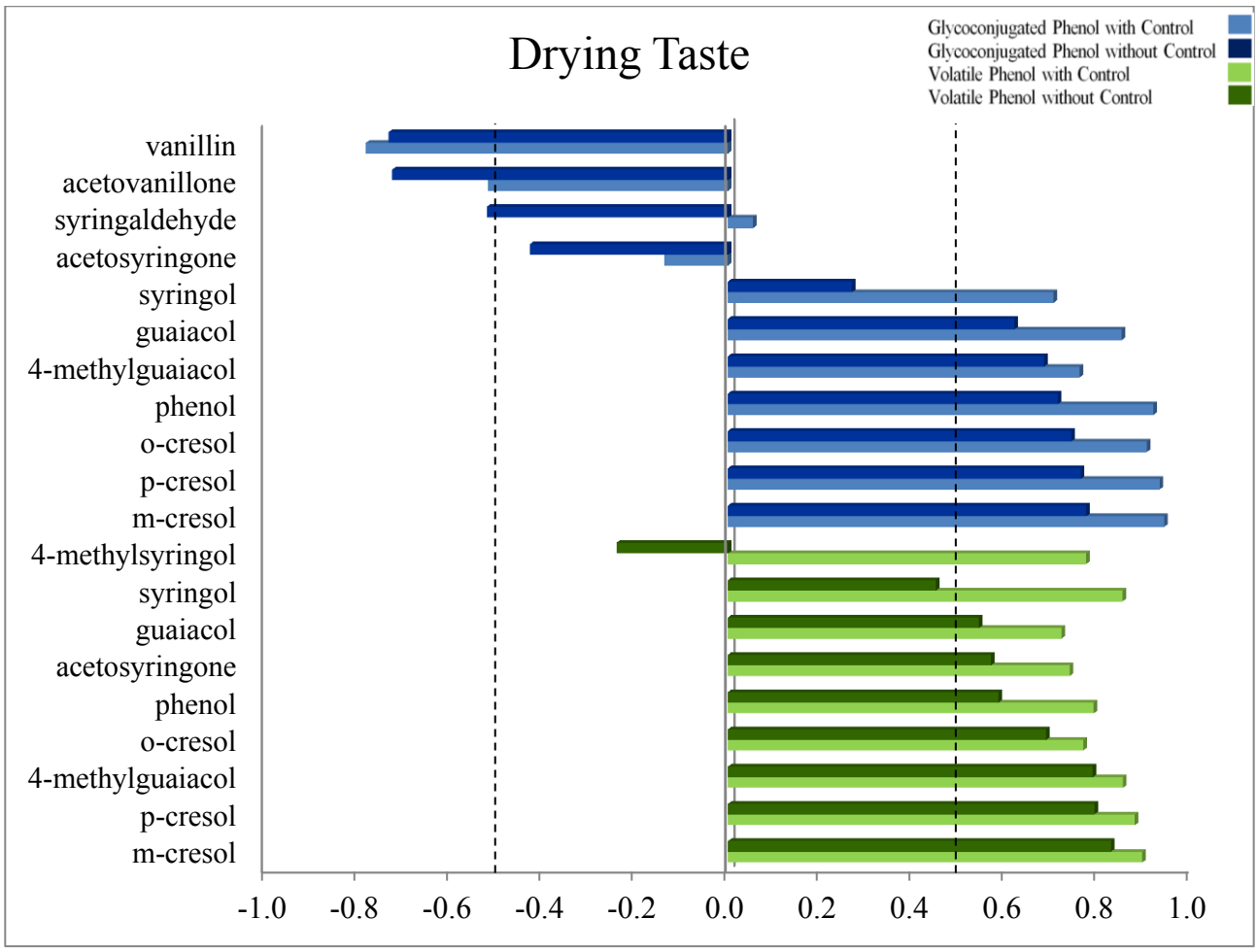
Drying Taste
Glycoconjugated Phenol with Control
Glycoconjugated Phenol without Control
Volatile Phenol with Control
Volatile Phenol without Control
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acetosyringone
syringol
guaiacol
4-methylguaiacol
phenol
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p-cresol
m-cresol
4-methylsyringol
syringol
guaiacol
acetosyringone
phenol
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m-cresol
-1.0
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-0.4
-0.2
0.0
0.2
0.4
0.6
0.8
1.0

Appendix 6. List of abbreviations used in this thesis.

MLF	Malolactic fermentation.
PM 2.5	Particulate matter suspended in air with an aerodynamic diameter up to 2.5 μm.
ENDF	Enzymatic Neutral detergent fibre.
ADF	Acid detergent fibre.
GC-MS	Gas chromatography–mass spectrometry.
Py GC-MS	Pyrolysis–gas chromatography–mass spectrometry.
E-L	Eichorn and Lorenz numerical system of vine phenology.
μg/L	micrograms per litre
μg/kg	micrograms per kilogram
nmoles/kg	nano moles per kilogram
TD GC-MS	Thermal Desorption- gas chromatography–mass spectrometry.
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
PG	Pentosylglucoside
RG	Rhamnosylglucoside
GG	Glucosylglucoside
CH	Chardonnay
SB	Sauvignon Blanc
M	Merlot
LAB	Lactic Acid Bacteria

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Andrew

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Research Group Leader | Bushfire Dynamics and Applications
Senior Research Scientist | ResearcherID: E-9427-2010
CSIRO Ecosystem Sciences

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