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Bushfire Generated Smoke Taint in Grapes and Wine

FINAL REPORT to
GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

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Research Organisation: **Department of Agriculture and Food Western Australia**

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Cover Photograph: Commencement of fuel reduction burn in south-west Western Australia.

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Abstract

Smoke exposure to grapevines has been reported to produce smoke aromas in wine resulting in a sensory 'smoke taint'. Smoke was applied to field-grown grapevines to investigate vine factors associated with smoke uptake, chemical and sensory detection in grapes and wine. Grapevine sensitivity to smoke uptake varies depending on the timing of smoke exposure with a heightened sensitivity to smoke uptake from the time of 7 days post veraison to harvest. Repeated smoke applications result in accumulation of smoke compounds and aromas in subsequent wines. Strategies for reducing the incidence and severity of smoke taint have resulted from this study.

Executive Summary

Smoke derived taint in grapes and wine has resulted in a decline of produce quality and financial losses for many grape growers and wine producers within Australia. The issue of smoke taint in wine is increasing in Australia as climatic conditions change. To date, little information has been available on this subject. This report details initial research to understand the issue of smoke taint and to assist with the reduction of incidence and severity in grapes and wine.

This study implemented novel methodology for the application of smoke to field-grown grapevines and demonstrates the direct link between smoke application to grapevines and the development of smoke taint in subsequent wine. The timing of smoke exposure to grapevines is important with a low sensitivity to smoke uptake from the growth period of shoots 10cm to flowering, a variable smoke uptake period from berries pea size to 3 days post veraison with a heightened period of sensitivity to smoke uptake from 7 days post veraison to harvest.

The duration, or number, of smoke exposures to grapevines is also critical for the development of smoke taint in wine. Repeated applications of smoke to grapevines result in an accumulation of smoke compounds and aromas in wine. Furthermore, repeated smoke applications result in the decline of grapevine leaf functioning and ripening capabilities of the fruit.

The phenol compounds guaiacol and 4-methylguaiacol have been reliable indicators to detect the presence of smoke taint in grapes and wine during this study. Although, this study demonstrates these compounds to not be solely responsible for the taint. Further investigation of alternative compounds, and methods of sample preparation, are required to provide accurate estimations of smoke taint potential. Additionally, grapevine leaf material has been demonstrated to indicate the presence of smoke exposure to vines and may have potential for the analysis of smoke taint in the future.

This study presents research to suggest that smoke compounds can be assimilated by grapevine leaf blades and translocated to the fruit. This has implications for methods of management and prevention of smoke taint and therefore warrants further investigation. Smoke has not been demonstrated to exhibit a carry-over effect and characteristically appear in fruit one year post excessive smoke exposure.

Research outlined in this report has been achieved in collaboration with Assoc. Prof. Mark Gibberd and Dr Kerry Wilkinson from Curtin University of Technology, Margaret River.

Background

As Australia is facing a warming climate with increasing bushfire incidences the issue of smoke derived taint in grapes and wine has become a regular occurrence. Losses from smoke taint vary from year to year due to the unpredictable nature of bushfire events. Where significant smoke exposure occurs during sensitive periods of vine development the resultant wine is unfit for purpose. Smoke taint in wine has resulted in financial losses and decline in product quality for several wine producers in Western Australia and other states. Smoke derived taint in the Pemberton region (located in the South-West of WA) in 2004 was estimated to cost the wine industry in excess of $7.5 m in lost revenue from unmarketable grapes. The Canberra bushfires of 2003 resulted in smoke damage to vineyards in North-East Victoria and South-Eastern NSW estimated to cost the Alpine Valley wine industry alone $4 million (Bunn, 2003). In 2007 smoke exposure to the King and Alpine Valleys effectively resulted in almost zero vintage from locally grown grapes estimated at a loss of 15,000 t.

Smoke is a complex substance containing a multitude of compounds including carbon monoxide, carbon dioxide, ozone, polycyclic aromatic hydrocarbons, oxides of nitrogen and sulphur, volatile and semi-volatile organic compounds and particulate matter (McKenzie et al. 1994, Radojevic 2003). Smoke can impact the organoleptic properties of foods and this is attributed to smoke derived volatile compounds, including phenols (Maga 1988, Guillén et al. 1995). Of these phenols, guaiacol and 4-methylguaiacol are implicit in smoke analysis due to their ‘smoky’, ‘toasted’, ‘ash’, ‘chemical’ and ‘phenolic’ aromas in food and in wines originating from oak barrel fermentation and storage (Baltes et al. 1981, Boidron et al. 1988, López et al. 1999). The quantitative analysis of guaiacol and 4-methylguaiacol in grapes, wine and vine material provides a mechanism for detecting the presence of smoke taint.

Initial investigations into the nature and amelioration of taint in grapes and wine caused by bushfire smoke were conducted by the Australian Wine Research Institute (AWRI) subsequent to the Canberra bushfires in 2003 (AWRI, 2003). The AWRI detected guaiacol and 4-methylguaiacol compounds in smoke-tainted wine and noted these compounds to contribute, in part, to the sensory smoke taint. The measure of guaiacol and 4-methylguaiacol subsequently became a useful tool for indicating the potential presence of smoke taint in grapes and wine although grapevine mechanisms leading to the uptake and development of smoke taint in wine are unknown.

In fact, very little information is known on the effects of smoke exposure on grapes and wine which lead to difficulties in minimising the risk of smoke exposure and effectively responding to smoke exposure events. The current research project was therefore developed to identify possible key periods, during grapevine growth and development, when smoke exposure may be conducive to the development of smoke taint in grapes and wine. Furthermore, the potential mechanism by which grapevines assimilate and translocate smoke is a focus of this study. The effects of smoke on the winemaking process and sensory qualities of wine are investigated leading to a comprehensive understanding of the effects of smoke on grapes and wine.

This study was funded by GWRDC as a Regional Development Initiative project in response to the incidence of smoke taint to grapes and wine in regional Western Australia.

Project Aims and Performance Targets

As detailed in the project proposal, the objectives of the project were to:

1. Identify possible key periods during grapevine growth and development when smoke may be of negative effect and correspond this with timings for prescribed burning and historical bushfire events for viticulture areas in the South-West of WA.
2. Identify grapevine mechanisms of bushfire generated smoke assimilation, translocation and storage within organelles with an emphasis on grape berries.
3. Determine the effect of bushfire generated smoke applied at various phenological stages of grapevine growth and development.
4. Determine the effect of variation in bushfire generated smoke concentration and exposure durations on grapevines.
5. Determine the sensory smoke taint effect on grape juice and wine of both red and white varieties from bushfire generated smoke applied at various phenological stages of grapevine growth and development.
6. Determine the sensory smoke taint effect on grape juice and wine of both red and white varieties of grapevines exposed with various smoke concentrations and durations.
7. Identify and isolate volatile organic compounds in smoke that produce sensory smoke taint characteristics in grapes and wine by use of mass spectrometric analysis.
8. Investigate possible vineyard based amelioration techniques applied to grapevines after bushfire smoke events.

As also detailed in the project proposal, the outputs and performance targets of the project were:

Outputs and Performance Targets 2005-06	
Outputs	**Performance Targets**
1. Establishment of Trial	November 2005
2. Trial report for first season	May 2006
3. Progress reports in industry press and presentation to regional associations	June 2006

Outputs and Performance Targets 2006-07	
Outputs	**Performance Targets**
1. Trial report for second season	May 2007
2. Progress reports in industry press and presentation to regional associations	June 2007

Outputs and Performance Targets 2007-08	
Outputs	**Performance Targets**
1. Trial report for third season	May 2008
2. Final report	October 2008
3. Articles in industry press	November 2008
4. Production of Factsheet/Farmnote	November 2008
5. Presentation of information to regional associations	November 2008

Method

A series of experiments were undertaken to address the various objectives of the project over three years (2005 to 2008).

Smoke Application

1. Grape Bunches

Initial research into smoke effects on grapes and wine concentrated on smoke application to grape bunches alone. The earliest grape bunches to achieve ripeness for winemaking in WA were obtained (Verdelho) from a commercial vineyard. These bunches were placed on wire racks in a purpose-built smoke house (3 m long x 3 m high x 3 m wide) located at the Kings Park and Botanic Gardens (Perth, Western Australia) and similar to that described by Dixon et al. (1995) and Kennison at al. (2007) (Figure 1). These bunches were exposed to smoke for a period of 1 hour. The temperature within the smoke facility was maintained at 25 ^{o}C with the bunches were randomly mixed post smoke exposure to avoid variations in smoke content.

In all experiments in this study, smoke was generated from the combustion of dry barley straw. Dry barley straw was selected as the fuel source for all smoke treatments due to its basic cellulose / lignin composition, reproducibility and to reduce the variation associated with other fuel types. Additionally, straw was utilised to limit interference of smoke compounds from a multitude and variety of sources. Further investigation of smoke application from a range of forest fuels is currently being undertaken in separate research.

2. Field Grown Grapevines

For the application of smoke to field-grown grapevines, field based tents (measuring 6 m long x 2.5 m high x 2 m wide) were constructed of galvanised steel to enclose vines (Figure 2). These tents were covered with a greenhouse grade plastic (Solaweave$^{©}$) that enabled light to transfer through to vines to enable plant photosynthesis and functioning. Smoke was generated by the combustion of dry barley straw in a 50 L lidded drum and pumped into the smoke tent through a metal hose. Three replicates of each smoke treatment were applied to grapevines (3 per replicate) for a period of 30 min. Unsmoked (control) vines were situated away from smoked vines and were also enclosed within tents to replicate conditions. Merlot was the variety selected for all field-based applications of smoke to grapevines due to its subtle aroma characters in comparison to other major winegrape varieties. All field trials were sited in the Geographe area of Western Australia, away from forested areas and were not known to be exposed to external smoke events for the duration of experiments.

Figure 1. Verdelho bunches placed on wire racks in a purpose built smoke facility for the application of smoke.

Smoke duration and density was monitored within the tent with a DustTrack© laser photometer (TSI Model 8520) and VESDA LaserFOCUS™ nephelometer (VLF-250).

The application of smoke to field-grown grapevines was conducted in 2005-06, 2006-07 and 2007-08. Smoke was applied to field grown grapevines at various Eichhorn-Lorenz (E-L) (Eichhorn and Lorenz 1977) growth stages in:

- 2005-06: Single smoke applications were applied to separate field-grown grapevines. Due to the timing of the commencement of this project smoke application commenced at E-L 35 (veraison) and was then applied at either E-L 35 + 3 days (3 days post veraison), E-L 35 + 7 days (7 days post veraison), E-L 35 + 10 days (10 days post veraison), E-L 36 (intermediate sugar), E-L 36 + 3 days (3 days post intermediate sugar), E-L 37 (berries not quite ripe), or at E-L 38 (harvest).

- 2005-06: to investigate the effect of repeated smoke exposures on subsequent wine production, applications of smoke were applied to the same field-grown grapevines at E-L 35 (veraison), E-L 35 + 3 days (3 days post veraison), E-L 35 + 7 days (7 days post veraison), E-L 35 + 10 days (10 days post veraison), E-L 36 (intermediate sugar), E-L 36 + 3 days (3 days post intermediate sugar), E-L 37 (berries not quite ripe), and at E-L 38 (harvest). Treatments were assessed in 2006. Fruit was also harvested from these same vines one year post repeated smoke exposure to investigate the potential assimilation, ‘carry-over’ and release of smoke compounds by grapevines.

- 2006-07: field-grown grapevines were subjected to additional single smoke applications to investigate smoke effects on a broader range of grapevine growth stages. Smoke was applied to grapevines at either E-L 12 (shoots 10cm), E-L 23 (flowering), E-L 31 (berries pea size), E-L 32 (bunch closure), E-L 35 (veraison), E-L 37 (berries full colour) or E-L 38 (harvest).

- 2007-08: in order to validate results gained thus far, single smoke applications were applied to field grown grapevines at significant grapevine growth stages. Smoke was applied at either E-L 31 (berries pea size), E-L 35 (veraison), E-L 35 + 7 days (7 days post veraison) and E-L 38 (harvest).

Figure 2. Field smoke application apparatus showing smoke tent with vines enclosed (left) and smoke generator (right).

3. Assimilation and Translocation Studies

A key objective of this study was to gain an understanding of the location of smoke compounds within the grape berry, and the potential mechanisms by which smoke comes to reside in the berry (i.e. mechanisms of smoke assimilation and translocation). Investigation was undertaken initially by the segmentation of mature grapes produced from unsmoked (control) vines and grapes produced from vines exposed to smoke at E-L 35 (veraison). Berries were peeled and separated into components of skin, pulp and seeds for the analysis of smoke compounds.

Treatments of smoke and smoke-water were applied to separate grapevine organelles in order to investigate the potential assimilation and translocation of smoke compounds into fruit. For this experiment, leaves of field-grown grapevines were sealed in plastic and smoke was applied (for 30 min) to bunches alone (Figure 3). A separate treatment was conducted with bunches from field-grown grapevines sealed in plastic with smoke applied (for 30 min) to grapevine leaves only (Figure 3). Smoke was applied as per field smoke application outlined above. Separate treatments of smoke-water were also applied as a spray to fruit and leaves of field-grown grapevines with grapevine organelles sealed as previously mentioned. Smoke-water was prepared by producing smoke in the smoke generator (Figure 2) which was forced by vacuum through a 30 L drum of water for a period of 1 hour. The result was a brown coloured liquid (smoke-water) with a detectable smoke aroma. Fruit samples were taken from all treatments at harvest for the analysis of guaiacol and 4-methylguaiacol.

Figure 3. Vine components sealed within plastic for studies to investigate the assimilation and translocation of smoke. (1) Grapevine leaves sealed within plastic and grape bunches exposed for smoke application (left) and (2) grape bunches sealed within plastic and leaves exposed for smoke application (right).

All fruit samples were homogenised prior to analysis for guaiacol and 4-methylguaiacol. In some cases, guaiacol and 4-methylguaiacol were not detected in samples and were therefore, as per previous methodology (Kennison et al. 2008a), hydrolysed at a lower pH (pH 1) in order to release these compounds.

In addition to smoke application to separate grapevine organelles, further studies were undertaken to investigate the influence of the wax bloom on grapes on the uptake of smoke compounds. Chardonnay grapes were utilised for this study with these grapes harvested for treatment 2 times prior to verasion (at E-L 31 berries pea size and E-L 32 beginning of bunch closure) and 2 times post veraison (at E-L 35 veraison and E-L 36 intermediate sugar). The wax bloom was removed from harvested grapes by dipping in chloroform for 30 seconds with the berries left to dry on paper towel for 2 min. For exposure to smoke, berries were placed on wire racks and enclosed within a purpose built small scale smoke tent (1 m long x 1 m high x 1 m wide) with smoke applied by a smoke generator as explained previously (Figure 4). Both waxed and unwaxed berries were exposed to smoke either for 0, 5, 10, 20, 40 or 80 min duration. Immediately post exposure to smoke the berries were homogenised and frozen at -80 ^{o}C for 1 week prior to gas chromatography-mass spectrometry analysis for guaiacol.

Figure 4. Small scale model for the application of smoke to grape berries. Apparatus utilises smoke generating equipment and a small scale tent.

Amelioration and protective treatments

A heightened interest in the prevention and amelioration of smoke taint exists and a number of vineyard based amelioration and protective treatments trialled during this project in order to reduce the severity of smoke damage. Previous research has detailed the successful reduction in visible damage to plants from air pollutants and ozone by the application of protective chemicals (Archambault et al. 2000, Musselman 1985, Pandey et al. 1993). From this previous research, a number of chemical products were selected for application to grapevines prior to smoke exposure and were evaluated to determine their effectiveness in reducing smoke compounds in grapes. Protective chemicals evaluated in this study included chemical products registered for use on grapevines for the control of pests and diseases, surfactants and anti-transpiration chemicals utilised in the nursery industry. All chemicals were applied as per label rates to field-grown Chardonnay vines at least 2 hours prior to smoke exposure. These vines were then enclosed within the purpose built smoke tents (as explained previously for the application of smoke to field grown grapevines) and exposed to smoke for 30 min. Post smoke exposure, grape berries were sampled, homogenised and frozen at -80 ^{o}C prior to analysis for guaiacol and 4-methylguaiacol.

In order to reduce the presence of smoke taint in wine, this project also investigated the effect of amelioration treatments applied to vines post exposure to smoke. Previous research has been conducted in this area (AWRI 2003) with the high volume water wash applied to vines post exposure to smoke, used in the previous research, included in this study in order to compare the methodology employed with that of protective treatments.

Winemaking

Each fruit replicate, from the smoked and unsmoked treatments, produced approximately 15 kg of fruit that was made into wine using small lot winemaking methods (Rankine 2004). Fruit (Verdelho) that had received smoke exposure to grape bunches alone was made into two wine treatments. The first wine treatment was produced by the fermentation of free-run juice. To gain an understanding of the influence of skin contact on smoke taint development in wine, a second wine treatment was produced by the fermentation of free-run juice on skins.

Merlot fruit produced from grapevines subjected to field based smoke applications was also made into wine. The methodology for the production of wine in this project is outlined below.

1. Fermentation of free-run juice

Fruit was harvested at a total soluble solids (TSS) of approximately 24 ^{o}Brix, smoke treatments were applied in purpose built facilities as outlined previously and the fruit was transported to the winemaking laboratory for processing. Smoked and unsmoked (control) grapes were made into wine in separate environments to avoid smoke contamination. Grapes were crushed, de-stemmed, pressed from skins and must decanted into 15 L enclosed glass demijohns. Sulfur dioxide was adjusted to 30 ppm and 0.05 g/L of pectic enzyme (Clarex P150) was added. Musts were cold settled at 2 ^{o}C for 48 hours. Musts were allowed to adjust to room temperature, racked into clean fermentation vessels and inoculated with EC1118 yeast. On the completion of fermentation, wines were racked off lees, free SO_2 was adjusted to 30 ppm, wines were cold stabilised for 28 days at 2 ^{o}C, wines were filtered (at 5 micron) and bottled.

2. Fermentation of free-run juice on skins

Fruit was harvested at a TSS of approximately 24 ^{o}Brix. Smoke treatments were applied in purpose built facilities as outlined previously and the fruit was transported to the winemaking laboratory for processing. Grapes were crushed, de-stemmed and fermented in 15 L open fermentation vessels with EC1118 yeast (Lallemand Inc., Montreal, Canada). Musts were plunged twice per day and were pressed from skins when approaching 0 ^{o}Baume. Subsequently, these wines were stored in 15 L glass demijohns and when residual sugar approached 0 g/L were racked from lees and inoculated with malolactic culture (Leuconostoc oenos, Vinaflora Oenos, Chr. Hansen, Denmark). Malolactic fermentation was monitored by malic acid presence and on completion, wines were racked from lees, free sulphur dioxide was adjusted to 30 ppm and the wines were cold stabilised for 28 days at 2 ^{o}C. Wines were then filtered (at 5 micron) and bottled.

3. Production of red wine

Fruit from Merlot grapevines subjected to field-based applications of smoke was made into red wine and fermented as per ‘fermentation of free-run juice on skins’ method previously outlined. Samples of fermenting musts were taken at various stages throughout fermentation in order to measure the development and release of guaiacol and 4-methylguaiacol during the fermentation process.

Wine Sensory Analysis

Wine sensory analysis was conducted on all wines produced from treatments within this study. All wine sensory analysis conducted in this study concentrated on the aroma of wines only, and not the taste, to avoid potential deleterious effects to human health associated with the tasting of smoke tainted wine.

1. Difference testing

Difference testing of smoked and unsmoked wine was conducted on Verdelho wines (produced from both ‘free-run juice’ and ‘free-run juice on skins’ fermentation) using the triangle test method (Meilgaard et al. 2007). A panel of 24 people all being regular wine consumers, equal numbers of male and female participants, all of European origin and aged between 18 and 55 years were recruited for the sensory. Smoked and unsmoked wines were presented to each panellist using a balanced, randomised presentation order. Panellists were required to assess two sets of wines – one was Verdelho produced from the fermentation of free-run juice, the other was Verdelho produced from the fermentation of free-run juice on skins. Panellists were required to identify the sample within each set that was different.

2. *Aroma detection threshold*

The detection threshold for smoke aroma in Verdelho wines (produced from both ‘free-run juice’ and ‘free-run juice on skins’ fermentation) was conducted according to the American Society for Testing and Materials (ASTM) method 679E and as described by Meilgaard et al. (2007). A panel of 33 regular wine consumers were selected for this sensory on the basis of interest and availability. All panellists were aged between 18 and 55 years, of European origin, non-smokers with similar numbers of male and female participants. Smoked wines were presented at various concentrations (0.11, 0.33, 1.0, 3.0, 9.0, 27.0 and 81.0 mL) diluted with unsmoked (control) base wine to 250 mL. Wines were presented as part of a triangle test in ascending concentration order. Panellists that could detect the smoke aroma in all concentrations were tested at lower dilutions and those that could not detect smoke aroma at any concentration were tested at higher dilutions. The group smoke aroma detection threshold was calculated as the geometric mean of each panellist’s best-estimate aroma threshold.

3. *Quantitative descriptive analysis*

Quantitative Descriptive Analysis (QDA) of wine aroma was conducted on all wines (smoke and control) produced from field smoke applications to Merlot grapevines as per methodology described by Meilgaard et al. (2007). QDA consisted of thorough training of 8 panellists comprised of 4 males and 4 females aged between 21 to 30 years. Panellists were selected based on their interest and availability, being non-smokers, regular wine consumers and having experienced at least 100 hours of sensory wine education/experience. All panellists were of good health and able to detect the smoke aroma of Verdelho wines as predetermined by the aroma detection threshold activity.

As previously mentioned, all wines were assessed for aroma only to avoid any potential health impacts associated with tasting smoke tainted wines. All panellists underwent 8 QDA training sessions (2 per week) prior to formal evaluations that lead to the generation of 6 descriptive terms by panel consensus. For each descriptive term, each panellist was trained to rate the aroma presence and intensity on a 100 point unstructured line scale.

Final QDA testing of two replicates of wines produced from grapevines exposed to (i) single, (ii) repeated and (iii) phenological applications of smoke (i.e. 38 wines in total) was conducted over 6 sessions. Wines (20 mL) were presented to each panellist in a completely randomised order so that no two panellists received the same wine at the same time. Wines were presented at room temperature, in 3 digit coded ISO standard tasting glasses and covered with glass covers to avoid contamination of testing area and other samples. After evaluating each sample, panellists were required to leave the testing area to an external environment for a period of 10 min to avoid sensory fatigue.

Quantitative Gas Chromatography-Mass Spectrometry Analysis

Grape, wine and leaf samples were analysed for key smoke indicator compounds of guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, furfural, 5-methylfurfural, eugenol and vanillin by gas chromatography-mass spectrometry by stable isotope dilution assay methods (described by Spillman et al. 1997, Pollnitz 2000, Pollnitz et al. 2000, Pollnitz et al. 2004, Kennison et al. 2008a). Compounds were selected for analysis based on their known presence in smoke (Maga 1988) and contribution to smoke aromas in smoked foodstuffs and barrel aged wine (Baltes et al. 1981, Boidron et al. 1988).

Statistical Analysis

All data was analysed by two-way analysis of variance (ANOVA) using GenStat (9th Edition, VSN International Limited, Herts, UK). Wine sensory data was analysed by ANOVA and principal component analysis (PCA). Mean comparisons were performed by least significant difference (LSD) multiple comparison tests at $P < 0.05$. Wine data from the phenological smoke application experiments was analysed by the residual maximum likelihood (REML) procedure that was utilised to fit a Linear Mixed Model (Fixed effect = smoke treatment).

Results and Discussion

Smoke Application to Grape Bunches

Initial experimental studies that applied smoke to grape bunches alone demonstrate the direct link between smoke exposure and the development of smoke taint in grapes and wine (Kennison et al. 2007). Smoke exposure was shown to directly influence the chemical composition and sensory characteristics of wine leading to an apparent ‘smoke taint’. Wine made from smoked grape bunches by fermentation of (i) free-run juice and (ii) free-run juice on skins showed elevated levels of key smoke indicator compounds guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, eugenol and furfural (Table 1). In this case, the presence of these chemical compounds in wine is directly attributed to the application of smoke as these compounds were not present in wines made from unsmoked (control) fruit. 5-Methylfurfural and vanillin were not detected in any of the Verdelho wines (smoked and unsmoked) in this study.

A variation in the quantity of smoke-like compounds between winemaking treatments was evident with fermentation of free-run juice containing 1470 µg/L of guaiacol and free-run juice on skins fermentation containing 969 µg/L of guaiacol (Table 1). The fermentation of free-run juice on skins was expected to contain higher levels of smoke-derived compounds as previous studies have indicated smoke-derived guaiacol and 4-methylguaiacol to accumulate in the skins of grapes (AWRI 2003). This is not the case in our study which shows the different fermentation conditions for free-run juice and free-run juice on skins to influence compound release and accumulation in wine. Regardless, the enhanced levels of smoke-derived compounds are evident in all wines made from smoked grape bunches and were not detected in wines made from unsmoked (control) fruit.

Table 1. Concentrations of guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, eugenol, furfural, 5-methylfurfural and vanillin detected in smoked and unsmoked wines made from the fermentation of Verdelho free-run juice and free-run juice on skins (from Kennison et al. 2007).

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

Values followed by a different letter within rows are significantly different. n.d. = not detected. Mean values are from three replicates ($P \leq 0.05$).

The effects of smoke exposure to grape bunches were further evident in the rate of wine fermentation. The fermentation of free-run juice from unsmoked grapes was completed in 22 days whereas the fermentation of free-run juice from smoked grapes was completed in 13 days. This phenomenon was not reproduced in the fermentation of smoked and unsmoked free-run juice on skins that were both completed in 4 days. Although the subject of ongoing study, the increase in fermentation rate may be a result of smoke compounds causing damage to the membrane integrity of grape berries and skin. As such cellular enzymes associated with an injury response may have been released resulting in the increased rate of fermentation.

With all grapes harvested on the same day at a TSS of 24 ± 0.5 ^{o}Brix a variation between ethanol concentrations in final wines was present with smoked and unsmoked wines respectively containing 14.3 and 14.1% ethanol when fermented from free-run juice and 14.7 and 13.8% ethanol when fermented from free-run juice on skins. The higher ethanol content of smoked wines demonstrated the higher attenuation of sugars to ethanol during the fermentation process. Furthermore, as expected, the level of brown pigments in the colour of smoked wines was higher in wine made from smoked fruit (0.097 au free-run to 0.203 au free-run on skins) than wines made from unsmoked fruit (0.06 au free run to 0.142 free-run on skins) with fermentation on skins further increasing this level (Kennison et al. 2007).

A clear difference between the aroma of wine made from smoked and unsmoked Verdelho fruit was established by sensory analysis. Panellists participating in sensory analysis difference tests were able to correctly detect the difference between smoked and unsmoked wines from the fermentation of both free-run juice and free-run juice on skins treatments at the 99.9% confidence level. Due to the clear and notable difference between smoked and unsmoked wines, further sensory analysis was conducted to determine the aroma detection threshold of smoke taint in wine and the subsequent potential for reduction of this taint via blending.

The best-estimate aroma detection threshold of smoke taint in wine was determined by the presentation of smoked wine diluted (to 250 mL) with unsmoked (control) wine. The aroma thresholds were calculated to be 3.9 mL for the smoked free-run wine and 1.9 mL for the smoked free-run on skins wine which corresponds to dilutions of 1.6 and 0.8% of original concentration, respectively (Kennison et al. 2007) (Figure 5). Due to the high level of unsmoked wine required to dilute smoke effected wine the potential for blending is greatly reduced.

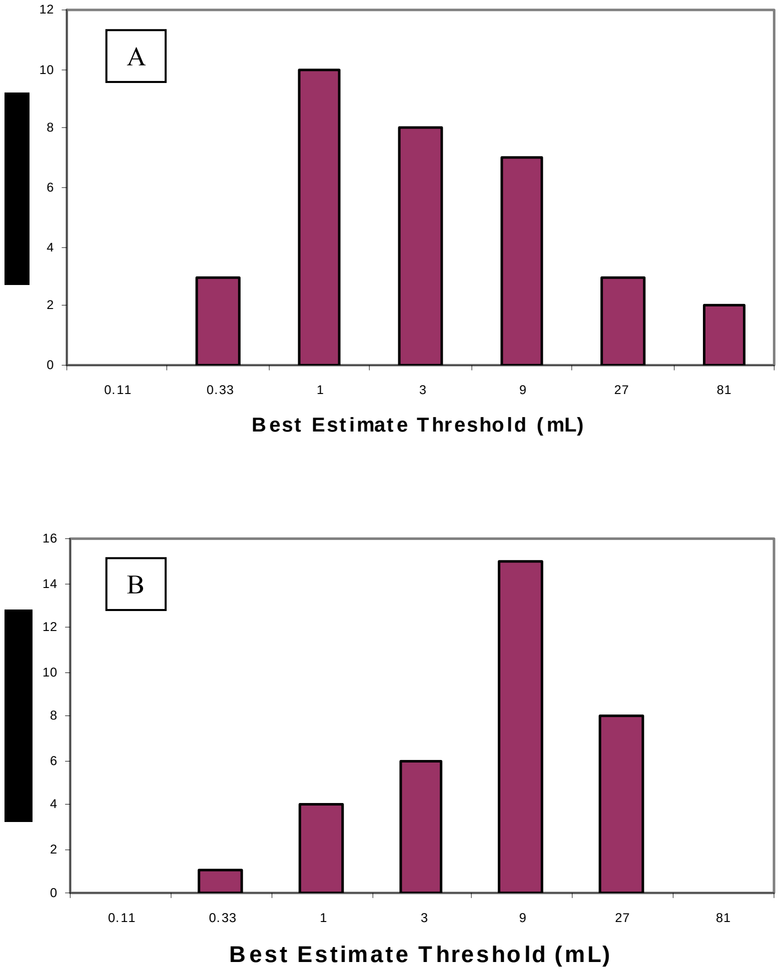

Figure 5. Best-estimate detection threshold distributions for smoke aroma in wine from smoked grape fermentation of (A) free-run juice on skins and (B) free-run juice (from Kennison et al. 2007).

Panellist determination of the best-estimate aroma detection thresholds corresponded to guaiacol concentrations of 23 and 7 µg/L, and 4-methylguaiacol concentrations of 5 and 2 µg/L for the smoked free-run and smoked free-run on skins wines respectively. Conjecture regarding the aroma detection threshold for guaiacol in white wine exists with Boidron et al. (1988) stating the concentration to be 95 µg/L whilst Simpson et al. (1986) states the concentration to be 20 µg/L. Boidron et al. (1988) further states the aroma detection threshold for guaiacol in red wine to be 75 µg/L and the aroma detection threshold for 4-methylguaiacol to be 65 µg/L for both red and white wines. Generally, the aroma detection thresholds for guaiacol and 4-methylguaiacol for the smoked free-run and free-run on skins wines in this study are below published thresholds therefore concluding that neither is solely responsible for the sensory smoke taint. Guaiacol and 4-methylguaiacol have been effective compounds for the detection of smoke taint in this study although smoke taint is not limited to the compounds investigated in this study.

Smoke Application to Field-Grown Grapevines

1. Effect of smoke timing

As described in the methodology, single smoke applications were applied to field-grown grapevines at a range of grapevine growth stages over the three years of the trial. Smoke applications were applied to investigate the critical timing of grapevine susceptibility to smoke uptake. Over the period of the trial, single applications of smoke were applied to grapevines at the following stages of growth and development: E-L stage 12 (shoots 10cm), E-L 23 (flowering), E-L 31 (berries pea size), E-L 32 (beginning of bunch closure), E-L 35(a) (veraison), E-L 35(b) (3 d post veraison), E-L 35(c) (7 d post veraison), E-L 35(d) (10 d post veraison), E-L 36(a) (15 d post veraison / intermediate sugar), E-L 36(b) (18 d post veraison / intermediate sugar), E-L 37 (berries not quite ripe) and E-L 38 (harvest).

1.1 Effect on yield parameters

Low levels of significant effects and interactions were found in the fruit yield, average bunch weight and average number of bunches for vines subject to a single smoke application over the three year trial period. An example of yield parameters from smoke application to a range of grapevine phenological stages has been presented in Table 2. Fruit yield (at harvest) ranged from a high of 15.9 kg/treatment from vines that received a smoke application at E-L 31 (berries pea size) to a low of 10.9 kg/treatment from vines exposed to smoke at E-L 32 (bunch closure). Average bunch weight followed similar trends with a high of 163.5 g/treatment from a smoke application at E-L 31 (berries pea size) to a low of 110.4 g/treatment from a smoke application at E-L 32 (bunch closure). Smoke application did not decrease yield or average bunch weight when applied post bunch closure, nor did smoke adversely impact fruit set and production when applied to vines at flowering.

Table 2. Fruit yield, average bunch weight and average bunch number measured at harvest from grapevines exposed to a single smoke application at E-L 12 (shoots 10 cm), E-L 23 (flowering), E-L 31 (berries pea size), E-L 32 (bunch closure), E-L 35 (veraison), E-L 37 (berries full colour) and E-L 38 (harvest).

Treatment	Yield (kg)	Ave bunch weight (g)	Average bunch No.
Control	12.7^{ab}	113.3^{bc}	117^{ab}
Smoke applied to vines at E-L stage:			
12	14.9^{ab}	125.4^{b}	120^{a}
23	12.6^{ab}	136.5^{ab}	92^{bcd}
31	15.9^{a}	163.5^{a}	99^{abcd}
32	10.9^{b}	110.4^{bc}	99^{abcd}
35	11.9^{ab}	135.1^{ab}	88^{cd}
37	12.7^{ab}	113.4^{bc}	109^{abc}
38	12.8^{ab}	130.6^{ab}	97^{abcd}

Means followed by the same letter within columns are not significantly different at $P \leq 0.05$, results are per treatment replicate = 3 vines, mean values are from 3 replicates.

1.2 Compounds detected in wine

The timing of smoke application to field-grown grapevines influenced the levels of smoke taint marker compounds detected in resultant wines. Initial trial results from a single smoke application to field grown grapevines commencing from the growth stage of E-L 35 (veraison) through to E-L 38 (harvest), showed detectable levels of guaiacol, 4-methylguaiacol, 4-ethylphenol and 4-ethylguaiacol in all resultant wines (Figure 5) (Kennison et al. 2009b).

Interestingly a peak in vine susceptibility to smoke was detected at E-L 35(c) (7 days post veraison) notable by the rapid increase of smoke compounds detected in smoke wines in comparison to control wines being 91.7 and 6.7 µg/L guaiacol, 22 and 0 µg/L 4-methylguaiacol, 15.3 and 2 µg/L 4-ethylphenol and 22 and 0 µg/L 4-ethylguaiacol, respectively. The reasons for the increase in smoke compounds in wines from the E-L 35(c) treatment is uncertain although the onset of veraison signals changes in berry physiology, sugar uptake, metabolism (Conde et al. 2007) and changes in grape cell walls (Nunan et al. 1998) that may contribute to this phenomena (Kennison et al. 2009b).

Smoke application to grapevines from E-L 35(d) (10 days post veraison) to E-L 38 (harvest) did not produce the high smoke compound levels that were detected in wines made from the E-L 35(c) smoke treatment (Figure 6). Although, in comparison to the control treatment, compound levels were significantly high from E-L 35(d) (10 days post veraison) onwards signalling high susceptibility to smoke uptake during this time.

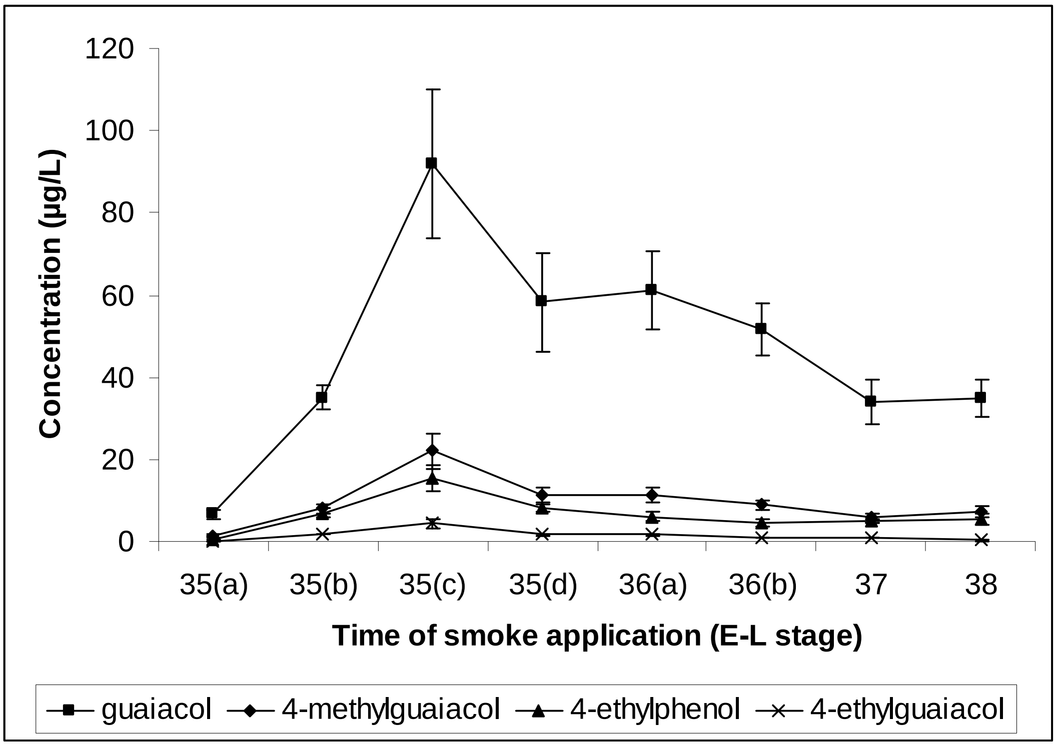

Figure 6. Guaiacol, 4-methylguaiacol, 4-ethylphenol and 4-ethylguaiacol concentrations in wine produced of grapes grown on vines subjected to a single smoke application at either E-L 35(a) (veraison), E-L 35(b) (3 d post veraison), E-L 35(c) (7 d post veraison), E-L 35(d) (10 d post veraison), E-L 36(a) (15 d post veraison / intermediate sugar), E-L 36(b) (18 d post veraison / intermediate sugar), E-L 37 (berries not quite ripe) or at E-L 38 (harvest) (from Kennison et al. 2009b). Data are means n = 3, error bars show 2 standard errors of the mean and may be obscured by symbols.

Additional single smoke applications were applied to field-grown grapevines in order to investigate the effects of smoke on a broader range of grapevine phenological stages in 2006-07 and again in 2007-08. In addition to the ongoing investigations of smoke treatment to vines from the period of E-L 35 (veraison) to E-L 38 (harvest), single smoke applications were also applied to vines either at E-L 12 (shoots 10 cm), E-L 23 (flowering), E-L 31 (berries pea size) or E-L 32 (beginning of bunch closure).

The analysis of this data from 2005 to 2008 is provided in Figure 7. The results clearly shows three distinct periods of vine response to the uptake of smoke compounds (Kennison et al. 2009a). Period 1 (P1) signals a period of low susceptibility to smoke uptake. During P1 no difference in the levels of guaiacol and 4-methylguaiacol concentration in control (unsmoked) wines to those made from fruit of grapevines exposed to a single application of smoke at E-L 12 (shoots 10 cm) and E-L 23 (flowering) can be established. Period 2 (P2) representing smoke application to grapevines from E-L 31 (berries pea size) to E-L 35(b) (3 d post veraison) resulted in a variable level of smoke uptake and accumulation in resultant wines. Heightened levels of guaiacol and 4-methylguaiacol were observed in wines made from grapes from vines exposed to smoke from E-L 35(c) (7 d post veraison) through to E-L 38 (harvest) as represented by Period 3 (P3) which demonstrates a peak period of sensitivity for smoke uptake.

Boidron et al. (1988) reports the aroma detection threshold for guaiacol and 4-methylguaiacol in red wine to be 75 and 65 µg/L respectively, 95 and 65 µg/L in white wine with lower detection limits for these compounds in water (5.5 and 10 µg/L) and model wine (20 and 30 µg/L). In this instance the detected concentrations of guaiacol and 4-methylguaiacol are below the published aroma detection threshold for all wines except for wines made from grapes of vines exposed to a single smoke application at E-L 35(c) (7 d post veraison) containing 91.7 µg/L guaiacol. Also, concentrations of 4-ethylguaiacol and 4-ethylphenol are well below the published aroma detection thresholds for all wines mentioned in this study (Boidron et al. 1988, Chatonnet et al. 1992). To clarify this discussion, further analysis of wine sensory attributes is provided in the following section.

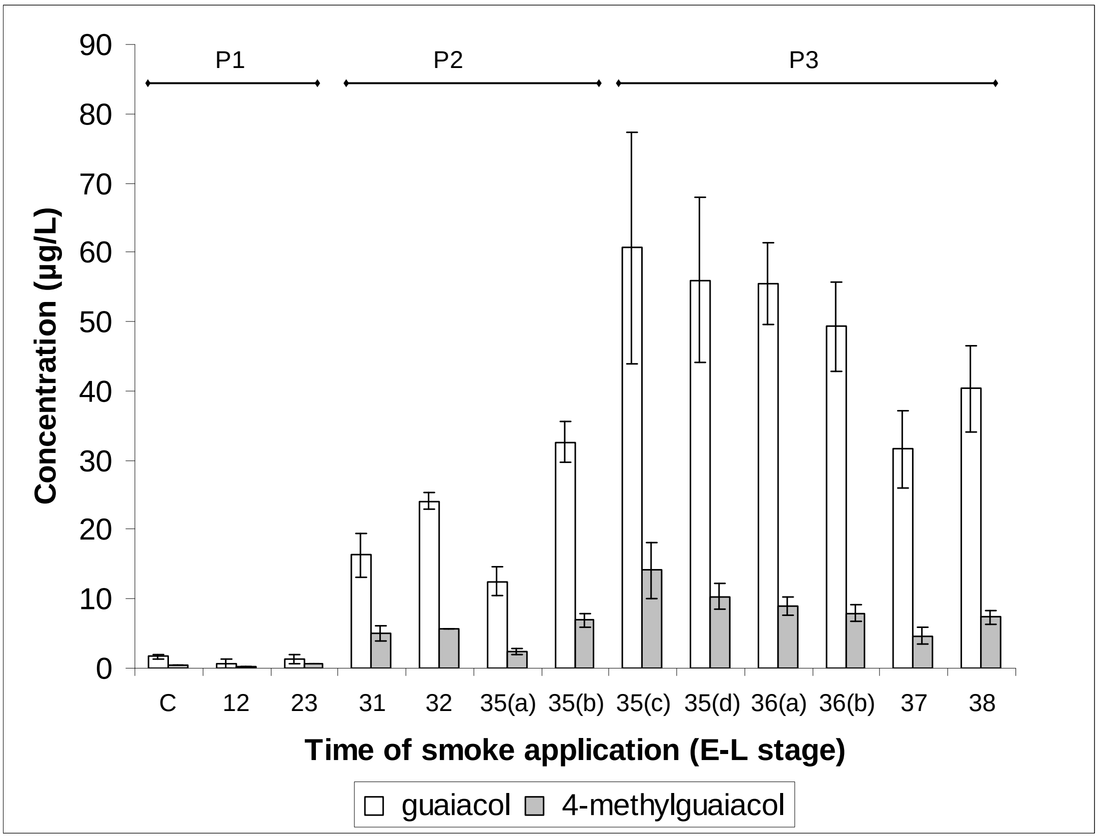

Figure 7. REML predicted means and standard errors of guaiacol and 4-methylguaiacol concentration in wine made of fruit from vines exposed to a single smoke application. Data are means from 2006 to 2008. Time of smoke application is indicated by E-L 12 (shoots 10 cm), E-L 23 (flowering), E-L 31 (berries pea size), E-L 32 (beginning of bunch closure), E-L 35(a) (veraison), E-L 35(b) (3 d post veraison), E-L 35(c) (7 d post veraison), E-L 35(d) (10 d post veraison), E-L 36(a) (15 d post veraison / intermediate sugar), E-L 36(b) (18 d post veraison / intermediate sugar), E-L 37 (berries not quite ripe), and E-L 38 (harvest). C = control, n = 3 to 9, error bars show 2 standard errors of the mean. Three separate periods of vine sensitivity to smoke are represented by P1 (low), P2 (variable) and P3 (high) (Kennison et al. 2009a).

1.3 Wine sensory analysis

Quantitative descriptive analysis (QDA) of wine aroma was conducted on all wines that were made from fruit of vines exposed to single smoke applications. Panellists were exposed to trial wines during QDA training, identified and agreed on the aromas to be used for the sensory evaluation. Aromas used in the study are the smoke-like aromas of ‘burnt rubber’, ‘smoked meat’, ‘leather’, ‘disinfectant / hospital’ and the wine aromas of ‘red berry fruits’ and ‘confection’. Panellists were trained to rank aroma intensity of all aromas on an unstructured line scale.

Analysis of variance showed both the wines and panellists to be sources of variation for all aroma attributes except for the wine aroma attribute of ‘confection’ (Table 3). Panellists showed consistency in their rating between sessions and wines however, once again, variation was present in the wine by replicate attribute of ‘confection’. Sources of variation existed in the wine by panellist interactions for the aromas of ‘burnt rubber’ ($P < 0.01$), ‘leather’ ($P < 0.05$) and ‘disinfectant / hospital’ ($P < 0.05$). Sources of variation in the aforementioned aroma attributes can indicate variations in panellist sensitivity to these aromas.

Table 3. Analysis of variance for wine sensory attribute ratings of ‘burnt rubber’, ‘smoked meat’, ‘leather’, ‘disinfectant / hospital’, ‘red berry fruits’, and ‘confection’ for wine (W), panellist (P), replicate (R), wine by panellist (W x P), panellist by replicate (P x R) and wine by replicate (W x R).

Aroma Descriptor	**Wine (W)**	**Panellist (P)**	**Rep (R)**	**W x P**	**P x R**	**W x R**
Burnt Rubber	4.922***	4***	2.133	2.21**	0.81	1.15
Smoked Meat	5.844***	2.994***	0.08	1.36	0.65	1.84
Leather	2.342*	3.747***	1.052	1.78*	0.33	1.31
Disinfectant/hospital	2.455*	9.265***	0.881	1.89*	0.66	0.53
Red Berry Fruits	2.399*	7.236***	1.813	0.97	1.35	1.45
Confection	1.812	8.161***	3.358	0.73	1.2	2.12*

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

Principal component analysis (PCA) was utilised to generate a biplot to describe the interrelationship of wines from single smoke treatments and their relationship to aroma attributes (Figure 8). The PCA displays the wines made in this study to encompass the range of wine-like and smoke-like aromas with the overall variation predominately accounted by Principal Component 1 (PC1) of 88.5% and to a lesser degree Principal Component 2 (PC2) accounting for 5.9% of variation. As discovered by Kennison et al. (2009b), PC1 is characterised by positive loadings on the smoke-like aromas of ‘burnt rubber’ (0.42), ‘smoked meat’ (0.41), ‘leather’ (0.41), ‘disinfectant / hospital’ (0.4) contrasted with the negative loadings on wine-like aromas of ‘red berry fruits’ (-0.41) and ‘confection’ (-0.4) (Table 4). PC2 is defined by a positive loading on the aroma attribute of ‘disinfectant / hospital’ with negative loadings on all other aroma attributes.

The placement and direction of arrows on the PCA biplot reveals the smoke-like aromas of ‘burnt rubber’, ‘smoked meat’, ‘leather’ and ‘disinfectant / hospital’ to be positively correlated ($r = 0.85$ to 0.98) (Figure 8). The aromas of ‘leather’ and ‘smoked meat’ have a high correlation ($r = 0.97$) that could potentially result in the reduction of one of these aromas for future sensory studies. The wine-like aromas of ‘confection’ and ‘red berry fruits’ are also highly correlated ($r = 0.96$). A strong negative correlation, displaying the opposing relationship between the wine-like aromas and the smoke-like aromas, exists ($r = -0.86$ to -0.91) and is evident by the converse positioning of attribute arrows.

Wines plotted on the PCA biplot encompass the broad range of aroma descriptors with their relative positioning in relation to aroma descriptors also providing insight into their character (Figure 8). Wines made from fruit of vines that received a single smoke application at E-L 12 (shoots 10 cm) and the unsmoked control (C) wine had elevated aromas of ‘confection’ and ‘red berry fruits’. Wines made from fruit of vines exposed to smoke at E-L 23 (flowering) displayed ‘confection’ and ‘red berry fruit’ aromas to a lesser degree. Whereas wines produced from vines that received a single smoke application at E-L 35(b) (3 d post veraison), E-L 35(c) (7 d post veraison), E-L 35(d) (10 d post veraison) and E-L 36(a) (15 d post veraison, intermediate sugar) exhibit elevated smoke-like aromas of ‘disinfectant / hospital’, ‘leather’, ‘burnt rubber’ and ‘smoked meat’ and low levels of wine-like aromas.

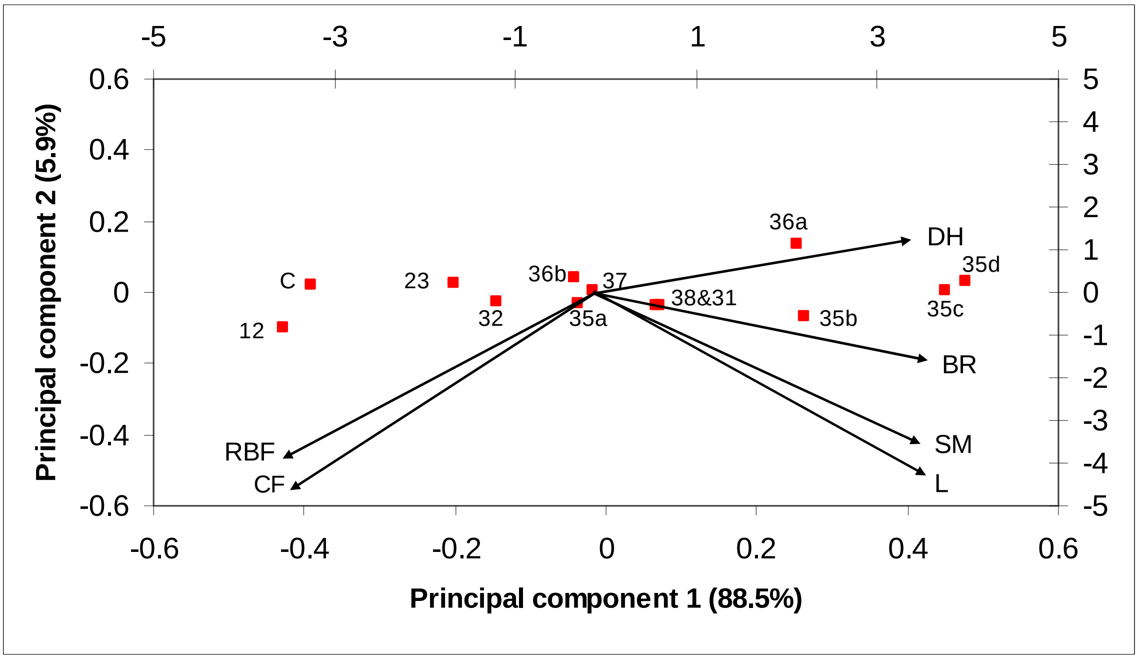

Figure 8. PCA biplot of mean wine sensory scores of wines made from fruit of vines exposed to single smoke applications. Treatments are indicated by symbols (■) and refer to E-L stages of 12 (shoots 10 cm), 23 (flowering), 31 (berries pea size), 32 (beginning of bunch closure), 35(a) (veraison), 35(b) (3 d post veraison), 35(c) (7 d post veraison), 35(d) (10 d post veraison), 36(a) (15 d post veraison / intermediate sugar), 36(b) (18 d post veraison / intermediate sugar), 37 (berries not quite ripe) and 38 (harvest). C = control (unsmoked). Aroma descriptors are indicated by arrows labelled BR ('burnt rubber'), SM ('smoked meat'), L ('leather'), DH ('disinfectant / hospital'), RBF ('red berry fruits') and CF ('confection') (derived from Kennison et al. 2009a and 2009b).

Table 4. Factor loadings for PC1 and PC2 for aroma descriptors of 'burnt rubber', 'smoked meat', 'leather', 'disinfectant / hospital', 'red berry fruits' and 'confection' for all wines produced from a single smoke application applied throughout the grapevine growth period (from Kennison et al. 2009b).

Aroma Descriptor	PC1	PC2
Burnt rubber	0.42	-0.19
Smoked meat	0.41	-0.43
Leather	0.41	-0.51
Disinfectant / hospital	0.40	0.15
Red berry fruits	-0.41	-0.45
Confection	-0.40	-0.55

As the determination of field-based smoke exposure timing is critical, further information detailing the contribution of individual aroma descriptors to each wine from a smoke application to grapevines at each developmental stage has been provided in Figure 9 (Kennison et al. 2009a). Aroma responses indicate a peak period of vine susceptibility to smoke uptake in the detection of smoke-like aromas of 'disinfectant / hospital', 'smoked meat', 'burnt rubber' and 'leather' in wines made from fruit of vines exposed to smoke at E-L 35(b) (3 d post veraison), 35(c) (7 d post veraison) and 35(d) (10 d post veraison). However, it is important to note that particular smoke aromas are notable in wines made from fruit of grapes exposed to smoke at other growth stages. For instance, when compared to the control (unsmoked) treatment, all wines made from fruit of vines exposed to smoke from E-L 31 (berries pea size) through to E-L 38 (harvest) clearly exhibit elevated smoke-like aromas of 'burnt rubber', 'smoked meat' and 'disinfectant / hospital'. Similarly, these same wines showed a reduction of 'red berry fruit' and 'confection' aromas. The overpowering nature of the smoke aromas in wines are noted, with these aromas present in wines made from fruit of vines exposed to smoke application from E-L 31 (berries pea size) through to E-L 38 (harvest).

In this study, smoke aromas were detected in wines that contained concentrations of key smoke compounds below the published aroma detection thresholds. Aroma detection thresholds have been published for guaiacol and 4-methylguaiacol alone in wine (Boidron et al, 1988). The majority of compound concentrations detected in smoked wines from this trial were below levels previously published except for wines made from fruit of vines exposed to smoke at E-L 35(c) (7 d post veraison) that contained guaiacol above the reported detection threshold. As expected, this wine did display elevated smoke-aromas of 'disinfectant / hospital', 'burnt rubber', 'smoked meat' and 'leather'. However, with the exception of control (unsmoked) wines and those wines produced from fruit of vines exposed to smoke at E-L 12 and E-L 23, wines produced from vines that received a smoke application at all other times produced noticeable smoke aromas whilst containing compound concentrations below reported aroma detection thresholds.

It is important to note that published aroma detection thresholds concentrate on the aroma of a single compound only and not the aromas potentially generated from the complexity of compounds that are present in smoke. A complexity of smoke compounds in wine could perceivably result in different wine aromas to those generated from one compound alone. This can therefore result in the detection of smoke-like aromas in wines even when they contain guaiacol and 4-methylguaiacol below published aroma detection thresholds. For the purpose of this study, guaiacol and 4-methylguaiacol have been effective markers for the detection of smoke taint in wine although it is important that further research is conducted to investigate the range of smoke-like compounds attributable to smoke taint.

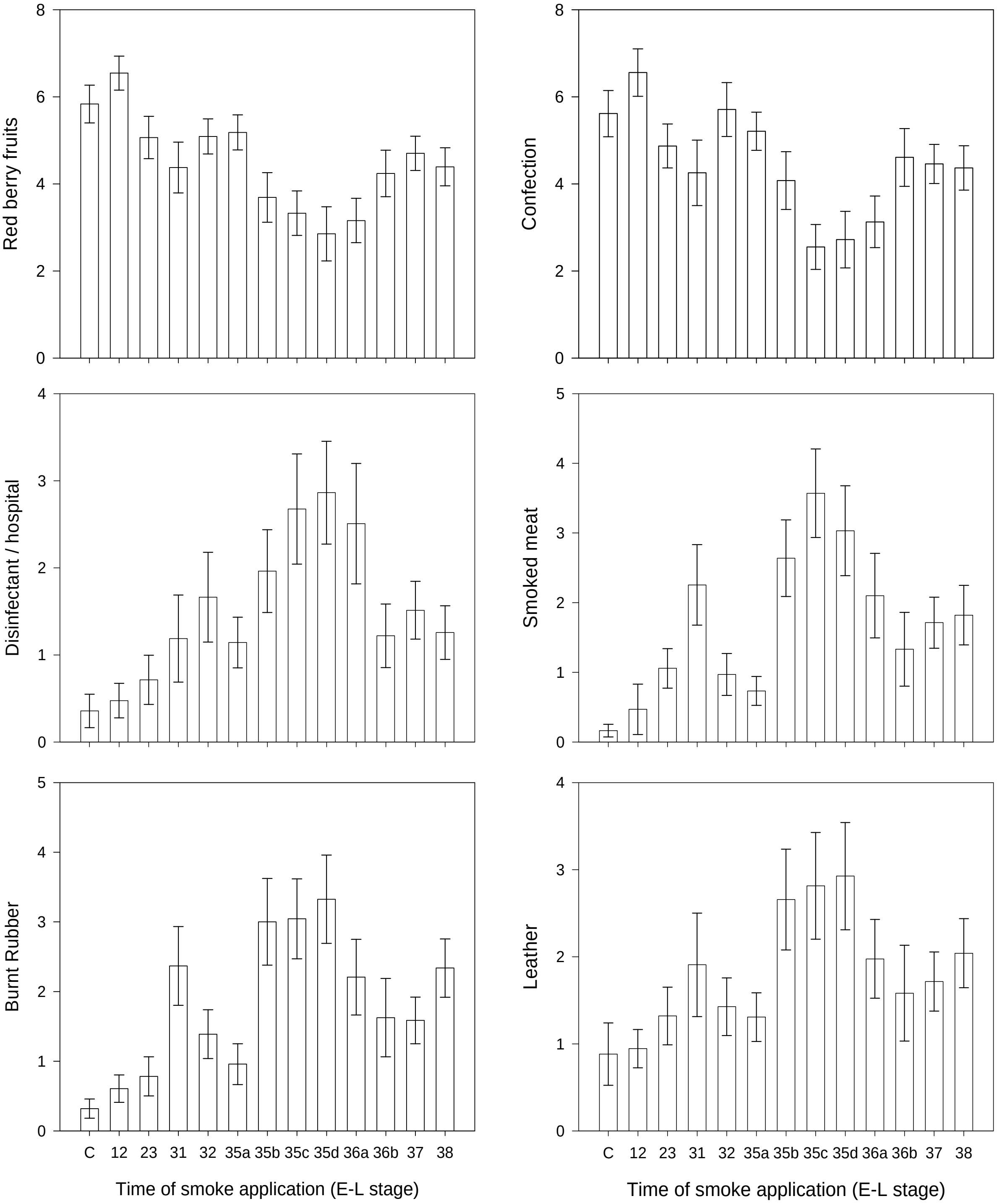

Figure 9. Aroma descriptor scores of ‘burnt rubber’, ‘smoked meat’, ‘leather’, ‘disinfectant / hospital’, ‘red berry fruits’ and ‘confection’ detected in wines made from grapes of vines exposed to a single smoke application at either E-L stage 12 (shoots 10 cm), 23 (flowering), 31 (berries pea size), 32 (beginning of bunch closure), 35(a) (veraison), 35(b) (3 d post veraison), 35(c) (7 d post veraison), 35(d) (10 d post veraison), 36(a) (15 d post veraison / intermediate sugar), 36(b) (18 d post veraison / intermediate sugar), 37 (berries not quite ripe) or 38 (harvest). Error bars indicate 2 standard errors of the mean (figure from Kennison et al. 2009a).

2. Effect of smoke duration

As outlined in the methodology, the influence that many smoke exposures can impart on wine was investigated by the application of smoke to the same field-grown grapevines on numerous (8) occasions. Smoke was applied to the same vines at E-L 35 (veraison), E-L 35 + 3 days (3 days post veraison), E-L 35 + 7 days (7 days post veraison), E-L 35 + 10 days (10 days post veraison), E-L 36 (intermediate sugar), E-L 36 + 3 days (3 days post intermediate sugar), E-L 37 (berries not quite ripe) and at E-L 38 (harvest). Wine was made from fruit of vines exposed to numerous smoke exposures and evaluated for chemical and sensory characteristics.

2.1 Effect on vine growth and fruit production

Repeated smoke applications to grapevines resulted in the reduction of fruit yield and alteration of fruit composition at harvest. With smoke treatments encompassing 3 vines, the average yield from the control (unsmoked) vines was 17 kg/treatment. This is significantly higher than vines subjected to repeated smoke applications that resulted in a yield of 11 kg/treatment. Average bunch weight was also decreased by repeated smoke applications with smoked vines yielding an average bunch weight of 84 g, and control (unsmoked) vines yielding a much higher average bunch weight of 146 g. Interestingly, no significant difference between the average number of bunches harvested from the smoked and control treatments existed (average of 129 and 122 bunches/treatment respectively). Therefore, whilst repeated smoke applications did not reduce the bunch numbers per vine, in comparison to the control vines, the smoked vine’s ability to accumulate fruit weight was certainly reduced by smoke treatment.

Furthermore, repeated smoke applications to grapevines resulted in the reduction of total soluble solids (TSS) content in fruit at harvest. Fruit from all treatments was harvested on the same day with fruit from vines exposed to repeated smoke applications averaging a TSS of 19.3 ^{o}Brix at harvest, compared to the control (unsmoked) treatment averaging TSS content of 22.3 ^{o}Brix (Kennison et al. 2009b). Therefore, the grapevines capacity to ripen fruit was decreased due to numerous smoke applications. The reduction of fruit ripening capacity may be explained by the potential reduction of photosynthetic capacity of those vines repeatedly exposed to smoke. This reduction in photosynthetic capacity is further hypothesised due to the visual evidence of necrotic lesions that developed on leaves of vines repeatedly exposed to smoke (Figure 10). Necrotic lesions on leaves reduced the vine photosynthetic capacity and therefore the accumulation of sugar in the fruit. Such lesions were only present, at harvest, on those vines repeatedly exposed to smoke, and were not present on control (unsmoked) vines or those vines that were exposed to a single smoke application only. As such, necrotic lesions on leaves are directly attributable to repeated smoke exposures.

Figure 10. Necrotic lesions evident on leaves exposed to 8 repeated smoke applications (left) in comparison to vines that were not exposed to smoke with undamaged leaves (right). Photos were taken at harvest.

Interestingly, repeated smoke exposures to grapevines resulted in fruit from these vines containing a higher Free Amino Nitrogen (FAN) content at harvest (Kennison et al. 2009b). In comparison to fruit from control (unsmoked) vines that had a FAN content of 87 mg/L, fruit from vines exposed to repeated smoke applications contained a FAN content of 134 mg/L. The fermentation rate of must from grapes produced from repeatedly smoke vines (with increased levels of FAN) was faster with completing fermentation in 8 days in comparison to musts produced from fruit of control (unsmoked) vines (with lower FAN content) that completed fermentation in 12 days. It is unclear as to what caused the increase in FAN in fruit from repeatedly smoked vines although it may be a result of an injury response (Heath 1980) that was evident from the necrotic lesions that developed on leaf blades.

2.2 Compounds detected in wine

Repeated smoke applications to field-grown grapevines resulted in the accumulation of smoke compounds in subsequent wines (Kennison et al. 2008b, 2009b). Repeated smoke exposures applied to grapevines at E-L 35 (veraison), E-L 35 + 3 days (3 days post veraison), E-L 35 + 7 days (7 days post veraison), E-L 35 + 10 days (10 days post veraison), E-L 36 (intermediate sugar), E-L 36 + 3 days (3 days post intermediate sugar), E-L 37 (berries not quite ripe) and at E-L 38 (harvest) resulted in high concentrations of guaiacol (388 µg/L), 4-ethylguaiacol (16 µg/L), 4-ethylphenol (58 µg/L) and 4-methylguaiacol (93 µg/L) in resultant wines (Table 5). The levels of compounds detected in these wines were extreme in comparison to wines made from fruit exposed to a single smoke application that resulted in an maximum compound concentration in any one wine of 91.7 µg/L guaiacol, 4.3 µg/L 4-ethylguaicol, 15.3 µg/L 4-ethylphenol and 22 µg/L 4-methylguaiacol (resulting from a single smoke application at E-L 35(c)). In fact, when the number of compounds detected in all wines made from fruit of vines exposed to a single smoke application are added together, the total compound concentration is comparable to the level detected in wine made from the repeated smoke treatment (Table 5). Therefore the accumulative nature of smoke exposure on the increase in phenol compound concentration in resultant wines is demonstrated. This phenomenon has implications for understanding the potential effect that many smoke exposures, or smoke exposures for long durations, may have on resultant wines.

Table 5. Sum of concentration of guaiacol, 4-ethylguaiacol, 4-ethylphenol and 4-methylguaiacol detected in all 8 wines made from fruit of vines exposed to single smoke applications and mean concentration of compounds detected in wines subject to repeated smoke applications (from Kennison et al. 2008b, 2009b).

Treatment	**Concentration (µg/L) of**			
	guaiacol	**4-ethyl guaiacol**	**4-ethyl phenol**	**4-methyl guaiacol**
sum of (8) single smoke applications	373	12	52	76
repeated smoke application	388	16	58	93
Control	0	0	0	0

As expected, the compound concentration detected in wines made from grapes of vines exposed to repeated smoke treatments are well above the lowest published aroma detection thresholds for red wine. Boidron et al. (1988) state the lowest aroma detection threshold for guaiacol in red wine of 75 µg/L and 4-methylguaiacol of 65 µg/L, with Chatonnet et al. (1992) reporting the lowest detection threshold for 4-ethylguaiacol and 4-ethylphenol in red wine of 110 µg/L and 605 µg/L respectively. Concentrations of these volatile phenols in wines from the repeated smoke treatment are well above these thresholds which indicate the high attenuation of smoke compounds from repeated smoke exposures. The potential for the detection of smoke-like aromas in these wines is also high.

2.3 Wine sensory analysis

Smoke-like characters in wines made from fruit of vines exposed to repeat smoke applications were extreme and far overpowered the wine-like aromas of control (unsmoked) wines. Quantitative descriptive analysis (QDA) by a trained sensory panel was utilised to isolate smoke-like aroma descriptors of ‘burnt rubber’, ‘smoked meat’, ‘leather’, ‘disinfectant/hospital’ and the wine-like aroma descriptors of ‘confection’ and ‘red berry fruits’ (as outlined in the methodology). Sensory panellists were trained to rate the intensity of these aromas (reported as 0 = non detectable aroma to 8 = highly detectable aroma) and demonstrate wines made of grapes from vines exposed to repeated smoke applications to have elevated aromas of ‘burnt rubber’ (7.5), ‘smoked meat’ (7.8), ‘leather’ (6.2), ‘disinfectant / hospital’ (5.2) and low aromas of ‘confection’ (0.8) and ‘red berry fruits’ (0.6) (Figure 11) (Kennison et al. 2009b). This was in stark contrast to wines made from fruit of vines from the control (unsmoked) treatment that had elevated aromas of ‘confection’ (7.7) and ‘red berry fruits’ (7.0) with limited detection of any smoke-like aromas.

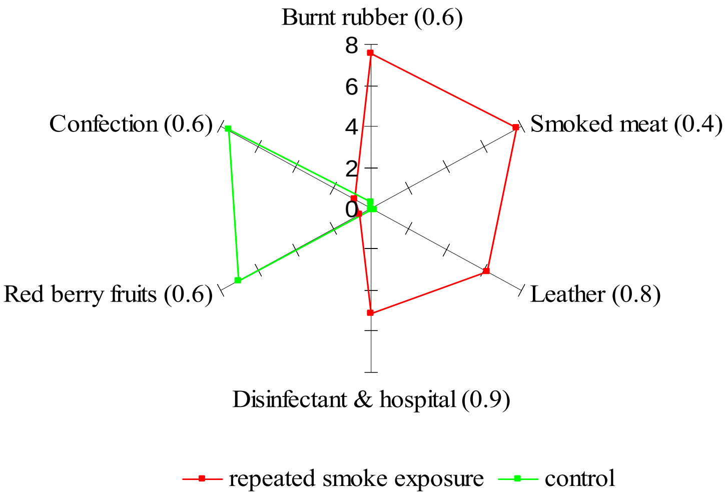

Figure 11. Mean intensity ratings of smoke-like aromas of 'burnt rubber', 'smoked meat', 'leather', 'disinfectant / hospital' and wine-like aromas of 'confection' and 'red berry fruits' in wines made from grapes of vines exposed to 8 repeated smoke exposures (from the period of veraison to harvest) and wines made from fruit of control (unsmoked) grapevines (from Kennison et al. 2009b). Scale represents 0 = non detectable aroma to 8 = highly detectable aroma.

3. *Release of compounds throughout fermentation*

Interestingly, throughout this study the concentration of guaiacol detected in grapes at harvest did not equate to the concentration of guaiacol in the final wine. Guaiacol in grapes showed a positive correlation for guaiacol in wine (r = 0.64) although it was difficult to postulate the level of guaiacol in final wines from the levels present in grapes. Guaiacol was measured in grapes (at harvest) and final wines made from fruit of vines subjected to a single smoke application at either E-L 12 (shoots 10 cm), E-L 23 (flowering), E-L 31 (berries pea size), E-L 32 (beginning of bunch closure), E-L 35 (veraison), E-L 37 (berries not quite ripe) or E-L 38 (harvest) (Figure 12). The concentration of guaiacol in both grapes and wine did not differ from a smoke exposure at E-L 12, although guaiacol concentrations were far less in grapes than in final wines from all other stages of smoke application. The greatest difference was notable from a single smoke application at E-L 37 (berries not quite ripe) that produced a 21-fold increase of guaiacol from the concentration detected in fruit (2.6 μg/L) to resultant wine (54.7 μg/L). A single smoke application at other growth stages presented various degrees of guaiacol increase from fruit to wine including a 7-fold increase at E-L 31, 8-fold increase at E-L 32, 8.5-fold increase at E-L 35 and only a 2-fold increase at E-L 38. Many winemaking factors could contribute to the increase of compounds throughout fermentation (including extraction from skin) although as the detection of guaiacol in grapes did not relate to the final guaiacol concentration in wine, further investigation was warranted.

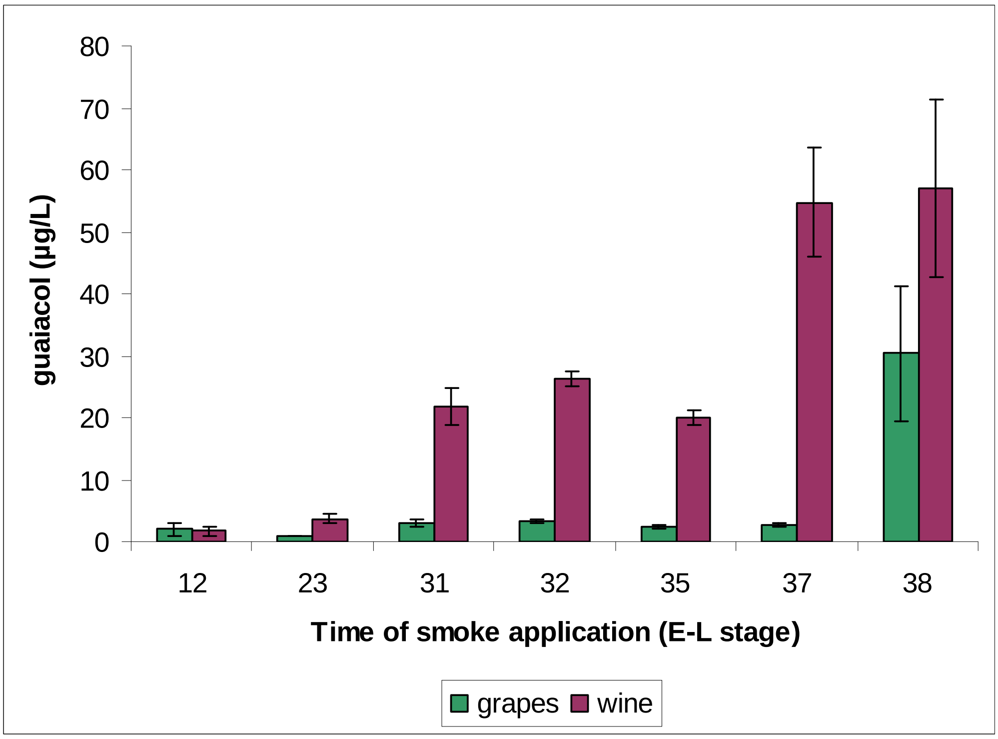

Figure 12. Concentration of guaiacol detected in grapes at harvest and in final wines made from grapes of vines exposed to a single smoke ether at E-L stage 12 (shoots 10 cm), 23 (flowering), 31 (berries pea size), 32 (beginning of bunch closure), 35 (veraison), 37 (berries not quite ripe) or 38 (harvest). Error bars show 2 standard errors of the mean, n = 3.

Levels of guaiacol, 4-methylguaiacol, 4-ethylguaiacol and 4-ethylphenol increased throughout the fermentation of fruit from vines exposed to repeated smoke applications (Table 6). Samples revealed compound levels to be minimal in the free-run juice of all samples and to increase to 249 µg/L guaiacol, 43 µg/L 4-methygauaicol, 8 µg/L 4-ethylguaiacol and 23 µg/L 4-ethylphenol post alcoholic fermentation of smoked grapes. Compound concentrations further increased post malolactic fermentation and cold stabilisation of smoked grapes to a high of 388 µg/L guaiacol, 93 µg/L 4-methylguaiacol, 16 µg/L 4-ethylguaiacol and 58 µg/L 4-ethylphenol in the final bottle. Wine made from grapes that were not exposed to smoke contained a total of 4 µg/L of guaiacol and trace levels (i.e. < 1 µg/L) of all other compounds in final wines. Studies conducted by the AWRI (2003) showed guaiacol and 4-methylguaiacol to accumulate in the skins of berries, rather than the pulp, which infers the longer skin contact time during fermentation to increase the accumulation of these compounds. Although, compounds also continued to accumulate post alcoholic fermentation during malolactic fermentation when wines were no longer on skin contact. The increased compound concentration post fermentation can signal the presence of potential precursor compounds (Kennison et al. 2008a) with research into the hydrolytic release of non-volatile precursors (e.g. glycoconjugates) an ongoing area of research (Hayasaka et al. 2008).

Table 6. Samples taken throughout the fermentation process detailing the concentration of guaiacol in wines made from grapes of unsmoked vines and concentration of guaiacol, 4-methylguaiacol, 4-ethylguaiacol and 4-ethylphenol in wines made of grapes of repeatedly smoked vines (from Kennison et al. 2008a).

Sample	Concentration (µg/L) unsmoked	Concentration (µg/L) smoked			
	guaiacol	guaiacol	4-methyl guaiacol	4-ethyl guaiacol	4-ethyl phenol
free-run juice	n.d.	1^{a}	tr.	n.d.	n.d.
Fermentation:					
after 1 day	tr.	68^{b}	11^{a}	10^{a}	5^{a}
after 3 days	tr.	168^{c}	26^{b}	8^{a}	5^{a}
after 5 days	tr.	203^{cd}	32^{bc}	9^{a}	15^{b}
after 7 days	tr.	249^{d}	42^{c}	9^{a}	17^{b}
After alcoholic fermentation	1	249^{d}	43^{c}	8^{a}	23^{c}
finished wine	4	388^{e}	93^{d}	16^{b}	58^{d}

Values followed by a different letter within columns are significantly different ($P < 0.05$), n = 3, n.d. = not detected, tr. = trace, (i.e. positive identification but < 1 µg/L). 4-Methylguaiacol, 4-ethylguaiacol and 4-ethylphenol were not detected or detected in trace amounts during fermentation of unsmoked wines.

4. Carry-over of smoke compounds to subsequent years

As the prevalence of smoke exposure to grapevines increase, vignerons and viticultural researchers have questioned the possibility of smoke-like phenol compounds being sequestered by grapevines during the season of smoke exposure and expressed in fruit, and subsequent wines, the following season. The potential for this to occur was investigated by evaluating vines, fruit and wine from grapevines 1 year post exposure to repeated smoke applications (n = 8). Field-grown grapevines were exposed to smoke at E-L 35 (veraison), E-L 35 + 3 days (3 days post veraison), E-L 35 + 7 days (7 days post veraison), E-L 35 + 10 days (10 days post veraison), E-L 36 (intermediate sugar), E-L 36 + 3 days (3 days post intermediate sugar), E-L 37 (berries not quite ripe) and at E-L 38 (harvest) and evaluated in the subsequent season to answer whether there is a ‘carry-over’ effect of smoke compounds.

4.1 Effect on vine growth and fruit production

The major carry-over effect to vines was the substantial reduction of fruit yield, bunch number and vine growth 1 year post heavy smoke exposure, in comparison to control (unsmoked) treatments that were both evaluated in the same season (Kennison et al. 2009a). Fruit yield 1 year post repeated smoke exposures was reduced to 6.4 kg/treatment (3 vines per treatment) which is extremely low when compared to the fruit yield of control (unsmoked) vines of 20 kg/treatment (Table 7). The average bunch number was also reduced to 73.7 bunches/treatment 1 year post smoke exposure in comparison to the control vines that yielded 115.3 bunches/treatment. As well as the reduced yield components, canopies of vines that were exposed to repeat smoke applications displayed shortened shoot growth 1 year post smoke exposure. Where control (unsmoked) vines reached the full height of the canopy and were trimmed during the course of routine vineyard management, vines that had received repeated smoke exposures in the previous year had reduced canopy shoot growth by approximately 20 cm.

Table 7. Fruit yield and average bunch number of vines 1 year post exposure to repeated (8) smoke applications and associated control (unsmoked) vines (Kennison et al. 2009a).

Treatment	Yield (kg)	Average bunch number
Control	20.0^a	115.3^a
1 yr post repeated smoke application	6.4^b	73.7^b

Means followed by the same letter within columns are not significantly different at $P \leq 0.05$, results are per treatment replicate (3 vines), mean values are from 3 replicates.

Due to the complex composition and influence of combustion conditions on smoke generation, the effect of smoke in reducing vine growth and fruit production capacity is unclear however parallels can be made with pollution studies on plants. Air pollution has been reported to reduce plant yields and more specifically, repeated applications of ozone, a component of smoke, have been demonstrated to reduce grapevine yield (Schempp et al. 2005). The yield loss is dependent on numerous factors including pollution duration, the interval timing between exposure and harvest and general plant health. Furthermore, other components of smoke, sulfur dioxide and nitrogen dioxide, have been responsible for inducing stomatal closure (Rosen et al. 1978) and creating 'water logging' (high concentrations of localised extra cellular water) in leaves that subsequently become necrotic lesions (Heath 1980, Schempp et al. 2005). Vine function and productivity are subsequently decreased, effects that were demonstrated by vines subsequent to smoke application in this study.

4.2 Compounds detected in wine

Wine made from fruit from vines 1 year post exposure to repeated (n = 8) smoke applications produced low levels of smoke-like compounds, levels that did not differ from wines produced from the control (unsmoked) treatment (Table 8). Wines made from fruit of vines 1 year post exposure to repeated smoke applications contained a low level of guaiacol (2 µg/L) and no detection of 4-methylguaiacol, 4-ethylguaiacol and 4-ethylphenol. This is in contrast to the extreme levels of compounds that were detected in wines during the same season that the repeated smoke applications were applied in 2006 being 388 µg/L guaiacol, 93 µg/L 4-methylguaiacol, 16.3 µg/L 4-ethylguaiacol and 58.3 4-ethylphenol.

As the compound levels detected in wine produced from fruit that had been exposed to repeated smoke applications 1 year previous are well below published aroma detection thresholds (Boidron et al. 1988, Chatonnet et al. 1992) and are not significantly different from wines produced from the control (unsmoked) vines, it is concluded that the likelihood and significance of sequestration and carry-over of smoke-like phenol compounds from one year to the next in grapevines is low.

Table 8. Concentration of guaiacol, 4-methylguaiacol, 4-ethylguaiacol and 4-ethylphenol in wines made from fruit of vines exposed to 8 repeated smoke applications from the period of veraison to harvest in 2006, in comparison to wines made from fruit of the same vines 1 year post repeated smoke exposure (2007) and wines from fruit of control (unsmoked) vines (Kennison et al. 2009a).

	Compound concentration (µg/L) in wine			
Treatment	**guaiacol**	**4-methyl guaiacol**	**4-ethyl guaiacol**	**4-ethyl phenol**
Repeated smoke application 2006	388^a	93^a	16.3^a	58.3^a
1 year post repeated smoke application 2007	2^b	0^b	0^b	0^b
Control	0^b	0^b	0^b	0^b

Means followed by the same letter within columns are not significantly different at $P \leq 0.05$, mean values are from 3 replicates.

4.3 Wine sensory analysis

As with other trial wines, QDA by a trained panel was conducted on wines made of fruit from vines 1 year post grapevine exposure to 8 repeated smoke treatments. Panellists did not detect any difference in the aromas of ‘burnt rubber’, ‘leather’, ‘disinfectant / hospital’, ‘red berry fruits’ and ‘confection’ between the control and the 1 year post smoke exposure treatment (Figure 13). Panellists detected a slightly elevated aroma of ‘smoked meat’ in wines made from fruit of vines that received repeated smoke applications 1 year prior, although it is important to note that these aroma detection levels are extremely low and are furthermore overpowered by the wine aromas of ‘red berry fruits’ and ‘confection’. The possibility for the carry-over of smoke compounds from one season to another is low although a further understanding of grapevine mechanisms of smoke assimilation and translocation would benefit understanding of this phenomenon.

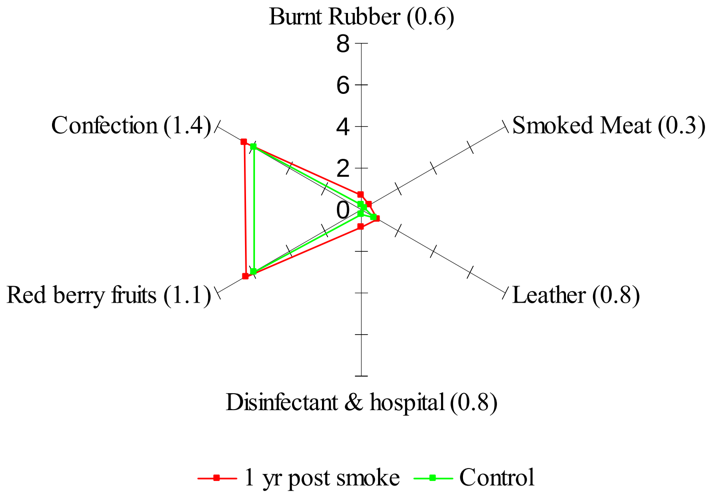

Figure 13. Mean intensity ratings of smoke-like aromas of 'burnt rubber', 'smoked meat', 'leather', 'disinfectant / hospital' and wine-like aromas of 'confection' and 'red berry fruits' in wines made from grapes of vines 1 year post exposure to 8 repeated smoke applications and wines made from fruit of control (unsmoked) grapevines. Scale represents 0 = non detectable aroma to 8 = highly detectable aroma (Kennison et al. 2009).

5. *Grapevine assimilation and translocation of smoke*

With heightened industry interest in how smoke compounds potentially form in the grape and subsequent wine, investigations into the potential mechanism of smoke uptake and assimilation by grapevines was conducted. Research included the analysis of grapevine organelles, including leaves, berries, berry skin and pulp, for the presence of smoke compounds from unsmoked (control) vines and those exposed to smoke. The influence of the wax coating on berry surface on the uptake of smoke compounds was also investigated. Smoke was applied to separate grapevine components (i.e. bunches only and leaves only) to investigate whether smoke uptake was direct from external smoke into the berry or whether smoke was translocated from the leaves to the berry.

5.1 *Smoke location within grapevine organelles*

Interestingly, the occurrence of smoke exposure to grapevines was evident in grapevine leaves and wines made from grapes from vines exposed to smoke. Strong relationships were evident between the levels of compounds detected in leaves and levels detected in wines. Initially, leaf samples were harvested from field-grown grapevines at fruit maturity (E-L 38) to investigate the presence of smoke compounds. Higher levels of guaiacol and 4-methylguaiacol were detected in leaf samples from a smoke application at E-L 35(c) (7 d post veraison) and E-L 38 (harvest) (Table 9). Guaiacol and 4-methylguaiacol were not detected in leaves of unsmoked (control) grapevines. Levels of 4-methylguaiacol and guaiacol were low (37 and 330 µg/kg) in leaves from vines exposed to smoke at E-L 31 (berries pea size) in comparison to levels detected in leaves at harvest (E-L 38) of 259 and 851 µg/kg respectively.

Similar trends were discovered with compound levels in wine, although compounds were at a lower concentration in wines than in the leaves of corresponding treatments.

A high level of correlation was found between compound concentrations detected in leaves with those concentrations detected in wines. From the same treatment, guaiacol concentration in the leaves showed a positive correlation ($r = 0.95$) with guaiacol concentration in wine. This correlation was also positive for levels of 4-methylguaiacol detected in leaves and wine ($r = 0.86$). The high level of correlation coupled with the non-detection of smoke compounds in unsmoked (control) leaves and wine indicates the relationship between the uptake of smoke compounds by grapevine organelles.

Furthermore, the high levels of guaiacol and 4-methylguaiacol detected within grapevine leaves has implications for fruit harvesting and winemaking. Leaves of grapevines can easily enter the load of harvested grapes throughout the mechanical harvesting process. Smoke exposed grapevine leaves entering the initial stages of the winemaking process could potentially result in the deposition and release of smoke-like compounds to the fermenting must. Research conducted by the AWRI (2003) discovered higher concentrations of guaiacol in must samples that were macerated with leaves. This led to the further recommendation for fruit harvesting to include leaf plucking, followed by a high volume and high pressure grapevine water wash followed by hand harvesting of grapes (AWRI 2003).

Table 9. Guaiacol and 4-methylguaiacol detected in leaf and wine samples from a smoke application to field-based grapevines either at E-L 31 (berries pea size), E-L 35(a) (veraison), E-L 35(c) (7 d post veraison) or E-L 38 (harvest) and the control (unsmoked) treatment (Kennison et al. 2009a).

Time of smoke application	**Concentration (µg/kg) in leaves**		**Concentration (µg/L) in wine**	
	4-methylguaiacol	**guaiacol**	**4-methylguaiacol**	**guaiacol**
E-L 31	37^{ab}	330^{ab}	2.33^{b}	8.7^{ab}
E-L 35a	64^{ab}	525^{bc}	2.33^{b}	11^{b}
E-L 35c	133^{b}	694^{bc}	6^{c}	27.3^{c}
E-L 38	259^{c}	851^{c}	5.67^{c}	29^{c}
Control	0^{a}	0^{a}	0^{a}	1^{a}

Means followed by the same letter within columns are not significantly different at $P \leq 0.05$; mean values are from 3 replicates.

Additional analysis of grapevine organelles was conducted to further isolate the location of smoke compounds within the grape berry. Samples of interest in this study included homogenised whole grapes, grape skins and grape pulp that were prepared from grapes at maturity. These samples were prepared from grapes of vines that were unsmoked (control) and from grapes of vines that had been exposed to smoke at E-L 35 (veraison). Guaiacol and 4-methylguaiacol were not detected in samples of homogenised grapes or grape pulp for fruit from both smoked and unsmoked vines (Table 10). A clear presence of guaiacol (3.8 µg/L) was detected in the skins of grapes from vines that were exposed to smoke. Guaiacol and 4-methylguaiacol were not detected in the skin and fruit from unsmoked (control) vines.

The location of smoke compounds are therefore present in the skins of grapes, a finding that supports previous research (AWRI 2003).

The location of smoke compounds within grapevine organelles can provide addition modes of potential analysis methods for the detection of smoke exposure to grapevines. In this study, guaiacol and 4-methylguaiacol were not detected in samples of homogenised grapes or grape pulp but were detected in the skins of grapes from the same smoke treatment (Table 10). Therefore, a dilution of the smoke compounds present in the skins occurred. Grapevine leaves indicated the exposure of smoke to grapevines at high concentrations, levels of which were not detected in leaves from grapevines that were not exposed to smoke. In this respect, the analysis of grapevine leaves provides a potential avenue for the detection of smoke exposure to grapevines and subsequent presence in wine.

Table 10. Concentration of guaiacol and 4-methylguaiacol detected in homogenised grapes, grape pulp, grape skin, grape leaves and final wines from field-grown grapevines exposed to smoke at E-L 35 (veraison) and unsmoked (control) vines (Kennison et al. 2009a).

	Concentration (µg/L) of			
	guaiacol		4-methylguaiacol	
	control	smoke	control	smoke
Grapes	n.d.	n.d.	n.d.	n.d.
grape pulp	n.d.	n.d.	n.d.	n.d.
grape skin	n.d.	3.81	n.d.	n.d.
Leaves	n.d.	525	n.d.	64
Wine	1	11	n.d.	2.33

Data are means from 3 replicates. N.d. = not detected. Grape and leaf samples were taken for analysis at harvest (fruit maturity).

5.2 Influence of grape wax bloom

Studies on the influence of the grape wax bloom on the berry surface and the uptake of smoke compounds were investigated to further understand potential modes of smoke assimilation and translocation. As discussed in the methodology, the wax bloom was removed from harvested grapes with chloroform and they were exposed to smoke either for 5, 10, 20, 40 or 80 min duration. Control treatments of berries with their wax layer intact were also smoked for the same durations. Research was conducted 2 times prior to veraison and 2 times post veraison with all grape samples homogenised for analysis of guaiacol.

Results clearly show the influence of the wax bloom on the grape surface in protecting the grapes by reducing potential smoke permeation (Figure 14). The enhanced uptake of smoke by grapes that have had their wax removed was evident. Smoke exposure to berries for either 5 or 10 mins revealed little difference in the uptake of guaiacol between waxed and unwaxed berries although smoke exposures for longer durations (20, 40 and 80 mins) resulted in the greater uptake of guaiacol by unwaxed berries. Interestingly, higher concentrations of guaiacol were detected in samples that were treated with smoke prior to veraison when berries were pea size (range of 2 to 901 µg/L) and when berries are hard and green (range of 1 to 750 µg/L). In comparison, berries exposed to smoke post veraison contained lower levels of guaiacol at veraison (range of 2 to 482 µg/L) and post veraison (range of 1 to 436 µg/L). The composition of the cuticle wax of grape berries is known to modify and increase in wax deposits from berry set to maturity (Commenil et al. 1997) however with the removal of wax from the surface of grape berries the uptake of smoke compounds was evident with this uptake furthermore enhanced in berries that were exposed to smoke prior to veraison.

It is important to note that this research was conducted on post harvest berries with analysis of homogenised berries only and is therefore limited in the investigation of the permeation of smoke compounds within berry components. Although, the experiment is of interest as it provided an insight as to the protective mechanism of the wax bloom on the uptake of smoke.

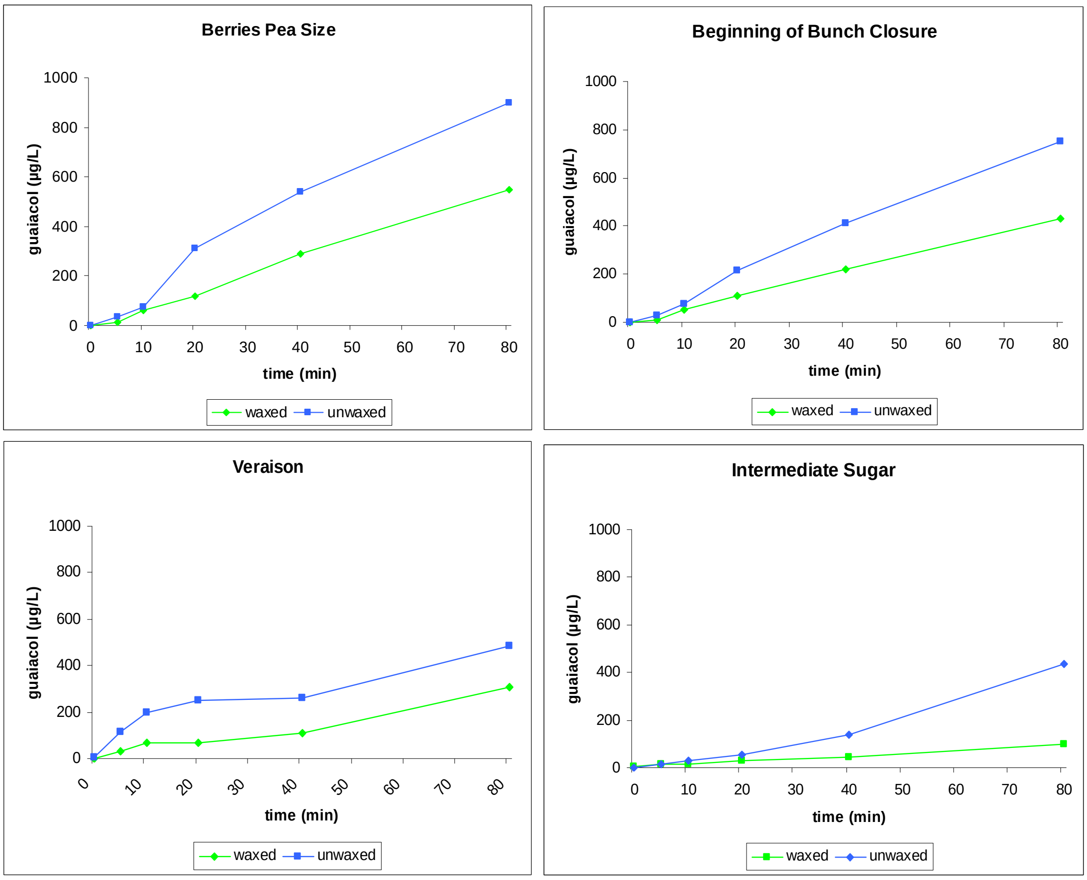

Figure 14. Levels of guaiacol detected in grapes that were exposed to smoke for either 5, 10, 20, 40 or 80 min at E-L 31 (berries pea size), E-L 32 (beginning of bunch closure), E-L 35 (veraison) and E-L 36 (intermediate sugar). Grape samples are either intact or have had wax layer removed.

5.3 Mechanisms of smoke assimilation

In order to investigate potential grapevine mechanisms of smoke assimilation and translocation, smoke was applied to separate grapevine components (i.e. bunches only and leaves only) to investigate whether smoke uptake was direct into the berry or whether smoke was translocated from the leaves through to the berry. As per methodology, smoke was applied prior to harvest to: (1) leaves only (with grape bunches sealed within plastic) and (2) grapes only (with grapevine leaves sealed within plastic). A separate treatment was conducted where smoke water was sprayed onto: (1) leaves only and (2) grapes only. Grape berries were homogenised and analysed for guaiacol and 4-methylguaiacol.

Surprisingly, analysis of berry homogenate samples from all smoke treatments did not result in the detection of guaiacol and 4-methylguaiacol. This was interesting considering that smoke exposure to grape bunches alone (post-harvest) had resulted in detectable levels of guaiacol and 4-methylguaiacol in subsequent wines (Kennison et al. 2007). Although, previous research has found smoke taint compounds to increase throughout the fermentation of smoked grapes with guaiacol and 4-methylguaiacol also released from berry samples that had undergone a strong acid hydrolysis (Kennison et al. 2008a). Therefore, the original berry homogenate samples, without detectable concentrations of guaiacol and 4-methylguaiacol, were hydrolysed with strong acid (pH 1) in order to release guaiacol and 4-methylguaiacol.

The hydrolysis of berry samples was successful in releasing guaiacol and 4-methylguaiacol to reveal the mechanisms of smoke assimilation and translocation. From this research, it is possible that components of smoke may be assimilated by grapevine leaves and translocated through to fruit. Smoke application to bunches alone resulted in high levels of guaiacol and 4-methylguaiacol, 30.7 and 7.7 µg/L respectively, demonstrating the likely deposition of smoke compounds onto the surface of the grape berry (Table 11). Interestingly, smoke application to grapevine leaves alone resulted in a high detection level of guaiacol (19.3 µg/L) and 4-methylguaiacol (2.7 µg/L) in resultant fruit. These compound levels are considerable when taking into account that the fruit was securely covered and not exposed to smoke at this time. Guaiacol was detected in the control samples, it is currently not clear why this occurred and further analysis will be conducted to confirm this observation.

The application of smoke-water to grapevines further evaluated the mechanism for smoke assimilation and translocation throughout the vine. Smoke-water contained extremely high levels of guaiacol (34996 µg/L) and 4-methylguaiacol (10664 µg/L) and was not absorbed by the grapes when applied to bunches alone (Table 11). Interestingly, smoke-water application to the leaves resulted in detectable levels of guaiacol (7.3 µg/L) and 4-methylguaiacol (2.7 µg/L) in fruit alluding to a mode of uptake through the leaf surface to the fruit.

An association between the exposure of smoke to grape leaves and subsequent detection of smoke compounds in fruit has been discovered in this research although it is currently unclear as to the mechanism of such action. The uptake of pollutant gases through the stomata of plant leaves has been demonstrated subsequent to the deposition of such gases onto the external surfaces of vegetation (Fowler 2002). However, the nature of particular aromas or flavours that pollutants or other gasses may impart to fruit are presently unknown. Possible mechanisms of smoke related compound entry into the plant may be by diffusion through the leaf cuticle into palisade and/or mesophyll cells. Further research is required in this area.

Table 11. Concentration of guaiacol and 4-methylguaiacol detected in berry acid hydrolysate samples from a smoke application or smoke-water application to either bunches or leaves prior to fruit harvest (Kennison et al. 2009a).

Sample	**Concentration (µg/L) of**	
	guaiacol	**4-methylguaiacol**
Smoke application		
bunches only		
strong acid hydrolysate	30.7^a	7.7^a
leaves only		
acid hydrolysate	19.3^b	2.7^b
Smoke-water application		
bunches only		
strong acid hydrolysate	n.d.	n.d.
leaves only		
acid hydrolysate	7.3^c	2.7^b
control		
strong acid hydrolysate	4^c	1.3^c

Means followed by the same letter within columns are not significantly different at $P \leq 0.05$, data are means from 3 replicates, n.d. = not detected.

6. Reducing the negative effects of smoke

An important aspect of this research has been the consideration of potential methods for reducing the negative effects of smoke taint within the vineyard. Such mechanisms for the reduction of smoke taint have included vineyard based amelioration treatments and more recently, the suggestion of agricultural chemicals applied to vines to protect against smoke exposure. Previous vineyard treatments have been applied to vines post smoke exposure (AWRI 2003) and this research was further investigated in this study by the application of high volume water washes to grapes post smoke exposure. This study has benefited by the presence of fruit from unsmoked (control) grapevines for comparison to fruit exposed to smoke.

Previous research has investigated the application of protective chemicals to prevent damage to plants from air pollutants (Pandey et al. 1993) and, in particular, to prevent damage to plants, including grapevines, by ozone (Archambault et al. 2000; Musselman 1985). This study investigated a range of chemical products that could potentially be utilised to protect grapevines from smoke. Chemical products registered for use on grapevines for the control of pests and diseases, surfactants and anti-transpiration chemicals were evaluated in this study. Very few chemical products and post smoke amelioration treatments were effective for the reduction of smoke compounds in grapes. Two key products are outlined for comparison of treatments in Table 12.

Application of an anti-transpirant compound (acrylic polymer) to grapevines prior to the exposure of smoke was discovered to reduce the uptake of smoke into grapes (Table 12). The protective nature of this chemical was significantly greater then other treatments such as Kaolin clay that resulted in the increase of guaiacol in grapes (20.9 µg/L) even greater than that of grapes from vines that were exposed to smoke only (4.2 µg/L). The mode of action of the anti-transpirant compound to reduce stomatal opening further explains the findings of previous studies in this project. That is, the anti-transpirant compound may have acted to reduce the assimilation and translocation of smoke from leaves to fruit. Alternatively, the anti-transpirant compound may act as a protective barrier to reduce smoke uptake and damage to fruit.

Amelioration treatments applied post smoke exposure were non-effective in reducing levels of smoke compounds with many treatments, such as high volume water applications, resulting in the promotion of guaiacol in grapes (11.9 µg/L) (Table 12). Previous research has concluded that levels of guaiacol in fruit samples were enhanced when contaminated with leaves and therefore recommended a post smoke vine treatment of leaf plucking, followed by high-volume, high-pressure cold water wash followed by hand picking of fruit (AWRI 2003). In our research, a high volume water wash was applied to grapes post smoke exposure without the removal of leaves. Fruit was hand harvested with subsequent analysis revealing enhanced concentrations of guaiacol (11.9 µg/L) and 4-methylguaiacol (1.1 µg/L) in this fruit. The mechanism of the high volume water wash was to therefore promote the accumulation of smoke in the fruit. As such, a high volume water wash is not recommended post smoke exposure whilst leaves are present.

Table 12. Concentration of guaiacol and 4-methylguaiacol detected in field-grown Chardonnay grapes post exposure to smoke and fruit from control (unsmoked) vines. Vines were sprayed with either kaolin clay or acrylic polymer prior to smoke application or with a high volume water wash post smoke exposure.

Treatment	**Concentration (µg/L) of**	
	guaiacol	**4-methylguaiacol**
Control	n.d.	n.d.
Smoke only	4.2^{a}	n.d.
Treatment prior to smoke		
Kaolin clay	20.9^{b}	2.1
Acrylic polymer	1.7^{c}	n.d.
Treatment post smoke		
Water wash	11.9^{d}	1.1

Means followed by the same letter within columns are not significantly different at $P \leq 0.05$, data are means from 3 replicates, n.d. = not detected.

It is important to note that this is a preliminary study on the investigation of protectant chemicals with chemicals trialled within this study not used for their intended purpose. This study is not an endorsement of any particular chemical for the protection of grapes from smoke. Further investigation of the mode of chemical action, effect of chemical on grapevine growth and production and the maximum residue limits for winemaking are required.

Outcomes and Conclusion

This project achieved all outputs and performance targets as outlined in the original project application with all project objectives successfully met with the methodology employed. From this research practical knowledge regarding smoke taint has been gained for the Australian grape and wine industry and it is recommended that this knowledge be used for the reduction of smoke taint to grapes and wine.

A major outcome of this study has been the establishment of a direct link between the smoke exposure of field-based vines and the subsequent development of 'smoke taint' aromas and phenol compounds in wine. The timing and duration of smoke exposure to grapevines is critical in the uptake of smoke compounds (guaiacol and 4-methylguaiacol) and the expression of these compounds and smoke-like aromas in subsequent wines. A heightened period of grapevine sensitivity to the uptake of smoke compounds has been established to occur from the growth stage of E-L 35(c) (7 days post veraison) to E-L 38 (harvest). Smoke uptake is also noted to occur in grapevines from E-L 31 (berries pea size) through to E-L 35(b) (3 days post veraison) although the uptake is variable during this period. Grapevines have a low sensitivity to smoke uptake when fruit is not present on the vine at E-L 12 (shoots 10 cm) and E-L 23 (flowering).

The duration of smoke exposure to grapevines is influential in the development of smoke taint in subsequent wines. Repeated smoke exposures to grapevines from the period of E-L 35 (veraison) to E-L 38 (harvest) result in an accumulation of smoke-like compounds and aromas in resultant wines. Furthermore, repeated smoke application resulted in the decline of grapevine leaf functioning visually evident by necrotic lesions on leaf blades. Subsequent fruit ripening capabilities of the vine were reduced.

This research study did not establish a carry-over effect of smoke-like compounds and aromas in wines made from fruit of vines one year subsequent to a heavy smoke exposure. However, heavy smoke exposures demonstrated negative effects on vine physiology. Vine yield and shoot growth were dramatically reduced in the growing season that followed repeated smoke applications.

Initial research has highlighted the potential mechanisms of smoke assimilation and translocation throughout the grapevine. From this study, smoke deposited on grapevine leaves alone has resulted in detectable levels of smoke compounds in homogenised grapes. This alludes to the uptake of smoke compounds by leaves and subsequent translocation to fruit. Anti-transpirant (acrylic polymer) sprays were discovered to provide a barrier against the uptake of smoke compounds that requires further investigation.

A number of key volatile compounds, detected in smoke and smoke wines, have been instrumental in the detection of smoke exposure to grapevines. Guaiacol and 4-methylguaiacol are key compounds measured in this study to detect the presence of smoke in grapevine leaves, grapes and wine. This study has discovered that the compound levels detected in fresh grapes at harvest do not equate to the levels that develop in subsequent wines. Guaiacol and 4-methylguaiacol increase during the fermentation process resulting in levels many fold higher than those measured in fruit. Therefore, analysis of guaiacol and 4-methylguaiacol in fruit can provide an indication of the presence of smoke taint, but is not a reliable determinant of the potential taint level in final wines.

Furthermore, guaiacol and 4-methyguaiacol have been reliable indicators of smoke presence in wine although it is probable that additional smoke compounds also contribute to the chemical and sensory smoke taint. The majority of wines produced from grapes of vines exposed to smoke within this study contain concentrations of guaiacol and 4-methylguaiacol below published aroma thresholds. These wines clearly exhibit smoke-like aromas with sub-threshold guaiacol and 4-methylguaiacol concentrations. Numerous compounds are detected in smoke. A true indication of the intensity of smoke taint in wine is not provided by current testing methods which do not account for potential combinations of compounds.

Smoke exposure to grapevines is also indicated by the detection of guaiacol and 4-methylguaiacol in grapevine leaves. During this study, guaiacol and 4-methylguaiacol were detected in leaves of grapevines exposed to smoke and were not detected in leaves from unsmoked vines. Although requiring further investigation, grapevine leaves could potentially be utilised for the detection of smoke exposure to grapevines.

It is concluded that the knowledge produced in this research can be used to develop strategies to reduce smoke taint in grapes and wine. Benefits for vignerons and improvements in the quality of grapes and wine are produced from this research.

Recommendations

A number of key recommendations are provided from this research. Initially, an understanding of the timing of grapevine sensitivity to the uptake of smoke compounds provides information to be used as a risk assessment tool in the planning of fires that may produce smoke events over vineyards. It is recommended that the sensitive timings outlined in this document be utilised for consideration when planning fires near vineyards. Furthermore, it is recommended that vignerons communicate the timing of seasonal vine phenology stages to those involved in planning fires as a potential consideration in fire planning and smoke management.

The knowledge of grapevine phenology timing on the uptake of smoke compounds furthermore provides vignerons with enhanced decision making capacity to minimise smoke taint in fruit and wine production. It is recommended that vignerons monitor the presence of smoke in vineyards to provide an indication of potential smoke damage. Smoke monitoring equipment, such as nephelometers, may be utilised.

Repeated smoke exposure to grapevines has demonstrated a cumulative smoke effect in wine. It is recommended that repeated smoke exposures, or exposures for long periods of time, to grapevines are minimised during sensitive periods. This will, in turn, reduce the accumulation of smoke compounds in grapes and subsequent wine.

Due to the multitude of compounds detected in smoke, this research recommends vignerons to be cautious with basing the detection and quantification of guaiacol and 4-methylguaiacol in grapes as the sole determinant for smoke taint potential in final wine. Levels of guaiacol and 4-methylguaiacol in grapes do not equate to the levels produced in final wines. Quantification of guaiacol and 4-methylguaiacol in grapevine leaves does provide an indication of smoke exposure to vines although the true level of sensory taint is only revealed in the final wine. It is recommended that further research for the accurate determination of smoke taint in grape samples is investigated.

This investigation of grapevine smoke exposure and the development of smoke taint in wine has provided a greater understanding of the issue however further research is required. In order to increase our understanding of smoke taint and to provide practical outcomes to minimise smoke taint further recommendations for research include:

- The development of a greater understanding of the complexity (density and duration) of smoke exposure from fires and the subsequent impact on the development of smoke taint in wine. Establishment of a minimal critical level of smoke duration and density exposure required to create smoke taint in wine would provide vignerons with a useful tool for risk analysis.

- Establishment of a model field-based smoke detection and smoke warning system to quantify levels of smoke present in vineyards. It is recommended that the smoke warning system incorporates field-based smoke detecting equipment and computer modelling software.

- Modelling of the seasonal timing of grapevine phenological stages within grape growing regions to provide an understanding of critical smoke exposure dates. It is proposed that the critical timings for smoke exposure be utilised for consideration in the planning of fires and smoke over vineyards.

- Development of an up to date database containing vineyard locations, vine varieties and manager contact information to assist with the collection of information of the timings of vine phenological stages and the communication of intended fire and smoke events.

- Understanding the effects of smoke on a range of grapevine varieties at key stages of grapevine growth and development. This investigation should include an investigation on the wine sensory attributes imparted by smoke to different grapevine varieties.

- Influence of various fuel types on the development of smoke taint in grapes and wine. Increase understanding of particular wine aromas imparted to grapes, and subsequent wines, from the smoke of various native forest fuels. Research particular compounds detected in the smoke from a variety of fuel sources and the implication of these compounds in wines made from fruit of vines exposed to this smoke.

- Improvement of grape analysis methodology to provide a better indication of the potential presence of smoke taint in final wines. Investigation of other compounds implicit in smoke that could contribute to smoke taint in wine. The development of a rapid test that provides an accurate indication of the smoke compound levels predictable in wines from an analysis of the fruit.

- Investigation of the mode of action of smoke compound assimilation and translocation within grapevines. Increased understanding would benefit the development and implementation of protective chemicals applicable to grapevines.

- Investigation of the link between compound levels detected in leaves and levels detected in final wines.

- Investigate the plant physiology smoke mechanisms within grapevines and the numerous factors that may affect this uptake (i.e. plant health, environmental considerations). Furthermore, gain an understanding of smoke effects on plant physiology and photosynthesis.

- Development of reliable vineyard based protective chemicals for the reduction of smoke uptake by grapes. The further development of cost effective vineyard and wine amelioration treatments will reduce the negative effects of smoke post exposure to grapevines. It is further recommended that amelioration treatments effective in reducing the severity of smoke damage in grapes and wine post exposure to smoke are investigated.

- Due to the heightened incidence of the smoke taint issue in Australia it is recommended that a national extension plan for this issue incorporating a national smoke effect working group be formed to foster the research and extension process.

Appendix

Appendix 1: Communication

Communication of the progress and outcomes of this research has continually been a focus of project contributors. Research outcomes have been delivered to industry and fellow researchers during presentations at:

- WA Smoke Effect Working Group 15th August 2008

Group is comprised of WA wine industry representatives, WA vignerons, Department of Environment and Conservation and smoke taint researchers. Working group has fostered the development of the current research with this project updated by presentation at each meeting.

- Smoke Taint meetings in Victoria 2007

Presented up to date smoke taint research to a total of approximately 130 people at four grower meetings held throughout Victoria at Knoxfield, Milawa, Oxley and Nagambie. An industry technical seminar was conducted in Milawa.

- National Smoke Taint Technical Industry Workshop

Presented up to date research at the GWRDC sponsored workshop held in Melbourne on 29th July 2008.

- Margaret River Wine Industry Association Seminars

Presented latest smoke taint research information at field day seminars in 2006 and 2007.

Written communication on this project has been conveyed to industry and a larger audience on a number of occasions. Research updates and factsheet have been included in the 'WA Wine Industry Newsletter' and 'Australian Viticulture' publications with poster presentations at the Australian Wine Industry Technical Conference 2007:

- Kennison K.R., Wilkinson, K.L., Pollnitz, A.P. and Gibberd, M.R. (2008) The timing and duration of grapevine exposure to smoke effects the chemical composition of wine. In *Proceedings of 13th Australian Wine Industry Technical Conference 2007,* Blair, R., Williams, P. and Pretorius, S. (Eds), Australian Wine Industry Technical Conference Inc., Glen Osmond, South Australia.
- Kennison K.R., Wilkinson, K.L., Williams, H.G. and Gibberd, M.R. (2008) Sensory characteristics and chemical composition of smoke-tainted wines. In *Proceedings of 13th Australian Wine Industry Technical Conference 2007,* Blair, R., Williams, P. and Pretorius, S. (Eds), Australian Wine Industry Technical Conference Inc., Glen Osmond, South Australia.

To convey this research to a scientific and wider industry audience, a number of refereed publications have been produced and include:

- Kennison, K.R., Gibberd, M.R., Pollnitz, A.P. and Wilkinson, K.L. (2008) Smoke-derived taint in wine: the release of smoke-derived volatile phenols during fermentation of merlot juice following grapevine exposure to smoke. *Journal of Agricultural and Food Chemistry* 56, pp. 7379-7383.
- Kennison. K.R., Wilkinson, K.L., Pollnitz, A.P., Williams, H.G. and Gibberd, M.R. (2009) Effect of timing and duration of grapevine exposure to smoke on the composition and sensory properties of wine. *Australian Journal of Grape and Wine Research* (accepted for publishing).

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

Appendix 2: Intellectual Property

No intellectual property has been generated as part of this research. This research has focused on the development of knowledge for industry which is contained herein.

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Appendix 4: Staff

Staff engaged on this project include:

- Ms Kristen Kennison, Viticulture Research and Development Officer, Department of Agriculture and Food, WA (0.6 FTE)
- Mr Glynn Ward, Project Manager for the Development of Premium Winegrapes, Department of Agriculture and Food, WA (0.05 FTE)
- Assoc. Prof. Mark Gibberd, Curtin University of Technology, School of Agriculture and Environment (0.05 FTE)
- Dr. Kerry Wilkinson, Curtin University of Technology, School of Agriculture and Environment. Present address: The University of Adelaide, School of Agriculture and Environment (0.05 FTE)
- Mr Robert Frayne, Senior Technical Officer, Department of Agriculture and Food, WA (0.4 FTE)

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Appendix 5

N/A

Appendix 6: Budget Reconciliation

Budget reconciliation is contained on GWRDC Form B as supplied in separate document.