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Understanding and mitigating the impact of smoke exposure on grape and wine composition and wine sensory properties



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1. Abstract

This project addressed important questions about the impact of smoke from the 2020 bushfires on wine. Vineyards with a range of smoke and fire damage were selected for an evaluation of the impact of early-season smoke. Grape composition was affected by the smoke, and wines had strong unpleasant smoky flavours that regular wine consumers strongly disliked. Concentrations of smoke marker compounds in grapes and wine related to unacceptably smoke-affected wine were determined for three major varieties. Strategies to reduce smoke taint were evaluated, and treatment of white juice with activated carbon gave the best results.

1. Executive summary

Smoke from the 2020 bushfires affected wine grapes across the ACT, NSW, SA and Victoria, with economic impacts estimated at $665 million. It is well understood that volatile phenols in smoke can be taken up by grapes and metabolised to form phenolic glycosides, which can accumulate in high concentrations during the growing season and contribute an unpleasant smoky aroma, flavour and aftertaste to wine. Smoke exposure can be detected in grapes and wine by measuring a suite of marker compounds (volatile phenols and phenolic glycosides) and comparing results with the concentrations found in non-smoke-exposed samples. However, the challenges of the 2020 vintage raised several questions that highlighted gaps in our knowledge about smoke taint. Firstly, does early-season smoke impact on wine quality in the same way as later in the season? What are the links between grape and wine composition and smoky flavours in wine, and what concentrations in grapes produce unacceptable wine? What are the most promising strategies to reduce smoke taint in wine?

The impact of early-season smoke was investigated using a comprehensive set of real smoke-exposed samples from the Adelaide Hills wine region. The Cudlee Creek fire in the Adelaide Hills occurred well before véraison, when winegrapes were small and green, approximately three months before harvest, at a phenological stage previously considered to be at low risk of smoke taint. Twenty-four vineyards with a range of smoke and fire damage were selected to evaluate the impact of early-season smoke. Grape composition was shown to be affected by the smoke. Volatile phenols and phenolic glycosides were elevated in the grapes at harvest, with concentrations matching anecdotal observations of smoke in the vineyards. Phenolic glycosides were also detected in the grapes sampled in January, four weeks after the fire, showing that early detection of smoke exposure is possible. Red wines from vineyards heavily affected by smoke had strong, unpleasant smoky flavours and were strongly disliked by regular wine consumers. White wines had less intense smoke flavour due to different wine production methods.

This study clearly showed that smoke early in the season can have a devastating effect on wine quality, particularly for red wine styles. This information continues to be communicated to growers and winemakers so they can be alert to the risk of smoke characters from early-season smoke and make informed decisions about vineyard management and wine production. Ultimately, producers should avoid producing unacceptable smoky wine that could damage their own reputation and Australia's reputation for quality wine. On a practical basis, the study showed that the time period during which wine grapes are at risk from smoke exposure is longer than previously thought. Ideally, controlled burns planned for fuel hazard reduction or for other reasons such as stubble management should not be undertaken near vineyards during the risk period. The wine sector should negotiate with those planning burns to ensure that bushfire risk is managed while minimising the impact of early-season smoke on wine grapes and avoiding preventable crop losses.

In addition to the early-season smoke event in the Adelaide Hills, grapes were exposed to smoke at various times during the 2019/20 vintage in many wine regions across Australia. Analysis of smoke marker compounds enabled non-smoke-exposed samples to be identified, which could be managed in the usual manner. However, for the samples with confirmed smoke exposure, it was not known what concentrations of smoke marker compounds in grapes would produce

unacceptably smoke-affected wine. For the first time, these links were established with a rigorous set of 65 wines made from Chardonnay, Pinot Noir and Shiraz grapes with a range of smoke exposure. The focus was on chemical composition of grapes and wines and objective assessment of smoke flavour. Smoke flavour in wine was predicted well using smoke markers in grapes. Marker compounds key to smoke flavour were identified. The varieties differed in grape chemistry, wine chemistry and smoke flavour. Interestingly, some wines, but not all, made from smoke-exposed fruit had strong smoke flavour. The concentrations are summarised in a decision tool to guide assessment of smoke-exposed grapes, which is attached to this report in Appendix 8.

Harvesting grapes, purchasing grapes or bulk wine, and choosing processing options for grapes suspected of smoke exposure are critical decisions, with significant financial and legal implications for growers and winemakers alike. Smoke taint was the top topic of queries through the AWRI helpdesk in 2019, 2020 and 2021 and in total over the last five years, covered approximately 25% of all winemaking queries since 2017. In 2019/20 alone, the economic impact of fires was estimated at $665 million across NSW, Victoria and SA. The decision tool will enable better decisions to be made about how to manage smoke-exposed grapes, alleviating uncertainty and stress. It will help protect brand reputation by avoiding the production of unacceptably smoky wine. It will minimise costly wine quality downgrades and unnecessary crop losses.

The establishment of unacceptable levels of smoke markers in Chardonnay, Pinot Noir and Shiraz grapes is a major development in the assessment of smoke-exposed grapes. These varieties represented 48% of Australia's wine grapes in 2022. It is important to note that the critical concentrations were different in the three varieties, so the concentrations may not be applicable to other cultivars or to rosé, fortified or sparkling wine styles. Further research is required to establish concentrations that lead to unacceptably smoky wines in other varieties and wine styles.

Strategies for reducing smoke taint in wine continue to be sought, and genuine solutions are elusive. Most treatments have negative impacts on wine flavour and colour, style and quality, and add costs and/or processes to manage smoke taint. This project focused on strategies to apply in the winery and evaluated yeast strains, glycolytic enzymes and activated carbons. The most successful strategy to date has been the treatment of white juices with activated carbon to remove phenolic glycosides and/or volatile phenols prior to fermentation. This practice was widely applied to white winemaking in 2020, reportedly with good outcomes. It was known that carbon products varied in their effectiveness, but the underlying reasons were unknown. The surface characteristics of activated carbon products were explored to allow insight into the reasons for their efficacy. Surface characteristics varied widely, and particular combinations of characteristics were required for optimal removal of volatile phenols or glycosides from a given matrix. There are few options for remediating red wine styles, and smoke taint remains a very significant risk for producers of premium full-bodied red wines. In contrast, some producers were more comfortable with a certain level of risk, and produced commercial grade white wine or non-wine products to recoup value from an otherwise lost crop. Innovative solutions are still required for red wine remediation.

Climate modelling predicts that fire events in southern Australia, where most Australian winegrapes are produced, will become more frequent and intense. Hazard reduction through prescribed burns is an important part of bushfire prevention, and the window of suitable weather and conditions for these activities is limited. It is therefore likely that prescribed burns will occur during the grapegrowing season, posing a risk to the crop. The knowledge from this project will support negotiations about prescribed burns and prepare the sector for bushfire events, increasing preparedness for climate change and reducing the risk from extreme environmental events. Increasing our knowledge about smoke taint represents an important tool to adapt to and mitigate against the effects of climate change.

2. Acknowledgements

This work was supported by Australia's grapegrowers and winemakers through their investment body Wine Australia with matching funds from the Australian Government. The AWRI is a member of the Wine Innovation Cluster in Adelaide, SA.

The project attracted a $100k co-investment from the South Australian State Government, in support of research towards an improved understanding of the consequences of early smoke exposure on grapes at harvest and wine composition and sensory attributes.

The project was supported by in-kind contributions from the State Governments of Victoria and New South Wales via Wine Victoria and NSW Wine.

3. Background

This project was initiated in early 2020 due to urgent stakeholder needs. The key stakeholders in this project were grapegrowers, winemakers, consumers and researchers. The main purpose of the research was to help producers affected by fires and smoke to assess the extent of any smoke damage and to help them decide whether or not to continue to spend money managing a vineyard, harvesting grapes and making and releasing wine to the market.

Since 2003, major fire events in 2003, 2004, 2007, 2009, 2013 and 2015 have resulted in wine industry losses of more than $400M as a result of smoke taint in Australia. The frequency of days with very high and extreme Forest Fire Danger Index (FFDI) are predicted to increase by 15-70% by 2050 under various climate change scenarios. In addition to natural bushfires, controlled burn activities by public land management agencies will continue. Thus, it is important that the wine industry manages this major financial and reputational risk.

With the major fires in the 2019/20 season, it was recognised that there is insufficient knowledge regarding the link between the concentration of smoke compounds in grapes from smoke-affected vineyards and the severity of smoke characters in wines made from those grapes. Many growers and winemakers quantified smoke compounds in grapes, but these data alone were often insufficient for commercial decisions about harvest and production. Knowledge of levels of smoke compounds that give unacceptable wine sensory properties, calibrated against consumer testing, was required. Knowledge of the effects of early-season, pre-véraison smoke exposure on wine was also lacking.

In addition, cost-effective remediation tools for smoke-affected grapes and wine were needed, to minimise the expression of smoke characters and retain the quality and integrity of wines in the marketplace.

4. Project aims

A major aim of this two-year project was to determine the relationship between the concentration of smoke-derived compounds (phenols and phenolic glycosides) in smoke-exposed grapes and wine and the intensity of smoke characters in wine assessed through sensory evaluation. In addition, the consequences of early-season smoke exposure and smoke exposure closer to harvest needed to be established, with multiple regions and both white and red grapes targeted with low, medium and high levels of smoke compounds. To establish these relationships, a large number of wines were made under standardised conditions, suitable for sensory assessments using a screened, qualified smoke sensory panel. The sensory intensity of smoke characters was related to chemical composition and consumer preference response.

The project also aimed to assess whether, following early-season exposure, the smoke phenolic glycoside concentration of unripe grapes and/or of grapevine leaves could be used to determine the extent of the smoke characters that would be expected in wine.

The project aimed to assess the effectiveness of treatments to remove or eliminate taint, notably using activated carbon products, as well as enzyme preparations and specific yeast strains.

Contracted deliverables and associated activities for the two years of this project are shown in Tables 1 and 2.

Table 1. 2020-21 Deliverables and associated activities

	Deliverables	**Activities**	**Target date**
A	Evaluation of the impact of early-season smoke exposure of grapes on grape composition and wine sensory attributes	1. Finish winemaking and complete bottling of Adelaide Hills trial wines. 2. Conduct chemical analysis of grapes and wine. 3. Conduct chemical analysis of leaves. 4. Conduct sensory analysis of trial wines. 5. Conduct data analysis. 6. Prepare a Final Project Report for PIRSA and provide copy to Wine Australia (note not end of project Final Report).	June 2021
B	Information regarding acceptable and unacceptable concentrations of smoke taint and taint marker compounds in grapes and wine, enabling specifications for grapes and wine.	1. Conduct analysis of smoke phenols and phenolic glycosides in microferments from Adelaide Hills and other relevant commercial and experimental wines and/or data sets obtained from across Australia. 2. Conduct sensory analysis of commercial and experimental wines with approaches such as taint rating, descriptive analysis, napping and/or dilution experiments. 3. Conduct data analysis of wine compositional data and comparisons with associated grape and sensory data. 4. Identify candidate wines for GC-MS-O and sensory reconstitution experiments. 5. Conduct sensory analysis and consumer testing of a dilution/blending study to assess consumer rejection level, and relate to wine composition in a white and possibly a red wine.	June 2021
C	Improved strategies for reducing smoke taint in wine	1. Characterise surface properties of a range of commercially available activated carbon products in relation to their fining performance. 2. Identify performance criteria for activated carbons through structure-activity relationship studies. 3. Develop recommendations for sourcing activated carbons with consistent and/or improved fining characteristics. 4. Characterise the impact of at least five yeast strains on smoke phenols and phenolic glycosides in benchtop experiments with red and white grape	June 2021

		juice. 5. If warranted by results from benchtop experiments, verify performance of yeast strains, including a sensory assessment of wine, through small-scale winemaking experiment. 6. Characterise the impact of glycolytic enzymes on selected wines with low free phenol but high phenolic glycoside concentrations.	

Table 2. 2021-22 Deliverables and associated activities

	Deliverables	**Activities**	**Target date**
A	Evaluation of the impact of early-season smoke exposure of grapes on grape composition and wine sensory attributes	1. Repeat chemical and sensory analysis of selected trial wines from the Adelaide Hills study with no taint or a low level of taint from earlier 2020/21 assessments, to track evolution of smoky characters over time. 2. Prepare extension materials and manuscript for peer-reviewed publication. 3. Investigate the potential presence of unknown volatile phenol metabolites in unripe berries to explain increases in glycosides that occurred without further smoke exposure, using techniques such as non-targeted LC-MS analysis of conjugates and analysis of released volatile phenols post-acid hydrolysis.	June 2022
B	Information regarding acceptable and unacceptable concentrations of smoke taint and taint marker compounds in grapes and wine, enabling specifications for grapes and wine	1. Conduct sensory analysis and consumer testing of a dilution/blending study to assess consumer rejection level, and relate to wine composition in a full-bodied red wine. 2. Repeat chemical and sensory analysis of selected trial wines from the 'national' sample set with no taint or a low level of taint from earlier 2020/21 assessments to track evolution of smoky characters over time up to 18 months post-bottling. 3. Conduct sensory experiments to assess masking effects on smoky characters in wine, investigating the effect of attributes such as 'green', 'minty' and 'tropical'. 4. Evaluate potential markers for predicting smoke taint in wine based on grape composition and/or wine composition. 5. Prepare extension materials including for the 18th AWITC and a manuscript for peer-reviewed publication. 6. Develop a practical adoption tool for communicating management strategies, such as a decision tree.	June 2022
C	Improved strategies for reducing smoke taint in wine	1. Conduct experiments aiming to identify potential ways to improve the performance (selectivity and/or efficacy) of activated carbon products. 2. Identify and source commercially available activated carbon products suitable for winemaking application based on their surface properties. 3. Benchmark activated carbon products in small-scale benchtop experiments and, if warranted, in winemaking at a scale suitable for obtaining wine for sensory evaluation.	June 2022

5. Methods

5.1 Grape samples

5.1.1 Adelaide Hills early-season smoke samples

During January to March 2020, Chardonnay, Shiraz and Pinot Noir grapes were sampled from eight blocks per variety in the Adelaide Hills region, following the 2019 Cudlee Creek smoke event. Sites were selected based on the range of smoke exposure. The location of sites and grape sampling protocol are described in full detail in Jiang et al. (2022).

5.1.2 Smoke-exposed grapes sourced from various regions and smoke events

In addition to the Adelaide Hills early-season project described in 5.1.1, at least 12 grape samples of each of the varieties Chardonnay, Shiraz and Pinot Noir were harvested from regions in Victoria, NSW, ACT and SA during February-March 2020. For each variety, the samples represented a wide range of smoke and bushfire scenarios, including no known smoke exposure, mild smoke exposure and heavy smoke exposure. The Geographic Indications included Alpine Valleys, Beechworth and King Valley in Victoria; Adelaide Hills, Barossa Valley, Langhorne Creek and McLaren Vale in South Australia; Cowra, Mudgee, Orange and Riverina in New South Wales; and Canberra District. Approximately 60 kg of each grape variety was hand-harvested at commercial ripeness for table wine styles (approximately 20°Brix). The whole bunches were frozen due to logistical and biosecurity considerations, and transported to WIC Winemaking Services in April 2020 for winemaking commencing in late April. The impact of the SARS COVID-19 pandemic added to this already complicated task. Frozen grapes were thawed slowly at 4°C over the weekend prior to commencement of winemaking. Once thawed, prior to winemaking, a randomised subsample of 2 kg of bunches was taken for grape analysis. Approximately 50 kg of each sample was used for winemaking.

5.2 Winemaking protocol

For both projects, each batch of grapes was fermented into wine under the standard winemaking protocol detailed in Jiang et al. (2022). Chardonnay wines were made by fermenting clarified juice, with minimal skin contact, in line with standard commercial winemaking procedure. Shiraz and Pinot Noir wines were made according to full-bodied red wine style, with six days of skin contact. There was one fermentation replicate per sample, in order to capture as much variation as possible in smoke exposure at vineyard level, rather than replicating winemaking.

5.3 Analysis of volatile phenols and glycosides in grapes, juice, wine and leaves

Volatile phenols (guaiacol, 4-methylguaiacol, *m*-, *o*-, and *p*-cresols, syringol, and 4-methylsyringol) and phenolic glycosides including syringol gentiobioside (SyGG), 4-methylsyringol gentiobioside (MSyGG), phenol rutinoside (PhRG), guaiacol rutinoside (GuRG), cresol rutinosides (rutinosides of *o*-cresol, *m*-cresol and *p*-cresol) (CrRG), and 4-methylguaiacol rutinoside (MGuRG) were quantified in grapes, juice, leaves, and wines.

Grape, leaf and wine samples were prepared for phenolic glycoside analysis as reported previously by Hayasaka et al. (2010, 2013). Volatile phenols were analysed by AWRI Commercial Services (now Affinity Labs) by GC-MS as per Pollnitz et al. (2004). Phenolic glycosides were analysed by UHPLC-MS/MS by the method of Hayasaka et al. (2013).

5.4 Sensory assessment of smoke aroma and flavour in wine

Smoke aroma and flavour were rated by a trained panel known to be sensitive to smoke flavours, with non-smoke-exposed clean controls included in every sample set. Details of the sensory protocols are described in Appendix 6. Sensory evaluation report and Jiang et al. (2022).

5.5 Consumer liking test

For the Chardonnay test, 124 consumers aged 18-65 who were regular drinkers of Chardonnay were recruited from an external market research company database of consumers from Melbourne. The Shiraz study selected regular drinkers of Shiraz from an AWRI database of South Australian consumers (n=111, aged 18-65). For the Chardonnay assessments, five wines were provided to consumers, ranging from a heavily smoke-exposed wine to a clean wine with no smoke exposure, and several dilutions of the two wines. The Shiraz study assessed six unique wines from different vineyards, with different levels of exposure to smoke from bushfires. All consumers were asked to indicate their overall liking of the wines on a nine-point hedonic scale from 'like extremely' to 'dislike extremely'. Prior to each test consumers were asked to complete an entrance questionnaire to obtain demographic information, and on completion, an exit survey to investigate spending behavior and attitudes to food and wine occasions.

5.6 Surface properties of activated carbons

Fifteen different activated carbon products were investigated in the project (Table 3). Carbon PS1300 and FPS were coated with allylamine as a precursor to produce positively charged, amine-functionalised carbons. All the carbons were trialled in contact with 2019 Tasmania Chardonnay and Pinot Noir free run juice at 2 g/L dose rate for 24 hours at 23°C. The details of the juices and the experiment protocol were described previously (Herderich and Krstic 2020). The protocol in this experiment was the same apart from the addition of 42 µg/L to 50 µg/L of volatile phenols to both juices prior to carbon contact. After 24 hours contact, samples were collected for analysis of both volatile phenols and phenolic glycosides.

Table 3. Activated carbon products included in the study

Carbon	Code
PS1300	AC_1
CA50	AC_2
CASPF	AC_3
P1000	AC_4
PS1000	AC_5
Norit SX Plus	AC_6
Smartvin	AC_7
Carbochromas	AC_8
Bentonorit	AC_9
PC1000	AC_10
FPS	AC_11
PC900	AC_12
Toxical	AC_13
Norit D10	AC_14
Deobrett	AC_15

5.6.1 Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy was employed to investigate the functional groups in the activated carbon products. The IRTracer-100 FTIR spectrometer (Shimadzu), equipped with a liquid nitrogen-cooled MCT detector, was used for all measurements. Measurements were performed using the Quest Single Reflection ATR Accessory (Specac), equipped with a diamond ATR crystal. In each case, 128 scans were performed with a resolution of 4 cm^{-1} to achieve a satisfactory signal-to-noise ratio. The ATR effect and atmospheric contributions from carbon dioxide and water vapour were corrected by the background performed on an empty ATR device.

5.6.2 X-ray diffraction (XRD)

The crystallinity of the activated carbon products was analysed with powder X-ray diffraction measurements (PXRD). The PXRD patterns were recorded at room temperature using a

PANalytical X'Pert Pro MPD diffractometer in the Bragg-Brentano reflection configuration. Copper Cu Kα radiation from a sealed tube was used. Data were collected in the 2θ range of 5 - 90° with a step of 0.0167° and exposure per step of 27 s. Since the raw diffraction data contained some noise, the background during the analysis was subtracted using the algorithm of Sonneveld, E. J. and Visser (1975). The data were then smoothed with a cubic polynomial function.

5.6.3 X-ray photoelectron spectroscopy (XPS)

To determine the surface composition of the activated carbon products, XPS analysis was performed. The XPS spectra were obtained with a Specs SAGE XPS spectrometer using an Al Ka radiation source (hm = 1253.6 eV) at 10 kV and 20 mA. Elements present on the sample surface were identified from the survey spectrum recorded in the energy range 0-1000 eV with a pass energy of 100 eV and a resolution of 0.5 eV. The areas under the selected photoelectronic peaks in a broad scan spectrum were used to calculate the percentage of atomic concentrations. High-energy-resolution spectra (0.1 eV) were then recorded for the relevant photoelectronic peaks at passing energy of 20 eV to identify the possible chemical bonding environments for each element. All binding energies were referred to the neutral carbon C1s peak at 285 eV to compensate for the effect of surface charge. Data processing and curve fitting were performed with the Casa XPS software.

5.6.4 Scanning electron microscopy/Energy dispersive x-ray (SEM/EDX)

The SEM was used to determine the morphology of the activated carbon products, while the bulk elemental composition of the products was determined with EDX. An FEI Quanta 450 FEG-ESEM with an EDAX Apollo X energy dispersive x-ray (EDX) spectrometer was used for the analysis.

5.6.5 Zeta potential measurements

The electrophoretic mobility of activated carbons was determined using Zetasizer Nano ZS (Malvern, UK) and transformed into zeta potential using the Smoluchowsky equation for recognition. Measurements were carried out at 22°C in wine or juice.

5.6.6 Plasma deposition

Plasma polymerisation of allylamine was carried out in a custom-built, bell-chamber reactor. To allow for coating of the activated carbons, a shaking platform was added inside the plasma reactor that provided a random motion of the particles. Allylamine precursor was deposited at 2.3×10^{-1} mbar. The deposition time was 10 minutes. To achieve sufficient coating stability and functional group retention, allylamine was deposited using a power of 40 W.

5.7 Evaluation of yeast strains

Two juices made from smoke-affected Tasmanian grapes from 2019, a Chardonnay and a Pinot Noir free run juice, were used for the benchtop ferments. The details of the selection of smoke-affected grapes and the juice processing were described in Culbert et al. (2021a). All grape juices were defrosted overnight at 4°C and sterilised by filtration (0.2 µm sterile filter) before conducting fermentation. Yeast available nitrogen was adjusted to 250 ppm in the 2019 free-run Chardonnay juice by adding 14 mL of 37 g/L sterile diammonium phosphate (DAP).

Seven yeast strains were evaluated in both red and white juices. Five *Saccharomyces cerevisiae* yeasts: AWRI 1502, AWRI 1503, AWRI 1426, AWRI 1964 (SIHA3) and AWRI 1498 (EC1118) and two non-*S. cerevisiae* yeasts, *Wickerhamomyces anomalus and Rhodotorula glutinis,* were selected. All the *S. cerevisiae* yeast strains were sourced from the AWRI wine microorganism culture collection. The two non-*S. cerevisiae* yeasts had been previously isolated from uninoculated wine ferments. Each yeast strain was firstly grown in 5 mL of YPD medium in a 10 mL centrifuge tube at 28°C and 120 rpm agitation. A volume of 1 mL of yeast culture was then transferred into 20 mL of 50% diluted grape juice and grown overnight at 22°C. This culture was used to inoculate 80 mL of grape juice at 0.1 OD_{600} nm.

Fermentations were performed in triplicate in 100 mL Schott bottles containing 80 mL of grape juice. Ferments were weighed every day to monitor fermentation kinetics. Once ferments stopped decreasing in weight, they were tested for residual glucose and fructose to confirm the completion of fermentation. When all ferments had stopped decreasing in weight, all the three replicates of white ferments conducted by yeast AWRI 1426, AWRI 1502 and *R. glutinis* had residual sugar over 5 g/L in the must. The wines were transferred to 4°C temperature-controlled room for cold stabilisation and cold settling for three days before racking off gross lees. Samples were collected for analysis of basic chemistry, volatile phenols and phenolic glycosides, as previously described (Hayasaka et al. 2013).

5.8 Evaluation of glycolytic enzymes

Previous work had evaluated the efficacy of various enzyme preparations to hydrolyse phenolic glycosides, and identified enzymes that were particularly effective. Trenolin Bouquet Plus (Erbsloh) is described as a depsidase-free (free from cinnamoyl esterase activity), aroma-releasing special enzyme and Rapidase Relevation Aroma (Oenobrands) is a microgranulated pectolytic enzyme preparation with the four essential α and ß-glycosidases activities. Trenolin Bouquet Plus was particularly effective, able to hydrolyse gentiobiosides SyGG and MSyGG and rutinosides CuRG, CrRG, PhRG and MGuRG. Rapidase was effective at hydrolysing gentiobiosides SyGG and MSyGG, but had less activity towards rutinosides. All enzymes tested to date were more effective in white wines than red wines (Herderich and Krstic 2020). Wines high in glycosides but relatively low in volatile phenols were selected to conduct the enzyme trial, in order to be able to observe the effect on both volatiles and glycosides, and sensory characteristics. A 2019 Tasmanian Chardonnay wine made from free-run juice, a heavily smoke-affected 2020 Adelaide Hills Chardonnay (201722—CHA_K) and two Shiraz wines from Orange, NSW (201826 and 201827) were selected. The winemaking details were described previously (Culbert et al. 2021a, Jiang et al. 2022). Enzymes were tested in the wines according to the manufacturer's suggested addition rates.

All treatments were conducted in duplicate at 15°C for three weeks following the trial plan, as summarised in Table 4.

Table 4. Enzyme treatment scheme

Wine	**Treatment**	**MilliQ water addition**	**Trenolin Bouquet Plus addition**	**Rapidase solution (1 g/10 mL) addition**
Wine 1 – 2019 Tas CHA	Control (no addition)	15 mL/hL	-	-
	Trenolin Bouquet Plus	-	15 mL/hL	-
	Rapidase 5g/hL	-	-	5 g/hL
Wine 2 – AH CHA 22	Control (no addition)	15 mL/hL	-	-
	Trenolin Bouquet Plus	-	15 mL/hL	-
Wine 3 – Shiraz national 26	Control (no addition)	15 mL/hL	-	-
	Trenolin Bouquet Plus	-	15 mL/hL	-
	5g/hL Rapidase	-	-	5g/hL
Wine 4 – Shiraz national 27	Control (no addition)	15 mL/hL	-	-
	Trenolin Bouquet Plus	-	15 mL/hL	-

After the three weeks of enzyme contact at 15°C, 10 g/hL of 5% w/v bentonite solution was added to a 50 mL subsample of each treatment to remove the enzyme prior to chemical analysis. Supernatants were collected after centrifugation, and the volatile phenols and glycosides were analysed as described for wine samples above. The wines were assessed for smoke aroma and smoke flavour in an informal bench tasting by trained panellists experienced in smoke assessment.

5.9 Masking of smoke aroma by other sensory attributes

In various sensory evaluations of smoke-affected wines, masking of smoke aroma and flavour by other sensory attributes was observed. Examples include wines with strong eucalyptus, minty, or tropical characters that were unexpectedly low in smoke flavour. In order to test the masking effect of other sensory attributes on smoke aroma, a moderately smoky Pinot Noir wine was made by blending a strongly smoky 2019 Tasmanian Pinot Noir wine with a non-smoke-exposed commercial Pinot Noir (2021 Minchinbury Pinot Noir) in 50:50 ratio, which was

then spiked with various compounds at two concentrations as listed in Appendix 7. The spiked wines were assessed for aroma only in a bench tasting with a panel of experienced tasters known to be sensitive to smoke.

6. Results and discussion

6.1 Evaluation of the impact of early-season smoke exposure of grapes on grape composition and wine sensory attributes

All planned activities for financial year 2020/21 were completed and summarised in a final report and technical report to PIRSA South Australian Wine Industry Development Scheme on 27 May 2021. The final report and technical report were provided to Wine Australia on 30 August 2021 and are included in Appendix 5.

Highlights:

- The Adelaide Hills early-season smoke project was shortlisted for the 2020 SA Science Excellence Award for Excellence in Research Collaboration
- WenWen (Maddy) Jiang was awarded the best presentation at Crush 2021, the Wine and Grape Science Symposium, for her presentation 'Phenolic glycosides in leaves as biomarkers for pre-véraison smoke exposure'

Briefly, pre-véraison smoke exposure of vineyards was shown to have impacts on grape phenolic composition, wine composition and sensory attributes. A total of 23 wines were made, sourced from vineyards with varying degrees of exposure to fire and smoke. Grapes from three timepoints during ripening, and wines at key stages were analysed for smoke markers, including volatile phenols, glycosides and other chemical compounds relevant to the sensory impact of smoke in wine. Phenolic glycosides were present at elevated concentration in most of the grape samples suspected of smoke exposure, with a range that matched anecdotal observations of smoke plume. Leaves were also sampled during the grape ripening season and analysed for phenolic glycosides. Leaves from smoke-affected vineyards had concentrations around ten times higher than berries on a per mass basis at harvest. Some varietal differences were observed. Similar concentrations were observed in the leaves sampled four weeks after smoke exposure and at harvest.

Sensory assessment was conducted six weeks after bottling and repeated six months later, with similar results. Most wines were rated similarly or slightly lower in smoke flavour after six months of ageing.

The rank order of 'smoky' sensory attributes in the wines was consistent with the rank order of blocks throughout ripening, shown in Figure 1 for Pinot Noir. Chardonnay wines had much lower intensity of smoke flavour compared to the red wines, due to the reduced skin contact in white winemaking.

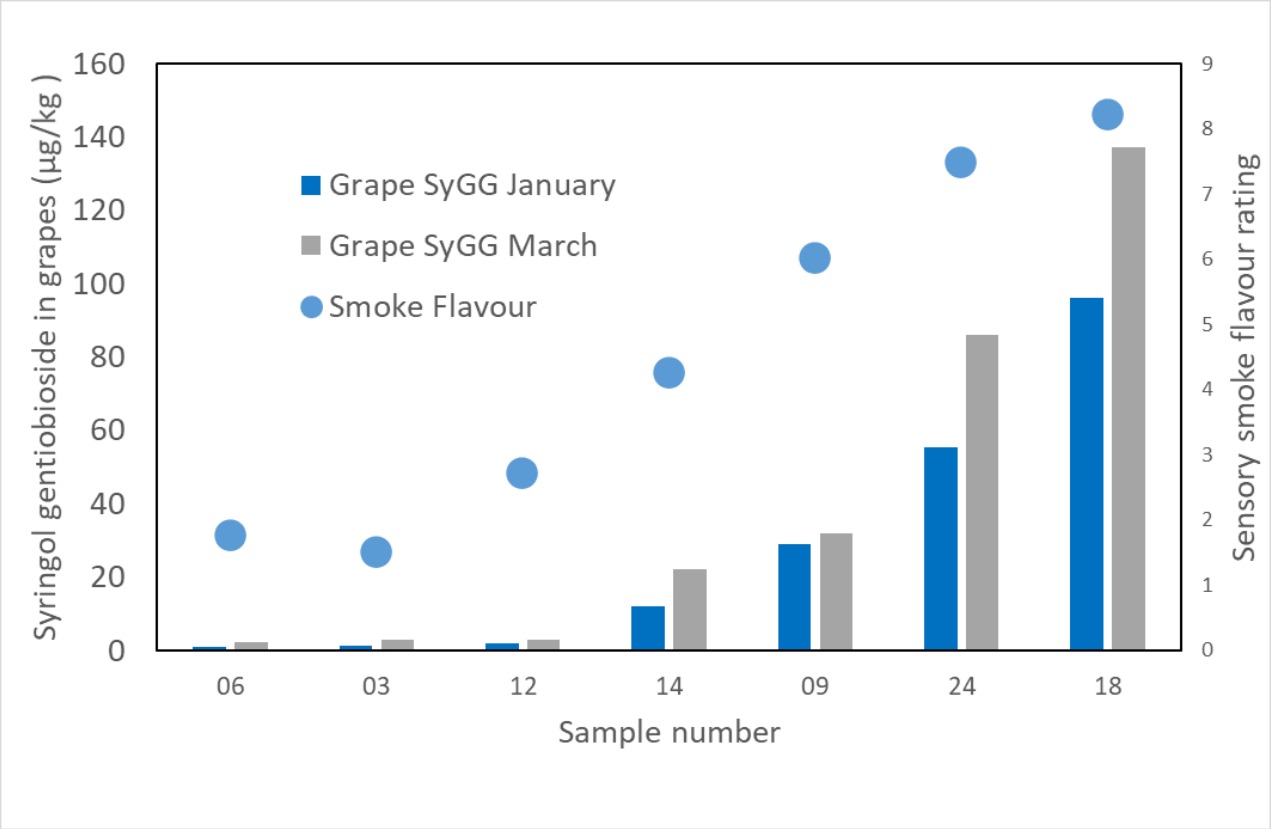


Figure 1. Key smoke exposure marker, syringol gentiobioside in Pinot Noir grapes increased in concentration (per kg) from January to March 2020, when the grapes were harvested, despite berry weight more than doubling from average 0.42 g/berry in January to 0.98 g/berry at harvest. Blue bars indicate syringol gentiobioside concentration in grapes in January, grey bars indicate syringol gentiobioside concentration in grapes in March, and blue dots indicate sensory smoke flavour rating. Sensory smoke flavour rating in wine corresponded to the concentration of syringol gentiobioside in grapes. Vineyards 3 and 6 were not exposed to smoke, vineyards 9, 12 and 14 had suspected smoke exposure, and vineyards 18 and 24 had smoke exposure and mild fire damage within vineyards.

6.1.1 Evolution of smoky characters over time

Chemical and sensory analysis was repeated on the entire Adelaide Hills wine set, to enable comparisons between the sensory results at the different time points. Most wines were rated similarly or slightly lower in smoke flavour after approximately 21 months of bottle ageing. Volatile phenol and phenolic glycoside concentrations remained consistent over this timeframe. These observations are in line with published data on aged smoke-affected red wines (Ristic et al. 2017), and indicate that differences in perception in smoke characters are likely to be due to changes in the wine matrix, such as decreased primary fruity characters which would be expected to mask smoke characters in young wines.

6.1.2 Extension materials and manuscript for peer-reviewed publication

A manuscript titled 'The effect of pre-véraison smoke exposure of grapes on phenolic compounds and smoky flavour in wine' has been accepted for publication in the *Australian Journal of Grape and Wine Research*.

Outcomes specifically related to early-season smoke exposure were presented at the 18th AWITC in a poster and in a workshop 'Smoke taint: the latest research'. Early-season smoke outcomes were presented during four (virtual) roadshows to Hunter Valley, Mornington Peninsula, Canberra and Orange wine regions. A full list of extension and communication activities is provided in Appendix 1.

6.1.3 Investigations into the potential presence of unknown volatile phenol metabolites in unripe berries

Pinot Noir grapes from fire and smoke-exposed vineyards showed increases in glycosides from January to March, despite no further smoke exposure. Replicated vineyard samples from eight different locations in the Adelaide Hills, sampled in January and March, were used to investigate the potential presence of other metabolites to explain these differences. The eight vineyards chosen represented control (2), suspect (3), and smoke-affected (3) vineyards. Note that the 'smoke-affected' vineyards also had signs of fire activity within the vineyard such as burnt grass in the swathe. Subsequent, targeted analysis identified that two 'suspect' vineyards were equivalent to the 'control' vineyards, while the remaining 'suspect' vineyard was most similar to the 'smoke-affected' group.

Extensive method development was undertaken to optimise methods for both reverse phase (RP) and hydrophilic interaction chromatography (HILIC) for the analysis of non-polar and polar metabolites, respectively. Separation and detection were performed using high-performance liquid chromatography coupled to orbitrap-high-resolution mass spectrometry (HPLC-HRMS). Pre-véraison green berries proved challenging to analyse, and the unusual matrix caused several major problems with instrumentation. Further method development will be required for any further analysis on pre-véraison grapes.

Nontargeted RP-HPLC-HRMS with positive electrospray ionisation identified 40,000 compounds in the grape samples. The compounds with best quality peaks, detailed mass spectral information (MS2), consistent in master mix quality control samples across the run, and abundance highest in fire and heavily smoke-affected vineyards compared to non-smoked controls were prioritised, resulting in 48 curated peaks. These peaks were compared with databases of structures and mass spectra and tentative identifications assigned where possible; further resources will be required to unambiguously confirm these identifications.

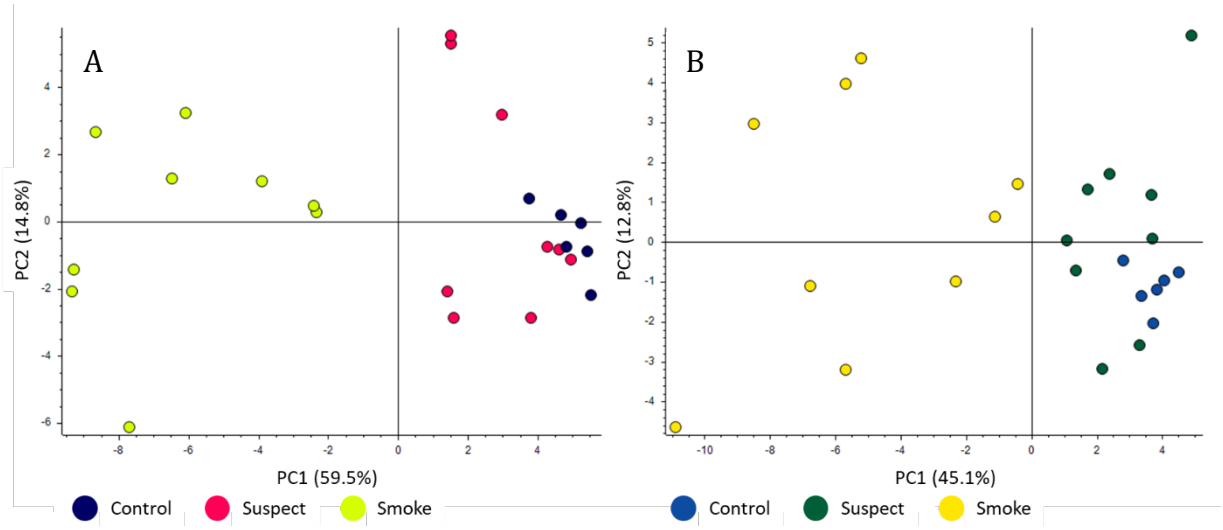


Figure 2. Principal component analysis of Pinot Noir grapes affected by early-season smoke sampled in A: January (TP1) (pale green circles) and at B: commercial harvest in March (TP3) (yellow circles), compared to non-smoke-exposed control samples taken at both time points (navy and dark blue circles). 'Suspect' grapes with intermediate levels of smoke exposure were also collected (red and dark green circles). Targeted analysis indicated that these grapes were more associated with the 'control' grapes, which was corroborated by the untargeted analysis.

Figure 2 clearly shows that the 48 identified compounds are important chemical identifiers for the separation of 'clean' vs 'smoke-affected' grapes at both pre-véraison (TP1) and commercial harvest (TP3). Of the tentatively identified 48 compounds, five of the most important have been identified and future work will further explore them.

Polar compounds were investigated using HILIC-HPLC-HRMS, resulting in 4,000 compounds detected using positive electrospray ionisation. Quality filters similar to those used above reduced that to 46 curated compounds. Again, tentative identifications using spectral databases were made where possible.

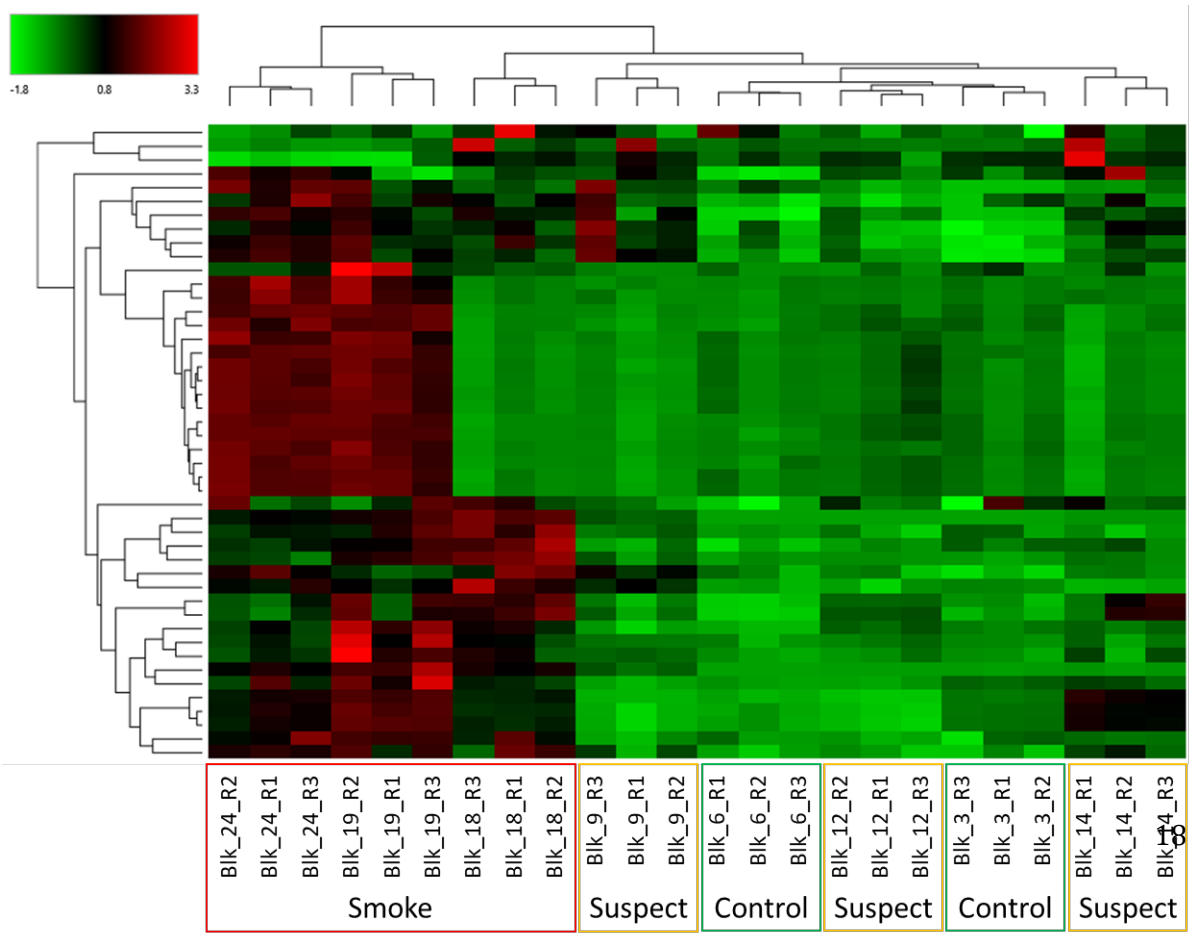


Figure 3. Two-way hierarchical cluster analysis of polar metabolites in Pinot Noir grapes affected by early-season smoke sampled in January (TP1), compared to non-smoke-exposed control samples (Blk 3, 6). Samples which had experienced a high amount of smoke (Blk 18, 19, 24) were well differentiated from control and suspect samples by the detected chemical markers (horizontal lines). Red cells indicate an increase in analyte for that sample and green indicates a decrease.

Figure 3 highlights differences between the samples, with January samples from smoke- and fire-affected vineyards (Blk 18, 19, 24) strikingly different from both the non-smoked controls (Blk 3, 6) and the suspected smoke-exposed samples (Blk 9, 12, 14). It was interesting to note that at least 16 compounds were abundant in the samples from two of the fire and smoke-exposed vineyards (Blk 24 and 19) but not the third fire and smoke-exposed vineyard (Blk 18) or the suspected smoke-exposed or non-smoke-exposed samples.

Compounds were better tentatively identified in the reverse phase extracts as the compound libraries used for assignment are more comprehensive for these analyses. Up-regulated compounds in smoke-exposed samples tentatively identified thus far have glycosylated and/or conjugated structures. Additionally, there is evidence to suggest increased amounts of some amino acids and associated compounds. It is evident that there is broader chemistry occurring beyond the current measured markers. Further research is required to confirm the identity of the compounds and understand their role in the plant and relevance to wine.

6.2 Information regarding acceptable and unacceptable concentrations of smoke taint and taint marker compounds in grapes and wine, enabling specifications for grapes and wine

6.2.1 Analysis of smoke-related volatile phenols and phenolic glycosides in smoke-affected grapes and wines

Several sample sets were sourced during vintage 2020, with a range of smoke exposure, including grapes exposed to bushfire smoke and non-smoke-exposed controls. Two large studies produced wine under controlled conditions at 50 kg scale, with no efforts to mitigate the impact of the smoke, enabling grape composition, wine composition and formal sensory analysis to be compared in a large number of samples. These were:

- The study on early-season (pre-véraison) smoke exposure in the Adelaide Hills described in section 7.1 above, with 23 wines produced under controlled conditions at 50 kg scale and bottled. (See Appendix 5 for results from this trial.)
- An additional 42 grape samples sourced from various regions and multiple smoke events, which were also made into wine under controlled conditions at 50 kg scale and bottled.

Volatile phenols and phenolic glycosides were analysed in subsamples from the 42 grape batches from various regions. The samples included Chardonnay, Pinot Noir and Shiraz with a range of smoke exposure including clean controls and moderately and heavily smoke-exposed samples. All control grape samples were consistent with non-smoke-exposed samples for all smoke markers, both volatile phenols and phenolic glycosides, with the exception of one of the Shiraz grape samples which had a slightly elevated concentration of guaiacol. In the smoke-exposed samples, the most abundant smoke marker was syringol gentiobioside (SyGG). Of the volatile phenols, guaiacol was the most abundant in the grapes, and *o*-cresol was also elevated in many of the smoke-exposed Chardonnay and Pinot Noir samples. Syringol and methylsyringol were at or below the limit of quantitation in all of the grape samples.

Volatile phenols and phenolic glycosides were analysed in 42 experimental wines from various regions, made under controlled conditions with no efforts to remediate the effect of the smoke. All control wine samples were consistent with non-smoke-exposed samples for all smoke markers, both volatile phenols and phenolic glycosides, with the exception of one of the Shiraz wine samples which had a slightly elevated concentration of guaiacol. In the smoke-exposed wines, the most abundant smoke marker was syringol gentiobioside (SyGG). Guaiacol was the

most abundant volatile phenol in the wines, and cresols were also abundant in the heavily smoke-exposed red wines. Syringol was elevated in many of the wines, but methylsyringol was rarely detected above values commonly seen in non-smoke-exposed wines. The Chardonnay wines had relatively low concentrations of volatile phenols compared to the red wines, likely due to the removal of skins during the winemaking process. The Pinot Noir wines had higher concentrations of cresols than the Shiraz wines, which had higher concentrations of guaiacol.

Volatile phenols and phenolic glycosides were analysed in an additional 110 micro-ferments from the Adelaide Hills, that had been produced and assessed by Adelaide Hills winemakers. Micro-ferments were generally conducted on grapes sampled at least two weeks prior to intended harvest data, and winemakers commented on the challenges associated with producing these ferments in open containers, finding them prone to oxidation and other wine faults. Winemakers also reported challenges in assessing the highly acidic young wines at the completion of alcoholic fermentation. Nevertheless, there was a good ability to predict taint from the compounds measured, with several volatile phenols, and some specific glycosides, being most important. Models for taint gave relatively low false positive and false negative rates. A higher rate of false positives (sensory classification of 'taint' in a sample classified as 'non-smoke-exposed' according to smoke markers) occurred in the micro-ferments compared to the 50 kg scale wines, due to the challenging nature of producing and assessing micro-ferments.

6.2.2 Sensory analysis of smoke-affected wines

Sensory analysis was conducted on the 42 experimental wines with varying degrees of smoke exposure six weeks after bottling. The sensory report is provided in Appendix 6. Briefly, the red wines had a wide range of smoke aroma and flavour scores, whereas the Chardonnay wines had lower scores for smoke aroma and flavour. Control wines generally had low scores for smoke aroma and flavour, showing the sensory panel was performing well. Not all smoke-exposed samples had smoke ratings significantly higher than the control wines, and some smoke-exposed wines had lower ratings of smoke flavour than the controls. Some wines exhibited strong 'green', 'eucalyptus' and 'tropical' notes which masked smoke flavour, and some wines had 'struck flint' and 'reduced' notes that could be confused with smoke.

6.2.3 Comparing wine composition with grape composition and sensory data

Models for predicting wine smoke flavour were generated from wine compositional data for each of the three cultivars. Separate models were constructed for the two studies (i.e. the Adelaide Hills early-season wines and the wines sourced from various regions/multiple smoke events). Smoke flavour was generally modelled well by PLS regression analysis based on wine guaiacol, cresols (*o*-, *m*-, and *p*-cresol) and phenolic glycosides, specifically guaiacol rutinoside and cresol rutinoside (R^2>0.90). Guaiacol and *m*-cresol were important predictors to all sets, and *o*-cresol, *p*-cresol, guaiacol rutinoside and cresol rutinoside were important for some sets. Syringol and syringol gentiobioside were not important. These results were in line with previous observations on smoke-affected wines from various regions, vintages and varieties (Mayr et al. 2014). The best models were obtained for Pinot Noir. Sensory scores for Chardonnay wines were quite low, which limited the model development.

The smoke flavour models were not significantly influenced by basic wine parameters such as alcohol, pH, TA, and residual sugar, indicating that the variation in basic wine composition was not generally problematic. The effect of the basic wine composition was of most concern with the Chardonnay wines sourced from various regions, because the two unsmoked control wines were both quite high in alcohol, and one also contained residual sugar. Smoke markers were highly correlated, with some varietal differences in line with previous observations (Hayasaka et al. 2013). The sum of (guaiacol + *m*-cresol + *o*-cresol + *p*-cresol) in wine enabled very good prediction of smoke flavour intensity, even when compounds were below individual thresholds. In summary, the compounds key to smoke flavour were identified as guaiacol, *o*-, *m*-, and *p*-cresol and glycosides, specifically guaiacol rutinoside and cresol rutinoside.

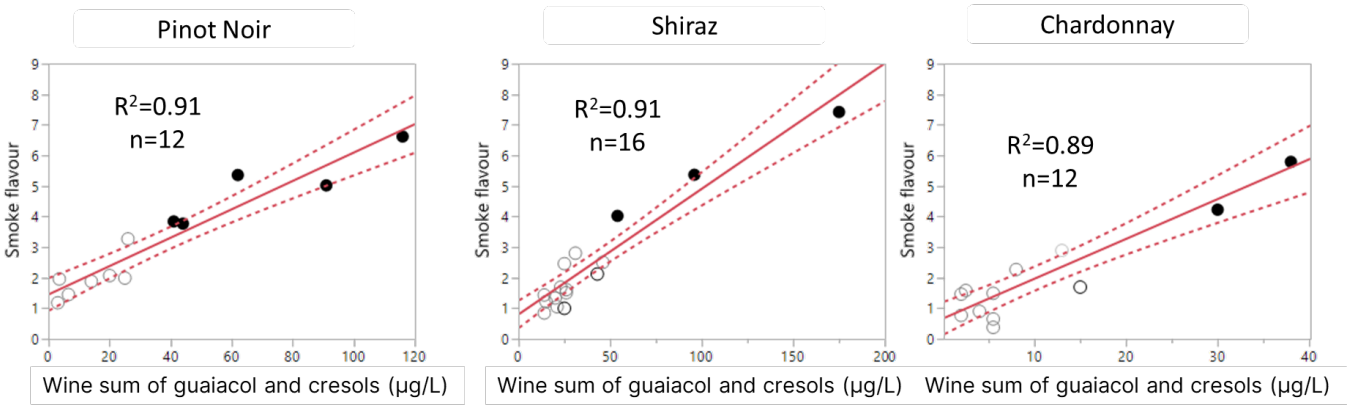


Figure 4. The sum of guaiacol and cresols (*m*-cresol, *o*-cresol and *p*-cresol) in wine was highly correlated with smoke flavour in each of the three varieties. Samples marked with black dots had smoke flavour rated significantly higher than non-smoke-exposed control wines. The smoke flavour was not significantly higher than controls for the samples marked with open circles.

Grape composition was compared to wine composition. For Chardonnay, ferments were conducted on clarified juice in line with commercial white wine production, so extraction from skins was minimised. This resulted in much lower concentrations of volatile phenols in wines compared to the concentrations found in grapes, because volatile phenols concentrated in grape skins were removed. Note that the analysis of grapes involves homogenisation and exhaustive extraction from the whole grapes. On the other hand, the concentrations of the glycosides in the wines were similar to those found in the grapes. A variable proportion of glycosides persisted in the wine compared to the concentration in the grapes: some glycosides were lower in concentration in the wines than in the grapes, and some were higher in the wine. Shiraz and Pinot Noir wines had similar concentrations of glycosides compare to the concentration in the juice, with slightly lower concentrations of syringol gentiobioside and methylsyringol gentiobioside and higher concentrations of rutinosides, with some individual samples showing other patterns. Volatile phenols were much higher in the Shiraz and Pinot Noir wines than the grapes. Guaiacol, cresols, guaiacol rutinoside and cresol rutinosides in grapes were strongly related to the concentrations of key phenols in Chardonnay and Pinot Noir wine, and the associations were weaker in Shiraz.

The relationships between the key markers in grapes (guaiacol, cresols, guaiacol rutinoside and cresol rutinosides) and smoke flavour in wine were explored. PLS regression models were developed for each variety (R^2>0.86). The compounds *m*-cresol and guaiacol were most important to all models, and *o*-cresol, guaiacol rutinoside, *p*-cresol and cresol rutinoside were also important to some models. Grape syringol and syringol gentiobioside were not important in predicting smoke flavour in wine yet are reliable smoke exposure markers in grapes.

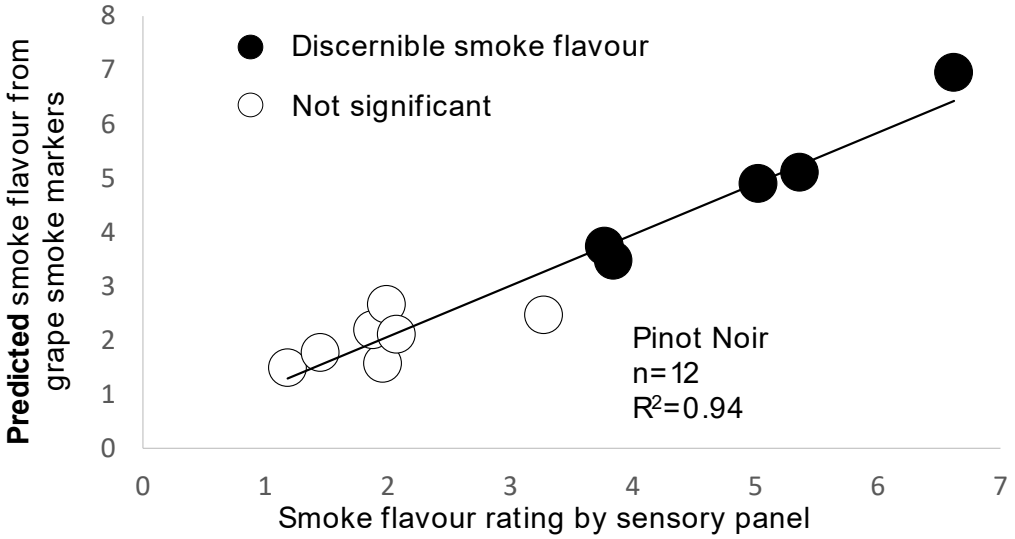


Figure 5. Smoke flavour in Pinot Noir wine predicted from smoke markers in grapes by partial least squares regression analysis

6.2.4 Consumer response to smoke-affected wines

Two consumer tests were conducted with smoky wines from the 2020 vintage, both of which clearly showed that consumer liking is negatively affected by smoke flavours in smoke-exposed wines.

A dilution study to assess consumer rejection threshold in Chardonnay was conducted with 124 consumers. The results showed that consumers responded negatively to 50% or more of the smoke-affected Chardonnay blended in a clean control wine, with many consumers also giving low liking scores to the 25% blend.

A second consumer liking test was conducted on a set of smoke-affected Shiraz wines from the 2020 Adelaide Hills study. Mean consumer liking scores were strongly negatively correlated with the smoke flavour ratings from the trained sensory panel, showing that consumers dislike bushfire smoke flavour. Most consumers (53%) were somewhat sensitive to the smoke flavour, with significantly lower liking for three of the wines with smoke flavour. The second biggest group of consumers (28%) was highly sensitive to smoke flavour, with low acceptance for all five smoke-affected wines. The liking scores of a small proportion of the consumers (19%) did not relate to the smoke flavour intensity.

A third consumer liking test using a smoke-affected 2019 Tasmanian Pinot Noir rosé dilution study was conducted prior to this project (Krstic and Herderich 2020) and had similar results.

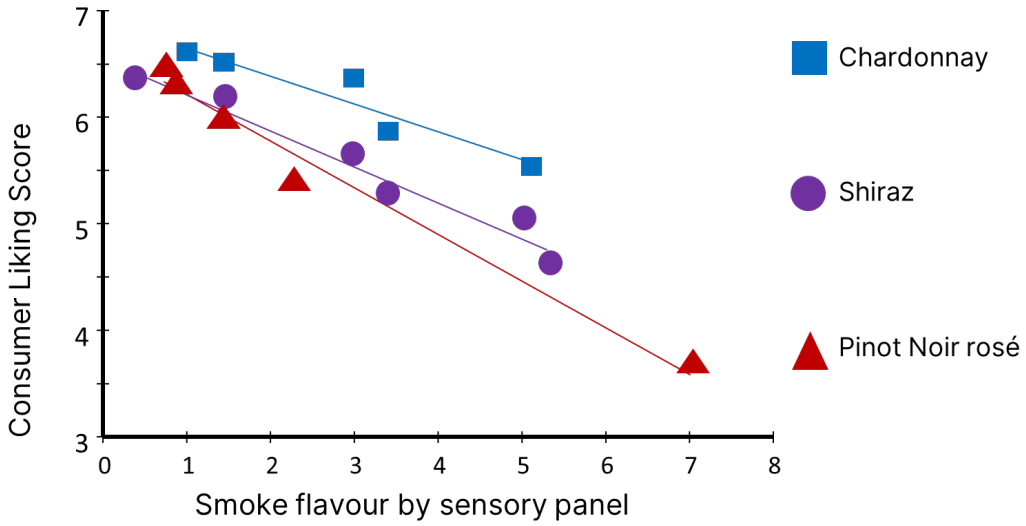


Figure 6. Consumer liking was negatively related to smoke flavour in three separate studies on unoaked Shiraz, unoaked Chardonnay and Pinot Noir rosé wines.

A manuscript has been prepared addressing the consumer response to smoke-affected wines. It includes results from three consumer liking studies: the smoke-affected Shiraz wines from 2020, the smoke-affected 2020 Chardonnay dilution study, and a smoke-affected 2019 Tasmanian Pinot Noir rosé dilution study conducted prior to this project (Krstic and Herderich 2020).

6.2.5 Evolution of smoky characters over time

Chemical and sensory analysis was repeated on all 63 wines after 21 months of bottle ageing and the results were compared to the young wines assessed six weeks after bottling. Two Pinot Noir wines were excluded from sensory analysis due to dominating 'nail polish remover' characters. Considering the sensory analysis first, the 'smoke flavour' rating did not increase in most wines over this time period. In the young wines, 21 had smoke flavour significantly higher than control wines, and 18 of those wines continued to have smoke flavour significantly higher than control wines after 21 months in bottle. 'Smoke' flavour decreased in three wines to become non-significant at 21 months. In 30 of the young wines, the smoke flavour was not significantly higher than control wines. Of these, 'smoke' flavour increased in five wines (four Chardonnay and one Shiraz) to become significant.

The concentrations of volatile phenols and glycosides were compared between the two time points. Concentrations of most volatile phenols and glycosides remained constant, within the method's measurement uncertainty. There was a trend towards increased volatile phenols and some glycosides (rutinosides) in highly smoke-affected wines, as well as a trend to increasing syringol. This is in line with published data on smoke-affected red wines aged for up to six years (Ristic et al. 2017). The increases in 'smoke' flavour are likely to be due to declining primary fruit flavour and other characters which mask low-level 'smoke' flavour in young wines, particularly in Chardonnay.

6.2.6 Masking of sensory smoky characters in wine

Wines exhibiting masking of smoke characters by 'green', 'eucalyptus' and 'tropical' notes were identified, along with wines exhibiting 'struck flint' and 'reduced' notes that can be confused with smoke. Smoke flavour was modelled well by the volatile phenols and phenolic glycosides, so GC-MS-O experiments were not deemed necessary. The masking of smoke characters was identified as an effect worthy of further investigation. Masking of smoke characters is important to take into account when assessing potentially smoke-exposed samples, and can also be used as a mitigation tool, when blending smoke-affected wine.

This has both positive and negative implications for industry. Some wines with sufficient fruit flavour intensity are likely to not be considered smoky by assessors, despite elevated concentrations of volatile phenols and phenolic glycosides. These wines may be appropriate for commercial sale. For other wines, the elevated concentrations of smoke compounds may have been high enough to be observed by assessors; however, if other characteristics (e.g. reduction or oxidation) are masking the smoke, this would result in a low smoky sensory score, but would not necessarily mean the wines would be appropriate for commercial sale.

A masking trial was conducted to assess the ability of certain compounds to obscure smoke characters. A variety of volatile compounds, at different concentrations, was included to determine if specific compounds (and hence production methods) could be used to mask any smoke character present in the wine. The results indicated that 'green', 'minty', and 'eucalyptus' characters from compounds such as iso-butyl methoxypyrazine and 1,8-cineole, and oak compounds such as vanillin, have potential to mask smoke characters in wine. See Appendix 7 for details.

6.2.7 Develop a practical adoption tool for communicating management strategies, such as a decision tree

A guide has been developed to assist the assessment of risk of producing smoky wine from smoke-exposed grapes. Twelve conversations across the sector have identified early adopters keen to support co-design and testing of decision tools.

The guide is attached in Appendix 8.

6.2.8 Extension materials and activities

A presentation titled 'Smoke flavour: linking chemical composition and sensory properties in smoke-affected wines' was presented in one of the Fresh Science plenary sessions at the 18th AWITC in Adelaide in June 2022.

A workshop titled 'Smoke taint: the latest research' was also convened at the 18th AWITC. The workshop was developed and delivered in conjunction with Leigh Schmidtke and Sijing Li from Charles Sturt University and Kerry Wilkinson from the University of Adelaide, alongside AWRI staff Mango Parker (convenor and presenter), Damian Espinase Nandorfy, Eleanor Bilogrevic, WenWen (Maddy) Jiang and Adrian Coulter.

Four posters directly relating to the project outcomes were presented at the 18th AWITC, and the poster titled 'Smoke flavour: linking chemical composition and sensory properties in smoke-affected wines' was awarded the prize for best oenology poster.

Key results were presented at four online presentations in NSW and Victorian regions.

A manuscript is in preparation to be included in the conference proceedings which will be published as a special edition of the *Australian Journal of Grape and Wine Research*. A manuscript focusing on the consumer response to smoke-affected wines, including the study on the Adelaide Hills Shiraz wines and the dilution series of smoke-affected Chardonnay wines from this project, as well as a consumer study conducted prior to this project, has been prepared.

In addition to these activities, project staff supported the finalisation of prior work undertaken during the previous smoke taint project (funded by the Australian Government's Rural R&D for Profit program), and collaborations with University of Adelaide researchers, resulting in six peer-reviewed publications and several fact sheets.

A list of communication and extension activities is provided in Appendix 1.

6.3 Improved strategies for reducing smoke taint in wine

Many strategies have been proposed to reduce smoke taint in wine, and there is a plethora of products and services available on the market. Published data have identified the strategies that have a direct effect on the concentrations of volatile phenols and glycosides as well as the sensory characters in the wine. Among these, yeast strain, glycosidase enzymes and activated carbons have shown the most promise, and are easily accessed and implemented by winemakers (Mirabelli-Montan et al. 2021, Herderich and Krstic 2020, Culbert et al. 2021a, Culbert et al. 2021b, Ristic et al. 2011). These were all addressed in the current project.

6.3.1 Surface properties of activated carbons related to fining performance

Fifteen commercially available activated carbon products were selected for surface characterisation based on their ability to remove smoke glycosides and/or volatile phenols from wine and juice. The carbon products are listed in Table 3, and their performance and physical characteristics are summarised in Figure 7. Three products, PS 1300, CA 50, and CASPF, performed best in removing smoke glycosides, and two, Carbocromos and FPS, performed best in removing volatile phenols. Norit D10 was ineffective in eliminating smoke glycosides but removed volatile phenols moderately. Smartvin removed smoke glycosides (not as well as PS1300, CA50, and CASPF) but was not as effective for volatile phenols. The other seven carbon products tested had relatively little effect on the removal of smoke compounds.

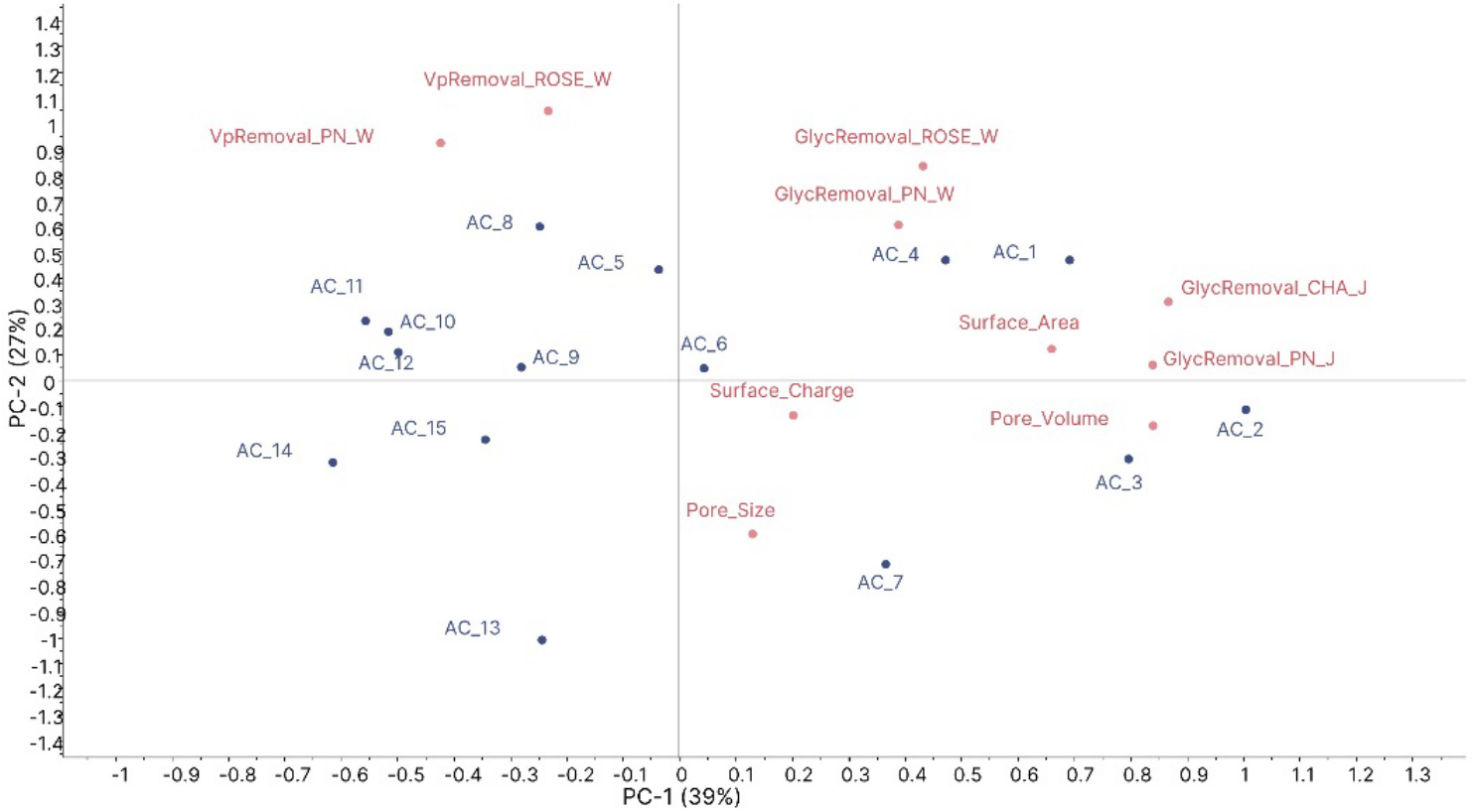


Figure 7. Principal component analysis biplot showing efficacy of fifteen activated carbon products (AC_1 to AC_15 blue dots) at removing volatile phenols (VpRemoval) and phenolic glycosides (GlycRemoval) from juice and wine and their associated physical properties (red dots). J=juice, W=wine, Vp=volatile phenols, PN=Pinot Noir, CHA=Chardonnay. The removal of volatile phenols and phenolic glycosides was determined by comparing the sum of seven volatile phenols and six phenolic glycosides in the treated matrix compared to the non-carbon treated matrix. The surface charge was measured in the Chardonnay juice only.

According to the X-ray diffraction (XRD), some carbon products were identified as amorphous (e.g. Carbocromos, CASPF and FPS), and others as semi-crystalline (e.g. Smartvin, PS 1300, Norit D10 and Ca 50). The surface and bulk elemental composition of the carbon products showed that all materials were predominantly carbonaceous (74-98%) and contained appreciable amounts of oxygen. Depending on the product, other elements were also detected (e.g. potassium, silicon, aluminium, calcium). The scanning electron microscope (SEM) images showed irregularly shaped surface morphology, which was consistent with the XRD results, showing that these materials were amorphous or semi-crystalline. Some examples are shown in Figure 8. To determine the surface charge of activated carbons, their zeta potential was measured in the juice after 24 hours of contact time. Overall, the results indicated that activated carbon products with higher zeta potential values were more effective in the removal of glycosides.

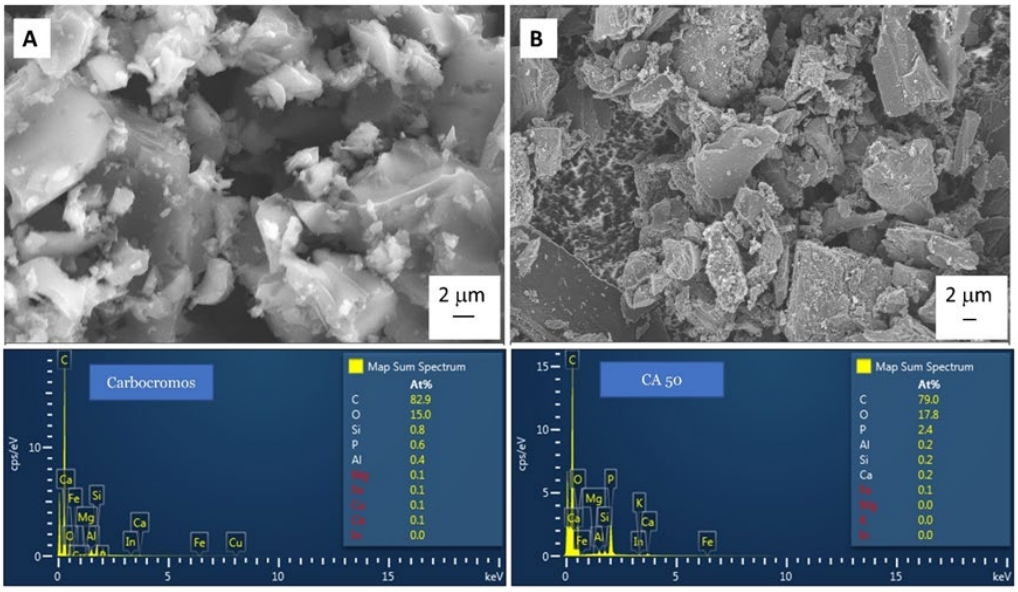


Figure 8. Scanning electron microscope images and elemental composition of (A) Carbocromos and (B) CA 50 activated carbon products

The surface functional groups of activated carbon products were analysed using FTIR spectroscopy. Figure 9 compares the FTIR spectra of seven carbon materials. The infrared transmission peak recorded at the wavelength range of 3200-3650 cm^{-1} exhibited a wide and broad transmission band with its maximum at ~ 3,430 cm^{-1} associated with O-H stretching vibration of hydroxyl groups. A peak that appeared at 1,632 cm^{-1} was attributed to C=O stretching vibrations of the conjugated hydrocarbon. A transmission band at around 1,032 cm^{-1} was attributed to stretching of the C-O group from alcohol, acids, phenols, ethers and/or ester functional groups. Finally, the peak around 500 cm^{-1} corresponds to aromatic ring deformation vibrations. All of the functional groups varied broadly among the carbons measured.

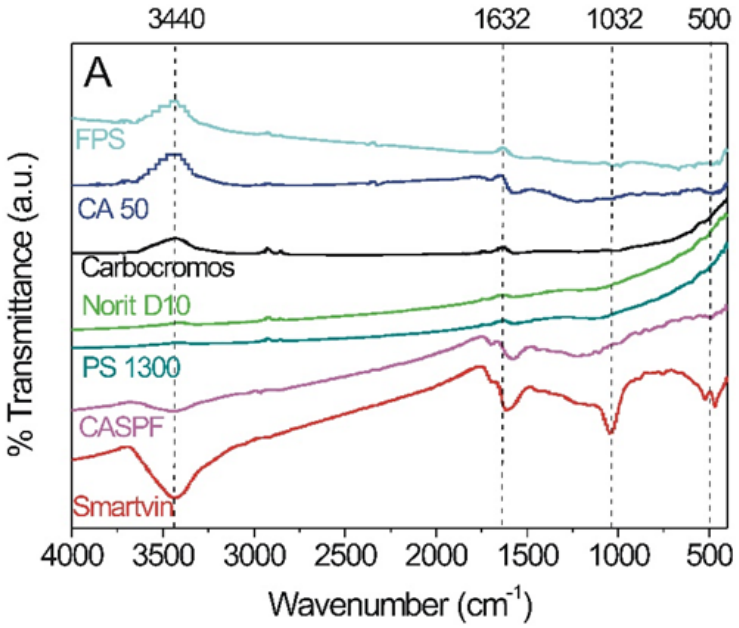


Figure 9. FTIR spectra of seven activated carbon products

The surface area (Brunauer-Emmett-Teller specific surface area) and porosity of the activated carbon products assessed are presented in Figure 10. The activated carbons with a larger surface area (above 1,150 m^2/g) and larger pore volume were more effective at removing smoke glycosides. Activated carbon products with smaller surface areas (below 1,150 m^2/g) and small pore sizes were more effective at removing volatile phenols. Based on the above, the specific surface area, porosity and zeta potential of activated carbons are likely indicators of fining performance.

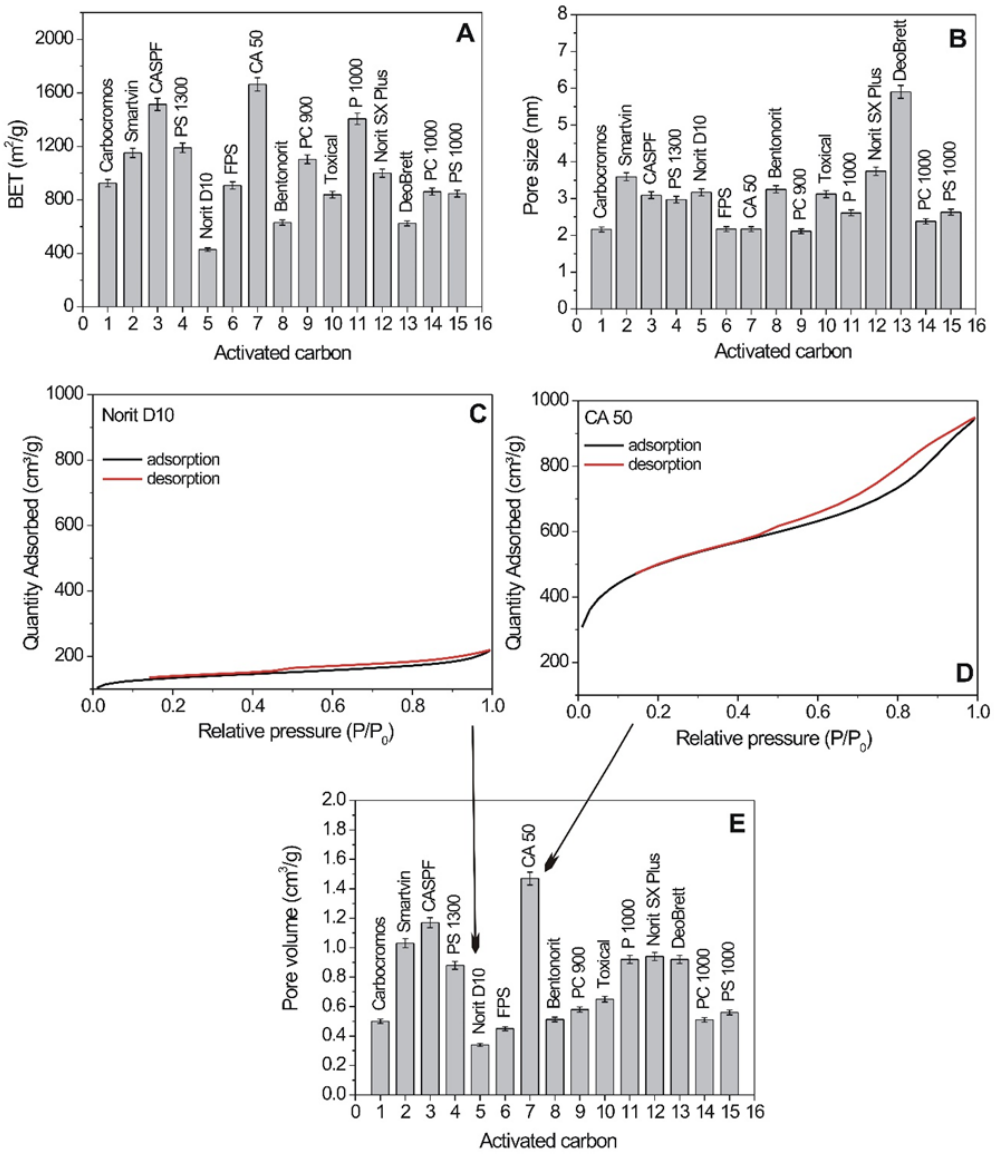


Figure 10. Comparison of (A) surface area (BET), (B) pore size, and (E) pore volume of all activated carbon products assessed. Panels (C) and (D) show examples of the nitrogen adsorption-desorption isotherm of two activated carbon products .

The surface area and porosity of the activated carbon products assessed are presented in Figure 10. The activated carbons with a larger surface area (above 1,150 m^2/g) and larger pore volume were more effective at removing smoke glycosides. Activated carbon products with smaller surface areas (below 1,150 m^2/g) and small pore sizes were more effective at removing volatile phenols. Based on the above, the specific surface area, porosity and zeta potential of activated carbons are likely indicators of fining performance.

The plasma polymerisation technique was identified as a potential way to alter the performance of activated carbon products. The PS1300 and FPS products were modified using allylamine as a precursor to produce positively charged, amine-functionalised carbons. An experiment was conducted with coated and uncoated (original) carbon products with juice to test the ability of the coated material to remove smoke compounds from smoke-affected Chardonnay and Pinot Noir juices sourced from the 2019 bushfire event in the Huon Valley, Tasmania. The results from the trial indicated that amine-coated carbon materials did not remove more smoke marker compounds than the original carbon products. The amine coated carbon materials were less effective at removing glycosides from both Chardonnay and Pinot Noir juice, and were also less effective at removing volatile phenols from Pinot Noir juice.

In summary, activated carbons were found to have diverse surface properties, and a different set of surface properties was required for optimal removal of volatile phenols or phenolic glycosides. The carbon products were most effective in white juice. Physical characteristics have been identified for optimal removal of volatile phenols or phenolic glycosides in particular matrices such as red or white juice or wine. Removal of glycosides and volatile phenols from red wine requires high dose rates, resulting in removal of important aroma, flavour, colour and texture components. Improved specificity of carbons for red wine treatment would be of particular benefit.

6.3.2 Impact of yeast strains on volatile phenols and phenolic glycosides

Five *Saccharomyces cerevisiae* yeasts and two non-*Saccharomyces* yeast strains were evaluated in red and white juice produced from grapes affected by 2019 Tasmanian bushfires. All *S. cerevisiae* strains performed similarly, corroborating findings by other researchers that differences in smoke taint ratings are likely due to sensory masking effects of yeast metabolites and not differences in volatile phenols and glycosides. The two non-*Saccharomyces* strains reduced SyGG more than the *Saccharomyces* yeasts in both white and red juice, alongside a small increase in free syringol. However, fermentation kinetics for these ferments were slower than for *S. cerevisiae*, so large-scale winemaking was not pursued.

6.3.3 Impact of glycolytic enzymes on smoke-affected wines

Previous research indicated that glycolytic enzymes could reduce the concentration of phenolic glycosides in wine (Herderich and Krstic 2020). However, the effect on the volatile phenols and wine aroma was not clear, because the wines had high concentrations of volatile phenols and strong smoke aromas and flavours prior to enzyme treatment. This study aimed to assess the effect of the most promising enzymes on volatile phenols and smoke sensory attributes, applying enzymes to wines with relatively low concentrations of volatile phenols and high concentrations of phenolic glycosides.

The results were in line with the previous study. Enzymes were more effective at decreasing concentrations of phenolic glycosides in Chardonnay than Shiraz wines. The Rapidase enzymes were found to cleave gentiobiosides with greater efficacy in Chardonnay than Shiraz wines. Trenolin enzyme also cleaved rutinosides. All enzyme-treated wines had increased levels of volatile phenols and increased smoke flavour. While enzymes are effective in reducing phenolic glycosides, a further treatment step is required to remove the liberated volatile phenols.

7. Outcome and conclusion

All planned outputs and performance targets in this project were achieved. The initiation of this project occurred on short notice and in response to the 2019/20 bushfire emergency that resulted in wide-spread and long-lasting smoke exposure of many vineyards, and led to rapid, robust and relevant outcomes. Research questions were clearly articulated by the local wine community, and a comprehensive sampling plan was rapidly designed by AWRI researchers, Wine Australia representatives and local landholders working together. The project was facilitated by support from South Australian Government through SAWIDS and in-kind contributions from Victorian and New South Wales state Governments via Wine Victoria and NSW Wine.

Early-season smoke exposure of grapes was shown to have significant negative impact on grape composition, wine composition and wine sensory characteristics. Significant smoke exposure early in the growing season (pea-size green berries) led to elevated phenolic glycosides in grapes at harvest and also to observable taint in many of the resultant wines. The Chardonnay wines had lower smoke flavour ratings than the red Shiraz and Pinot Noir wines in the study, due to the removal of skins in the white winemaking process.

Previous research had identified the pre-véraison ripening stages to be at low risk of smoke taint, but this study, combined with observations of smoke-exposed grapes from the Hunter Valley in the 2019/20 season, has expanded the risk period to be from fruit set to harvest. The incidence and severity of bushfires in the early stages of ripening are predicted to increase, and combined with pressure to undertake prescribed burns for hazard reduction, early-season smoke will have significant implications for grapegrowers and winemakers in the future, both in Australia and internationally. It will be important to detect early-season smoke exposure and manage vineyards and wine production accordingly.

The impact of smoke was detected early in the season, prior to véraison. There was a clear relationship between the detection of taint markers and compounds in fruit early in the season and observation of smoke characters in wine through sensory analysis. An unexpected result was that the concentration of phenolic glycosides in early-season grapes was not diluted during berry development and growth, despite berry size doubling and no further smoke exposure in the two months between sampling and harvesting for winemaking. Because the relationship between early-season composition and composition at harvest was not straightforward, early-season results cannot be directly related to concentrations at harvest, making interpretation difficult. Non-targeted analysis has revealed thousands of diverse chemical compounds in grapes exposed to early-season smoke, which should be investigated further.

Early-season smoke was also shown to affect leaves. There were elevated concentrations of phenolic glycosides in grapevine leaves exposed to early-season smoke soon after exposure and at harvest. Winemakers should avoid grape leaf MOG (matter other than grapes) when making wine from smoke-exposed vineyards, to minimise extraction of phenolic glycosides from leaves.

Significant progress was made regarding acceptable and unacceptable concentrations of smoke compounds in grapes and wine. A total of 65 batches of grapes were sourced from multiple regions and smoke events during the 2020 vintage. Wines were produced under standard small-lot (50 kg scale) winemaking conditions with no effort to mitigate the effect of the smoke. The relationships between grape and wine composition and sensory rating of smoke characters were explored. Reliable and rigorous sensory analysis was critical to the success of this project. The standard smoke markers, including seven volatile phenols and six glycosides, were shown to perform well in predicting smoke flavour. Further investigations to identify new smoke markers were not warranted. The most important compounds to model smoke flavour were guaiacol, *m*-cresol, *o*-cresol, *p*-cresol and the glycosides guaiacol rutinoside and cresol rutinoside, in both grapes and wine. The concentrations of these key compounds that resulted in wines with unacceptable smoke flavour have been summarised and communicated in a guide

for producers, which is attached in Appendix 8. These data will underpin better assessment of smoke-exposed grapes, avoiding unnecessary crop losses while avoiding production of smoke tainted wine. The data will also assist in grape purchase negotiations.

Consumers were shown to strongly dislike the smoky characters in smoke-affected unoaked Shiraz and unoaked Chardonnay wines, with similar results to a previous study with Pinot Noir rosé. Most consumers disliked wines with smoke flavour of moderate intensity, and a proportion of consumers disliked wines with a low level of smoke flavour, comparable to the lowest intensity discernible by the AWRI trained panel. These data will be of benefit to wine producers when assessing whether mildly smoke-affected wine is suitable for commercial release.

Strategies for reducing smoke taint in wine continue to be challenging, and most treatments have negative impacts on wine flavour and colour, style and quality. This project focused on strategies to apply in the winery, namely evaluating yeast strains, glycolytic enzymes and activated carbons. The most successful strategy to date has been the treatment of white juices with activated carbon, to remove phenolic glycosides and/or volatile phenols prior to fermentation, and this practice was widely applied to white winemaking in 2020. Physical characteristics of activated carbon products varied widely, and particular combinations of characteristics were required for optimal removal of volatile phenols or glycosides from a given matrix.

8. Recommendations

- The impacts and risks of early-season smoke should continue to be communicated to grapegrowers, winemakers and those planning burns near vineyards. Timing for planned burns should be influenced where possible to manage fire risk and minimise crop losses.

- Future research should seek strategies to effectively reduce smoke characters in red wine while maintaining wine quality, as limited options are currently available.

- Researchers at Oregon State University recently identified a new class of compounds (thiophenols) claimed to be important to ‘ashy aftertaste’ in smoke-affected wines. The relevance of thiophenols to Australian smoke-affected wines should be investigated, along with other targets identified through non-targeted analysis.

- Critical concentrations of smoke markers resulting in unacceptable smoky wines should be communicated to the sector, through extension activities and advice in the event of smoke exposure. More varieties and styles should be analysed to build more knowledge.

- Further research is required on the aerial transport forms of volatile phenols in smoke, which would allow better insights into the risk posed by a particular smoke event, modes of uptake and potential lead to improved detection, prevention and/or remediation strategies.

Appendix 1: Communication

8.1 Publications directly related to project outcomes

8.1.1 Journal articles published

Jiang, W., Parker, M., Hayasaka, Y., Simos, C., Herderich, M. 2021. Compositional changes in grapes and leaves as a consequence of smoke exposure of vineyards from multiple bushfires across a ripening season. *Molecules*. 26 (11): 14.

Jiang, W., Bilogrevic, E., Parker, M., Francis, I. L., Leske, P., Hayasaka, Y., Barter, S., Herderich, M. J. 2022. The effect of pre-veraison smoke exposure of grapes on phenolic compounds and smoky flavour in wine. *Aust. J. Grape Wine Res.* (Accepted)

Coulter, A., Baldock, G. A., Parker, M., Hayasaka, Y., Francis, I. L., Herderich, M. 2022. The concentration of smoke marker compounds in non-smoke-exposed grapes and wine in Australia. *Aust. J. Grape Wine Res.* 28 (3): 459-474.

8.1.2 Book chapters

Krstic, M., Culbert, J., Parker, M., Herderich, M. 2021. 17.4 Smoke taint and climate change. Reynolds, A. G. (ed.) *Managing wine quality.* Woodhead Publishing.

8.1.3 Reports

Herderich, M., Francis, I. L., Parker, M. and Jiang, W. 2021. Evaluation of the impact of early-season smoke exposure on grapes and wine composition and sensory properties project. Final Report to PIRSA, South Australian Wine Industry Development Scheme (see Appendix 5)

8.2 Publications arising from collaborations and past projects, supported by project staff

In addition to the publications directly addressing project outcomes listed above, project staff co-authored seven peer-reviewed publications and one industry journal article related to smoke taint more broadly, arising from collaborations and past projects supported by project staff.

Culbert, J.A., Jiang, W., Bilogrevic, E., Likos, D., Francis, I.L., Krstic, M. P., Herderich, M.J. 2021. Compositional changes in smoke-affected grape juice as a consequence of activated carbon treatment and the impact on phenolic compounds and smoke flavor in Wine. *J. Agric. Food Chem*. 69(35): 10246-10259.

Culbert, J.A., Jiang, W., Ristic, R., Puglisi, C.J., Nixon, E.C., Shi, H., Wilkinson, K.L. 2021. Glycosylation of volatile phenols in grapes following pre-harvest (on-vine) vs. post-harvest (off-vine) exposure to smoke. *Molecules*. 26(17): 5277.

Culbert, J.A., Krstic, M.P., Herderich, M. J. 2021. Development and utilization of a model system to evaluate the potential of surface coatings for protecting grapes from volatile phenols implicated in smoke taint. *Molecules*. 26(17): 5197.

Modesti, M., Szeto, C., Ristic, R., Jiang, W., Culbert, J., Bindon, K., Catelli, C., Mencarelli, F., Tonutti, P., Wilkinson, K. 2021. Potential mitigation of smoke taint in wines by post-harvest ozone treatment of grapes. *Molecules*. 26(6): 1798.

Modesti, M., Szeto, C., Ristic, R., Jiang, W., Culbert, J., Catelli, C., Mencarelli, F., Tonutti, P., Wilkinson, K. 2021. Amelioration of smoke taint in Cabernet Sauvignon wine via post-harvest ozonation of grapes. *Beverages*. 7(3): 44.

Szeto, C., Ristic, R., Capone, D., Puglisi, C., Pagay, V., Culbert, J., Jiang, W., Herderich, M., Tuke, J., Wilkinson, K. 2020. Uptake and glycosylation of smoke-derived volatile phenols by Cabernet Sauvignon grapes and their subsequent fate during winemaking. *Molecules*. 25(16): 3720.

Scrimgeour, N., Hirlam, K., Wilkes, E., Parker, M. 2021. A burning need: developing a rapid screening method for smoke-affected grapes and wine. *Wine Vitic. J.* 36(2): 33-35.

Wilkinson, K., Ristic, R., McNamara, I., Loveys, B., Jiang, W., Krstic, M. 2021. Evaluating the potential for smoke from stubble burning to taint grapes and wine. *Molecules*. 26(24): 7540.

8.2.1 Fact sheets

The following fact sheets were developed as part of this project:

- Grape smoke panel analysis results and sensory impact
- Sensory impact of smoke exposure

8.3 Presentations

A total of 26 presentations were given over the two years of the project.

Staff	Title of presentation	Presented to and where	Date
Markus Herderich	Insights into the effects of early ripening smoke exposure of vineyards and grapes	Australian Society for Viticulture and Oenology online seminar: Australian Wine - Winning the long game	30 June 2020
Mango Parker	Smoke taint research, lessons from Cudlee Creek	SA Central Viticulture Expo at Longview Wines, Macclesfield, SA to approximately 80 growers and winemakers face to face, and recorded presentation made freely available afterwards https://vimeo.com/user/39003402/folder/2677777	23 September 2020
Mango Parker	A summary of the early-season trials undertaken in the Adelaide Hills and Hunter Valley	AWRI smoke taint seminar, Rutherglen, Vic (virtual)	26 October 2020
Markus Herderich	A summary of the early-season trials undertaken in the Adelaide Hills and Hunter Valley	AWRI smoke taint seminar, Yarra Valley, Vic (virtual)	27 October 2020
Maddy Jiang	A summary of the early-season trials undertaken in the Adelaide Hills and Hunter	AWRI smoke taint seminar Gippsland, Vic (virtual)	28 October 2020

	Valley		
Maddy Jiang	A summary of the early-season trials undertaken in the Adelaide Hills and Hunter Valley	AWRI smoke taint seminar North East Victoria (virtual)	11 November 2020
Mango Parker, Maddy Jiang, Markus Herderich, and Peter Leske (Revenir, Adelaide Hills Wine Region)	Out of the smoke: experiences from the 2019/20 Australian bushfire season	1.5-hour seminar with Adelaide Hills Wine Region (virtual)	26 November 2020
Mango Parker and Peter Leske (Revenir, Adelaide Hills Wine Region)	Out of the smoke: experiences from the 2019/20 Australian bushfire season: early-season smoke exposure from Cudlee Creek fire, 20 December 2019	Wine tasting and discussion of all research wines and project outcomes with collaborative partners for 2.5 hours, with 22 attendees including representatives from Adelaide Hills Wine Region (both landholders and winemakers), representatives from Wine Australia, PIRSA, the AWRI and University of Adelaide	28 January 2021
Mango Parker	Pre-veraison smoke exposure: learnings from the 2019 Adelaide Hills fire event.	National Wine Sector Bushfire Conference, Adelaide Hills Convention Centre, Hahndorf, SA, and recording made freely available afterwards	25 May 2021
Damian Espinase Nandorfy	Smoke taint sensory evaluation: considerations to improve rigour and aid decision-making	National Wine Sector Bushfire Conference, Adelaide Hills Convention Centre, Hahndorf, SA, and recording made freely available afterwards	25 May 2021
WenWen (Maddy) Jiang	Phenolic glycosides in leaves as biomarkers for pre-veraison smoke exposure	Crush 2021 - the grape and wine science symposium, Adelaide SA • *Awarded prize for best presentation*	16 June 2021
Mango Parker	Glycosides and their role in flavour release	Interwinery Analysis Group Seminar, West Lakes, SA	18 June 2021
Markus Herderich	Early-season smoke exposure – Hunter Valley and Adelaide Hills	Smoke taint new knowledge seminar, Hunter Valley, NSW (virtual)	15 November 2021
Mango Parker	Predicting smoke taint in wine. Are we there yet?	Smoke taint new knowledge seminar, Hunter Valley, NSW	15 November 2021

		(virtual)	
Markus Herderich	Early-season smoke exposure – Hunter Valley and Adelaide Hills	Smoke taint remediation workshop, Mornington Peninsula, Vic (virtual)	17 November 2021
Mango Parker	Predicting smoke taint in wine. Are we there yet?	Smoke taint remediation workshop, Mornington Peninsula, Vic (virtual)	17 November 2021
Markus Herderich	Early-season smoke exposure – Hunter Valley and Adelaide Hills	Smoke taint new knowledge seminar, Canberra, ACT (virtual)	18 November 2021
Mango Parker	Predicting smoke taint in wine. Are we there yet?	Smoke taint new knowledge seminar, Canberra, ACT (virtual)	18 November 2021
Markus Herderich	Early-season smoke exposure – Hunter Valley and Adelaide Hills	Smoke taint new knowledge seminar, Orange, NSW (virtual)	30 November 2021
Maddy Jiang	Predicting smoke taint in wine. Are we there yet?	Smoke taint new knowledge seminar, Orange, NSW (virtual)	30 November 2021
M.Jiang	Seedling-plant your dream	Women in Chemistry Group (RACI SA Branch), Adelaide, SA	28 April 2022
M. Parker	Smoke taint: the latest research	18th AWITC workshop 29, Adelaide Convention Centre, SA	26 June 2022
D. Espinase-Nandorfy	Sensory assessment of wines made from smoke-exposed grapes	18th AWITC workshop 29, Adelaide Convention Centre, SA	26 June 2022
E. Bilogrevic	The consumer response to smoke-exposed wines	18th AWITC workshop 29, Adelaide Convention Centre, SA	26 June 2022
M. Jiang	Pre-veraison smoke exposure of vineyards affects wine flavour	18th AWITC workshop 29, Adelaide Convention Centre, SA	26 June 2022
A. Coulter	Answering your questions on smoke taint	18th AWITC workshop 29, Adelaide Convention Centre, SA	26 June 2022
Mango Parker	Smoke flavour: linking chemical composition and sensory properties in smoke-affected wines	18th AWITC Fresh Science plenary session, Adelaide Convention Centre, SA	28 June 2022

8.4 Workshops

A workshop was convened at the 18th AWITC in June 2022, in collaboration with the University of Adelaide, Charles Sturt University and La Trobe University

M. Parker, D. Espinase-Nandorfy, E. Bilogrevic, M. Jiang, A. Coulter, K.	AWITC workshop 29 Smoke taint: the latest research	18th AWITC Technical workshop program, Adelaide Convention Centre, SA	26 June 2022

Wilkinson (University of Adelaide), S. Li (Charles Sturt University), L. Schmitdke (Charles Sturt University), I. Porter (La Trobe University)			

8.5 Media interviews

Date	Staff member	Discussed	Media
February 2021	M. Parker	A fast and furious smoke taint project brings interesting results -Adelaide Hills early-season smoke exposure	Jacquie van Santen, *Wine Australia News*
25 May 2021	M. Parker	Pre-veraison smoke exposure	Cassandra Hough, *ABC Country Hour*
June 2021	M. Parker	High calibre career	Sam Squire, Australia and New Zealand Grapegrower and Winemaker
27 September 2021	M. Parker	Why are winemakers throwing away flavour? (Glycosides in grape marc and smoke-affected grapes and wine)	AWRI decanted podcast
6 December 2021	M. Parker and C.A. Simos	Smoke taint	Chris Scott -*UK Wine Show* podcast

8.6 Posters

Authors	Title of poster	Presented at	Date
Maddy Jiang, Mango Parker, Eleanor Bilogrevic, Leigh Francis, Peter Leske[1], Yoji Hayasaka, Sheridan Barter, Markus Herderich	Does pre-veraison smoke exposure of vineyards affect wine flavour?	18th AWITC, Adelaide	26-29 June 2022
Mango Parker, Maddy Jiang, Eleanor Bilogrevic, Leigh Francis, Adrian Coulter, Markus Herderich	How have the smoke-exposed wines from 2020 changed over the last two years?	18th AWITC, Adelaide	26-29 June 2022
Eleanor Bilogrevic, Mango Parker, Julie Culbert, WenWen Jiang, Desireé Likos, Markus Herderich, Leigh Francis	The consumer response to smoke flavour in wine	18th AWITC, Adelaide	26-29 June 2022
Mango Parker, Eleanor Bilogrevic, Maddy Jiang, Desireé Likos, Patricia Williamson, John Gledhill[2], Con Simos, Geoff Cowey, Adrian Coulter, Markus Herderich, Leigh Francis	Smoke flavour: linking chemical composition and sensory properties in smoke-affected wines • *Awarded prize for best oenology poster*	18th AWITC, Adelaide	26-29 June 2022

Eleanor Bilogrevic, Damian Espinase Nandorfy, Mango Parker, Patricia Williamson, Desireé Likos, Wes Pearson, Leigh Francis	Good sensory practices for assessing wines made from smoke-exposed grapes	18th AWITC, Adelaide	26-29 June 2022
Julie Culbert, WenWen Jiang, Eleanor Bilogrevic, Desireé Likos, Leigh Francis, Con Simos, Mark Krstic, Markus Herderich	Choosing the right carbon product for remediating smoke-affected juice and wine	18th AWITC, Adelaide	26-29 June 2022
Julie Culbert, WenWen Jiang, Eleanor Bilogrevic, Desireé Likos, Leigh Francis, Mark Krstic, Markus Herderich, Con Simos	Taking the smoke out: winemaking remediation options for smoke-affected juice and wine	18th AWITC, Adelaide	26-29 June 2022

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Appendix 2: Intellectual property

Program IP has been communicated through presentations and publications and resides in the public domain. Copyright in any reports, posters or presentations is held by the AWRI. Copyright in publications remains with the publisher. No inventions or patentable discoveries have been identified from the work. No Program IP has been commercialised or identified for commercialisation at the time of writing this report.

Appendix 3: References

Culbert, J. A., Jiang, W., Bilogrevic, E., Likos, D., Francis, I. L., Krstic, M. P., Herderich, M. J. 2021a. Compositional changes in smoke-affected grape juice as a consequence of activated carbon treatment and the impact on phenolic compounds and smoke flavor in Wine. *J. Agric. Food Chem.*, 69 (35): 10246-10259.

Culbert, J. A., Krstic, M. P., Herderich, M. J. 2021b. Development and utilization of a model system to evaluate the potential of surface coatings for protecting grapes from volatile phenols implicated in smoke taint. *Molecules.* 26(17): 5197.

Hayasaka, Y., Baldock, G. A., Pardon, K. H., Jeffery, D. W., Herderich, M. J. 2010. Investigation into the formation of guaiacol conjugates in berries and leaves of grapevine *Vitis vinifera* L. Cv. Cabernet Sauvignon using stable isotope tracers combined with HPLC-MS and MS/MS analysis. 58 (4): 2076-81.

Hayasaka, Y., Parker, M., Baldock, G. A., Pardon, K. H., Black, C. A., Jeffery, D. W. and Herderich, M. J. 2013. Assessing the impact of smoke exposure in grapes: development and validation of a HPLC-MS/MS method for the quantitative analysis of smoke-derived phenolic glycosides in grapes and wine. *J. Agric. Food Chem.* 61: 25-33.

Herderich, M., Krstic, M. 2020. Mitigation of climate change impacts on the national wine industry by reduction in losses from controlled burns and wildfires and improvement in public land management. Available from: https://www.wineaustralia.com/research/projects/mitigation-of-climate-change-impacts

Jiang, W., Bilogrevic, E., Parker, M., Francis, I. L., Leske, P., Hayasaka, Y., Barter, S., Herderich, M. J. 2022. The effect of pre-veraison smoke exposure of grapes on phenolic compounds and smoky flavour in wine. *Aust. J. Grape Wine Res.* (Accepted)

Mayr, C. M., Parker, M., Baldock, G. A., Black, C. A., Pardon, K. H., Williamson, P. O., Herderich, M. J., Francis, I. L. 2014. Determination of the importance of in-mouth release of volatile phenol glycoconjugates to the flavor of smoke-tainted wines. *J. Agric. Food Chem.* 62(11): 2327-2336.

Mirabelli-Montan, Y. A., Marangon, M., Graça, A., Mayr Marangon, C. M., Wilkinson, K. L. 2021. Techniques for mitigating the effects of smoke taint while maintaining quality in wine production: a review. *Molecules*. 26(6): 1672.

Pollnitz, A. P., Pardon, K. H., Sykes, M., Sefton, M. A. 2004. The effects of sample preparation and gas chromatograph injection techniques on the accuracy of measuring guaiacol, 4-methylguaiacol and other volatile oak compounds in oak extracts by stable isotope dilution analyses. *J. Agric. Food Chem.*,52(11): 3244-52.

Ristic, R., Osidacz, P., Pinchbeck, K. A., Hayasaka, Y., Fudge, A. L., Wilkinson, K. L. 2011. The effect of winemaking techniques on the intensity of smoke taint in wine. *Aust. J. Grape Wine Res.* 17(2): S29-S40.

Ristic, R., Van Der Hulst, L., Capone, D. L. and Wilkinson, K. L. 2017. Impact of bottle aging on smoke-tainted wines from different grape cultivars. *J. Agric. Food Chem.* 65(20): 4146-4152.

Sonneveld, E.J., Visser, J.W. 1975. Automatic collection of powder data from photographs. *J. Appl. Cryst.* 8: 1-7.

Appendix 4: Staff

Mango Parker, WenWen (Maddy) Jiang, Eleanor Bilogrevic, Sheridan Barter, Cristian Varela, Agnieszka Mierczynska-Vasilev, Flynn Watson, Julie Culbert, Leigh Francis, Adrian Coulter, Desireé Likos, John Gledhill, Lieke Van de Hulst, Damian Espinase Nandorfy, Geoff Cowey, Matt Holdstock, Patricia Williamson, Yoji Hayasaka, Yuki Takahashi, Robin Stegmann, Yevgeniya Grebneva, Jana Hildebrandt, Natoiya Lloyd, Luca Nicoletti, Con Simos, Markus Herderich, AWRI quality panel, AWRI sensory panel.

Appendix 5: Final Project Report: Evaluation of the impact of early-season smoke exposure on grape and wine composition and sensory properties project

Grant Recipient	The Australian Wine Research Institute Limited (ACN 007 558 296) Hartley Grove, Urrbrae, SA 5064		
Project Title	Evaluation of the impact of early-season smoke exposure on grape and wine composition and sensory properties project		
Report Number	3. Final project report	**Report Due Date**	28 May 2021
Progress Report / Milestone Purpose	Final Report		
Milestone Achieved Date	28 May 2021		
Report/Milestone Progress	The purpose of the project was evaluation of the impact of early-season smoke exposure on grape and wine composition and sensory properties. The project aimed to provide answers to significant research questions through analysis of grapes and wine from selected vineyards in the Adelaide Hills, following the 2019 Cudlee Creek bushfire. All outcomes were achieved as per Item 9 Attachment A (Grant Detail). **Smoke taint in wine after early-season smoke exposure of grapes** Research question a) Does significant smoke exposure early in the growing season (pea-size green berries) lead to phenolic glycosides in grapes at harvest, and/or to observable taint in wine? • Significant smoke exposure early in the growing season (pea-size green berries) led to elevated phenolic glycosides in grapes at harvest. • Significant smoke exposure early in the growing season (pea-size green berries) can lead to observable taint in wine. **Early-season smoke and berry growth** Research question b) Is the concentration of phenolic glycosides in early-season grapes diluted out by/during berry development and growth? • The concentration of phenolic glycosides in early-season grapes was not diluted out by/during berry development and growth. **Smoke exposure effects on leaves** Research question c) Are there phenolic glycosides in leaves of grapevines exposed to early-season smoke soon after exposure and are there phenolic glycosides in leaves at harvest following early-season exposure? • There were phenolic glycosides in leaves of grapevines exposed to early-season smoke soon after exposure and at harvest following early-season exposure. **Early detection of smoke taint in grapes** Research question d) Is there a relationship (or not) between the detection of taint markers and compounds in fruit early in the season, and observation (or not) of taint by sensory analysis of the wine made from such fruit? • There was a clear relationship between the detection of taint markers and compounds in fruit early in the season and observation of taint by		

	sensory analysis of the wine made from such fruit.
Demonstrated evidence	See attached Technical Report: Evaluation of the impact of early-season smoke exposure on grape and wine composition and sensory properties project.

I certify that the information contained in this Report is true and accurate in accordance with the Grant Agreement.

Name of signatory	Dr Markus Herderich
Position	Group Manager -Research
Organisation	The Australian Wine Research Institute
Signature	
Date	27 May 2021

Attachment 1. Technical Report

Background

When the Cudlee Creek bushfire started on 20 December 2019, grapegrowers and winemakers in the Adelaide Hills were concerned about the possibility of smoke affecting the quality of the wine-grape crop, as well as the immediate threat of the fire to homes and vineyards.

Smoke can contaminate wine-grapes due to accumulation of volatile phenols and glycosides, which can contribute to undesirable smoky aromas and flavours in the resulting wines. Previous research indicated that at the early stages of grape ripening, when the grapes are still hard and green, the risk of smoke taint is low to variable. However, the previous research had been conducted in the early 2000s, focused on model smoke experiments, and targeted free volatile phenols and smoky aromas in the wines, since the analysis of phenolic glycosides was not available at that time. Phenolic glycosides are now included in the routine analysis for smoke exposure marker compounds in grapes and wine, along with volatile phenols.

Grape leaves have been shown to be able to take up volatile phenols and form phenolic glycosides, similarly to berries. Leaves could be used as early markers of smoke exposure, particularly if the current suite of smoke exposure markers are shown to be inapplicable for unripe berries. Another aspect to grape leaves is that they can be accidentally included in the grape load during harvesting, particularly when machine harvesters are used, and therefore if leaves are high in phenolic glycosides they could contribute to smoke taint if included in the winemaking process.

While many of Australia's grapegrowing regions were affected by smoke from numerous bushfires during the 2019-2020 vintage, the Adelaide Hills region was only affected by one major smoke event, which coincided with the early stages of grape ripening (average stage E-L 27), and the region was clear of smoke for the rest of the ripening period. Thus in the 2020 vintage, the Adelaide Hills provided a unique opportunity to test the effect of early-season bushfire smoke exposure on wine-grapes, and the resulting wines, providing practical outcomes for growers and winemakers faced with early-season smoke.

In early January 2020, a meeting was held with representatives from Adelaide Hills Wine Region, PIRSA, the AWRI and Wine Australia, which kickstarted the project. A series of key questions were developed, vineyards were selected, and landowners, winemakers and researchers all came together very quickly, with the first vineyard samples collected on 14 January 2020. Additional funding was secured from Wine Australia, which is supporting further research.

Key questions to be addressed in the trial:

a) Does significant smoke exposure early in the growing season (pea-size green berries) lead to phenolic glycosides in grapes at harvest, and/or observable taint in wine?

b) Is the concentration of phenolic glycosides in early-season grapes diluted out by/during berry development and growth?

c) Are there phenolic glycosides in leaves of grapevines exposed to early-season smoke soon after exposure and are there phenolic glycosides in leaves at harvest following early-season exposure?

d) Is there a relationship (or not) between the detection of taint markers and compounds in fruit early in the season and observation (or not) of taint by sensory analysis of the wine made from such fruit?

Scope

Activities included collection of 216 grape berry samples and 99 leaf samples from January 2020 to March 2020 from 24 privately owned vineyards (Table 1). The samples chosen

represented the three major varieties grown in the Adelaide Hills (Chardonnay, Pinot Noir and Shiraz) and a range of suspected smoke exposure, from clean controls to heavily smoke exposed, and vineyards that were mildly affected by fire, such as stubble burnt within the vineyard. Figure 1 shows the location of the Pinot Noir vineyards. Phenolic glycoside smoke markers were analysed in all the grape and leaf samples. Wines were made from 23 of the vineyards according to research small-lot winemaking best practice. Unfortunately one vineyard was harvested commercially before trial fruit could be picked. All wines were bottled, analysed for phenolic glycosides and free volatile phenols as well as basic wine composition, and assessed at least twice using formal sensory assessment using a highly screened and trained panel demonstrated to be very sensitive to smoke taint, at two key timepoints, six-weeks post bottling and after six months of ageing in the bottle.

Table 1. Overview of vineyards sampled

Fire and smoke impact	Location	Vineyard code	Block number	Variety
Control sites unaffected by smoke or fire	Kuitpo	A	1	Chardonnay
			2	Shiraz
			3	Pinot Noir
	Macclesfield	B	4	Chardonnay
			5	Shiraz
			6	Pinot Noir
Suspected of smoke exposure	Woodside	C	7	Chardonnay
			8	Shiraz
			9	Pinot Noir
	Woodside	D	10	Chardonnay
			11	Shiraz
			12	Pinot Noir
	Forreston	E	13	Chardonnay
			14	Pinot Noir
	Gumeracha	F	15	Shiraz
Fire and smoke apparent	Lobethal	G	16	Chardonnay
			17	Shiraz
			18	Pinot Noir
	Charleston	H	19	Pinot Noir*
	Woodside	I	20	Shiraz
	Lobethal	J	21	Chardonnay
	Woodside	K	22	Chardonnay
			23	Shiraz
			24	Pinot Noir

*Wine was not made from this site H19 as the vineyard was harvested for commercial winemaking before trial fruit was harvested.

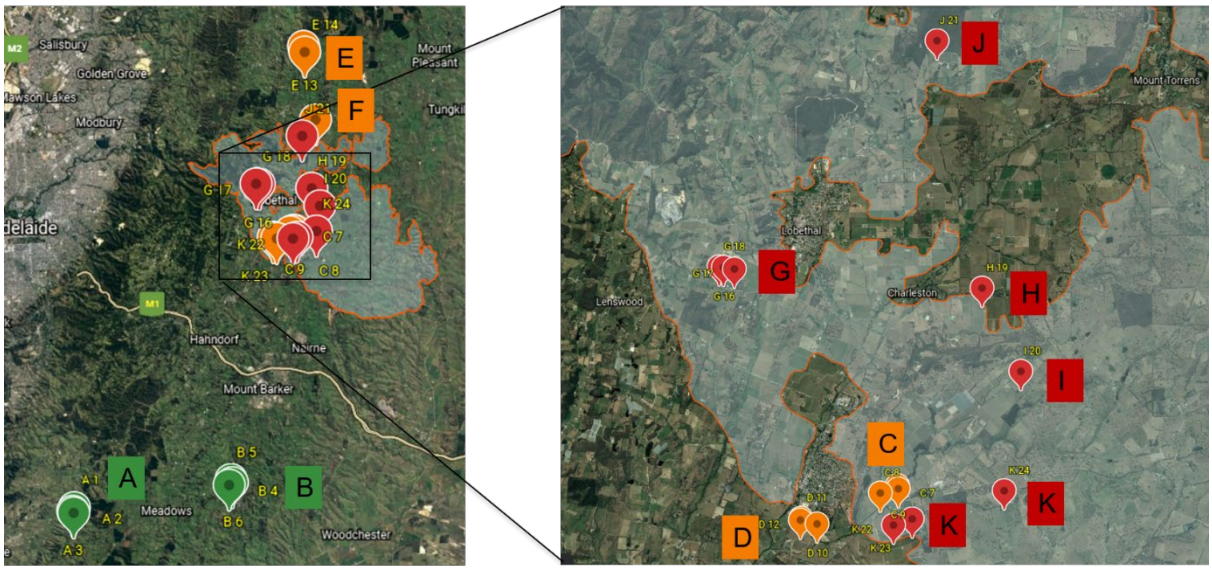


Figure 1. Locations of the vineyards included in the project. The fire zone is indicated and the colours relate to extent of smoke exposure: green - control sites unaffected by smoke or fire; orange – sites suspected of smoke exposure; red – sites with fire and smoke apparent

In addition to research supported by this Grant, the AWRI provided support for the formation of a sensory panel made up of Adelaide Hills winemakers to undertake rigorous assessments of smoke taint in small-lot wines. This included sensory standards, protocols, proformas, and data analysis and interpretation. The panel assessed 296 wine samples during vintage 2020 (February to April) and the results of the winemakers' sensory assessments formed a basis for decisions around suitability of smoke-affected grapes for commercial harvest. Comparison with the AWRI's smoke panel validated the results from the winemakers, and the model has been since applied to other regions across Australia.

Outcomes

The project achieved clear outcomes relating to the key research questions outlined in Item 9 of Attachment 1 (grant details).

- Significant smoke exposure early in the growing season (pea-size green berries) clearly led to elevated phenolic glycosides in grapes at harvest for most of smoke exposed vineyards studied.

    - This finding corroborates the use of phenolic glycosides in grapes as smoke exposure markers even during the early stages of grape ripening.
    - The effect was seen for all three grape varieties included in the study: Chardonnay, Shiraz and Pinot Noir. A range of concentrations of phenolic glycosides were quantified in the berries at harvest. The concentration of phenolic glycosides was not related to proximity to the fire scar, but matched better with the direction of smoke drift and anecdotal observations of smoke behaviour.

- The two control vineyards, located at Kuitpo and near Macclesfield far from the fires, had phenolic glycosides within typical concentrations found in non-smoke-affected grapes, consistent with vineyards that have not been exposed to smoke.
- One vineyard very close to the fire front in Woodside (labelled D in Figure 1, and sample 12 in Figure 2), which would have been expected to be smoke affected, did not have elevated phenolic glycosides at harvest. This vineyard was reported by the vineyard manager to have escaped the full force of the smoke plume due to a wind change. All three varieties at this vineyard were consistent with grapes that have not been exposed to smoke, which was also consistent with the wine chemical data and sensory assessment of the wines.
- On the other hand, results from Vineyard E, which is located 4 km away from the fire scar to the north, demonstrated elevated concentrations of phenolic glycosides (sample 14 in Figure 2), and the Pinot Noir wine made from that site had distinct smoky flavours, confirming smoke exposure. This matched with anecdotal observations of smoke at the vineyard, and demonstrated that smoke exposure may not correlate directly with proximity to the fire. Hence it is important to test for presence of smoke exposure markers in grapes from individual sites and not make generalisations about regions that have been exposed to smoke.

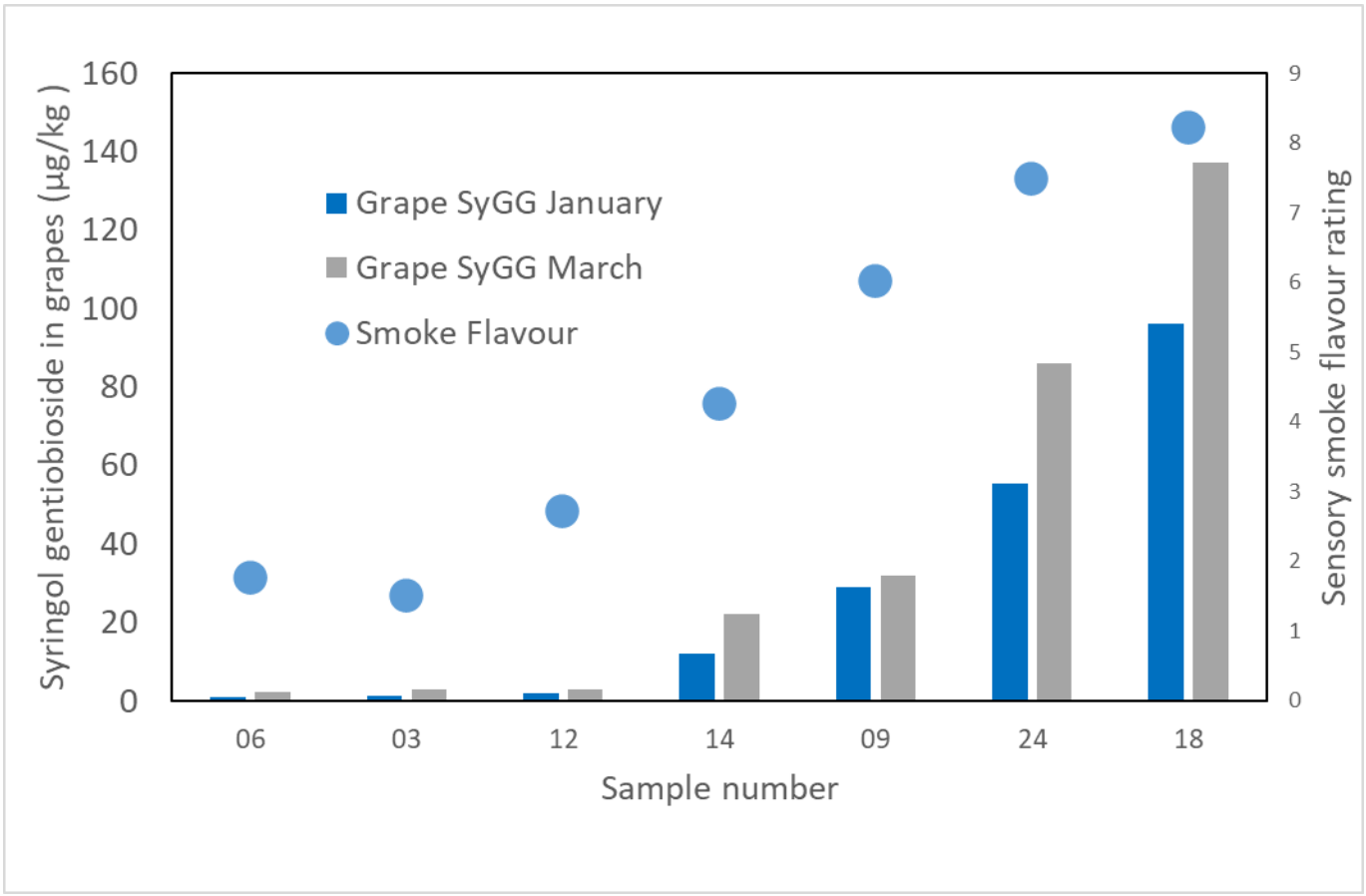


Figure 2. Key smoke exposure marker, syringol gentiobioside in Pinot Noir grapes increased in concentration (per kg) from January to March 2020, when the grapes were harvested, despite berry weight more than doubling from average 0.42 g/berry in January to 0.98 g/berry at harvest. Blue bars indicate syringol gentiobioside concentration in grapes in January, grey bars indicate syringol gentiobioside concentration in grapes in March (harvest), and blue dots indicate sensory smoke flavour rating. Sensory smoke flavour rating in wine corresponded to the concentration of syringol gentiobioside in grapes. Refer to Table 1 and Figure 1 for sample details.

- Significant smoke exposure early in the growing season (pea-size green berries) can lead to observable taint in wine.
    - Most red wines made from smoke-exposed grapes exhibited distinct smoky and ashy flavour six weeks post-bottling, and after six months of bottle ageing.
    - The smoky flavour was rated much lower in the Chardonnay wines, likely due to the lack of skin contact in white winemaking.
    - Wines of all three varieties made from the vineyard at Woodside (vineyard D) which was very close to the fire front but had very low levels of phenolic glycosides, did not show significant smoky aromas or flavours.

- The concentration of phenolic glycosides in early-season grapes was **not** diluted by the increase in berry size during ripening, contrary to expectations.
    - For all three varieties, phenolic glycosides were not diluted by/during berry development and growth.
    - Phenolic glycosides increased between January and harvest in March 2020, both on a per berry and per kg basis. This was unexpected due to the lack of smoke exposure during this period, and doubling of berry weight. The expectation was that the concentration of phenolic glycosides per berry would remain constant, and consequently the concentration per kg would halve as berry weight doubled. A potential explanation for this observation would be an on-going biochemical glycosylation reaction during ripening which might continuously convert volatile phenols to phenolic glycosides. However this is unlikely as samples had no detectable concentrations of volatile phenols when analysed in January 2020. Another explanation would be the presence of other yet-to-be-identified storage forms in unripe berries, which is a topic of ongoing research.

- There were elevated levels of phenolic glycosides in leaves of grapevines exposed to early-season smoke soon after exposure and at harvest following early-season exposure.
    - Comparing to control leaves, smoke-exposed leaves had elevated concentrations of phenolic glycosides when sampled in January 2020 and at harvest.
    - The level of phenolic glycosides in the leaves related to that of the grape berries, particularly for syringol gentiobioside.
    - There were differences between the varieties in phenolic glycoside concentration and pattern of abundance in the leaves.
    - Syringol gentiobioside and five other phenolic glycosides are commonly used as smoke markers in grape berries and these smoke markers had a similar pattern of abundance in Chardonnay and Shiraz leaves, but the pattern of abundance was different for Pinot Noir leaves, with rutinosides much more abundant than gentiobiosides.
    - The concentration of phenolic glycosides in the leaves was much higher than that found in the grape berries, and ranged up to 7,600 µg/kg. If leaves were incorporated into the grape crush at the winery at a rate of 3% by mass, this could contribute up to 230 µg/kg phenolic glycosides to the grape load.

- There was a relationship between the concentration of taint markers and compounds in fruit early in the season and observation of taint by sensory analysis of the wine made from such fruit.

- The ranking of marker compounds early in the season matched the ranking of smoke taint in the wines (see Figure 2). This relationship was particularly strong for Pinot Noir, which is a lighter style of red wine. While the ranking of marker compounds early in the season matched the ranking of smoke taint in the Shiraz wines in general, dominant green/eucalyptus characters seemed to mask the smoky flavour in one wine, particularly in the young wines (six weeks post-bottling).
- For the Chardonnay wines the ranking of marker compounds early in the season matched the ranking of smoke taint in the wines, but the wines were rated much lower in smoky flavour than the red wines. This is likely to reflect the winemaking process for white wine, which involves early removal of the grape skins, resulting in minimal skin contact, whereas analysis of grapes involves extraction from all grape components.

Reports and meetings

- Three industry briefings were held between June 2020 and May 2021, showcasing the project outcomes, with information about grape and wine data and tastings of wines with highly engaged Adelaide Hills wine sector stakeholders. The briefings were organised by the AWRI with assistance from the Adelaide Hills Wine Region. Further detail is outlined in Attachment 2.
- Six seminars targeted the broader Australian wine sector. Further detail is outlined in Attachment 3.
- Results were presented at the Australian National Wine Sector Bushfire Conference, 25 May 2021.

Highlights

- The team was a finalist for Excellence in Research Collaboration in the 2020 SA Science Excellence Awards. This nomination attracted a number of high-profile visits and associated publicity, including the preparation of a 2-minute video showcasing the project which can be viewed at the following link: 2020 SASEIA Finalist - Out of the Smoke (AWRI) - Excellence in Science and Industry Collaboration - YouTube
- A story about the project appeared in the Wine Australia RD&A News February 2021, titled 'A fast and furious smoke taint project brings interesting results' https://www.wineaustralia.com/news/articles/smoke-taint-project-brings-interesting-results

Attachment 2. Briefings with Adelaide Hills Wine Region stakeholders

Staff	Title of presentation	Presented to and where	Date
Mango Parker	Smoke taint research, lessons from Cudlee Creek	SA Central Viticulture Expo at Longview Wines, Macclesfield, SA to approximately 80 growers and winemakers face to face, and recorded presentation made freely available afterwards https://vimeo.com/user/39003402/folder/2677777	23 September 2020
Mango Parker, Maddy Jiang, Markus Herderich, and Peter Leske (Revenir, Adelaide Hills Wine Region)	Out of the smoke: experiences from the 2019/20 Australian bushfire season	1.5-hour seminar with Adelaide Hills Wine Region via Zoom, with full details of grape and wine data to date.	26 November 2020
Mango Parker and Peter Leske (Revenir, Adelaide Hills Wine Region)	Out of the smoke: experiences from the 2019/20 Australian bushfire season: early-season smoke exposure from Cudlee Creek fire, 20 December 2019	Wine tasting and discussion of all research wines and project outcomes with collaborative partners for 2.5 hours, with 22 attendees including representatives from Adelaide Hills Wine Region (both landholders and winemakers), representatives from Wine Australia, PIRSA, the AWRI and University of Adelaide	28 January 2021

Attachment 3. Briefings with the wider Australian wine sector

Staff	Title of presentation	Presented to and where	Date
Markus Herderich	Insights into the effects of early ripening smoke exposure of vineyards and grapes	Australian Society for Viticulture and Oenology online seminar: Australian Wine - Winning the long game	30 June 2020
Mango Parker	A summary of the early-season trials undertaken in the Adelaide Hills and Hunter Valley	AWRI smoke taint seminar, Rutherglen, Vic (virtual seminar via zoom)	26 October 2020
Markus Herderich	A summary of the early-season trials undertaken in the Adelaide Hills and Hunter Valley	AWRI smoke taint seminar, Yarra Valley, Vic (virtual seminar via zoom)	27 October 2020
Maddy Jiang	A summary of the early-season trials undertaken in the Adelaide Hills and Hunter Valley	AWRI smoke taint seminar Gippsland, Vic (virtual seminar via zoom)	28 October 2020
Maddy Jiang	A summary of the early-season trials undertaken in the Adelaide Hills and Hunter Valley	AWRI smoke taint seminar North East Victoria (virtual seminar via zoom)	11 November 2020
Mango Parker	Pre-veraison smoke exposure: learnings from the 2019 Adelaide Hills fire event.	National Wine Sector Bushfire Conference, Adelaide Hills Convention Centre, Hahndorf, SA, and recording made freely available afterwards	25 May 2021
Damian Espinase Nandorfy	Smoke taint sensory evaluation: considerations to improve rigour and aid decision-making	National Wine Sector Bushfire Conference, Adelaide Hills Convention Centre, Hahndorf, SA, and recording made freely available afterwards	25 May 2021

Appendix 6. Sensory Evaluation Report

Attention: Mango Parker/Con Simos		Report date:	20/11/2020
Analysis Report:	Desireé Likos, Eleanor Bilogrevic, Leigh Francis		

Materials and Methods

A panel of at least nine qualified and highly experienced AWRI judges was convened to assess the Chardonnay wines, and twelve for the Pinot Noir wines and ten for the Shiraz wines. Judges were chosen for their ability to perceive smoke flavour from phenol glycosides, and on the basis of previous experience and performance in smoke taint sensory panels. For the Shiraz and Pinot Noir assessments, judges were selected from the external descriptive panel, and for the Chardonnay assessment, judges were selected from both the external descriptive panel and internal technical quality panel. The judges were asked to rate *smoke aroma* (defined as any type of smoke aroma, including hickory or artificial smoke, phenolic, burnt aroma associated with ashes, ashtray, fire ash, including also medicinal, band aid) and *smoke flavour* (defined as including bacon, smoked meat and ashy aftertaste) and *overall fruit aroma* (defined as any type of citrus fruit, stone fruit, and tropical fruits including pineapple) for the white wines and (any type of red fruit, dark fruit, red berry, strawberry, raspberry and cherry) for the red wines. O*verall fruit flavour* was also included. Judges also had an '*other'* term for both aroma and palate to capture any additional noteworthy characteristics in the wines.

The samples were presented separated by variety. The Chardonnay assessment was carried out on 30 September 2020, with assessors rating twelve wines in duplicate in one day, with a 30 minute break between the first and second presentation replicate. An additional sample of non-smoke exposed control wine from Adelaide Hills 2020 vintage was also included, '201704-CHA'. The Pinot Noir assessment was carried out on the 13 and 14 October 2020, with assessors rating twelve wines in duplicate across the two days. The Shiraz assessment was carried out on 15 and 20 October 2020, with assessors rating sixteen wines in duplicate across the two days. The tests were supervised by Eleanor Bilogrevic and Desireé Likos.

Samples were presented to judges in 30 mL aliquots in 3-digit-coded, covered, ISO XL5 standard wine glasses at 22–24 °C, in isolated booths under colour-masking sodium lighting, with randomised presentation order - a modified Williams Latin Square design generated by Compusense Cloud sensory evaluation software (Compusense Inc., Guelph, Canada). Samples were assessed in duplicate. The judges were forced to have a 2-minute rest between samples. Water was provided for palate cleansing.

The intensity of each attribute was rated using an unstructured 15 cm line scale (0 to 10), with indented anchor points of 'low' and 'high' placed at 10% and 90% respectively. Data were acquired using Compusense Cloud sensory evaluation software. The results were analysed by analysis of variance (ANOVA), for the fixed effects of wine and presentation replicate, the random effect of judge, and their two-way interactions, followed by a Fisher's Least Significant Difference (LSD) and Dunnett's means comparison test to determine whether the samples were rated significantly higher than the corresponding controls.

Results

For each of the three sets, from the ANOVA there were highly significant differences between the wines for *smoke aroma* and *smoke flavour*. The mean data for each wine is shown in Tables 1-3 below.

Table 1. Smoke mean scores, rated on a 0 to 10 scale, for the Chardonnay wines

Wines	Smoke Aroma	Smoke Flavour	Significantly different from all three controls[ǂ]?	Significantly different from one control[†]?	Notes
201704_CHA_AH (Clean Control)	0.35	0.86	-	No	
2018-02 (Clean Control)	0.93	1.46	-	No	
2018-06 (Clean Control)	0.45	0.77	-	-	
2018-01	0.57	2.27	No	Yes, Flavour only	
2018-03	1.65	2.89	No	Yes, Aroma and Flavour	Several comments for rubber, wet dog and cooked vegetable (reduction)
2018-04	0.04	0.65	No	No	Many comments, but no consensus
2018-05	0.48	0.90	No	No	Many comments for sweaty, box-hedge, rubber aromas (could be thiol related)
2018-07	0.18	1.58	No	No	Several comments for cooked vegetable/green tomato leaf terms
2018-08	0.40	0.38	No	No	
2018-09	0.81	1.49	No	No	Many comments for sweaty, fungal, cheesy/vomit aromas
2018-10	2.88	4.23	Yes, Aroma and Flavour	Yes, Aroma and Flavour	
2018-11	1.00	1.69	No	No	Rated highest in Overall Fruit, but also had several comments for dirty drains (reduction) aromas
2018-12	3.77	5.79	Yes, Aroma and Flavour	Yes, Aroma and Flavour	

ǂDunnett's (left-sided) means comparison test, using the higher of the three control wines as the 'control'.
[†]Fishers's least significant difference (LSD) means comparison test.

Table 2. Smoke mean scores, rated on a 0 to 10 scale, for the Pinot Noir wines

Wines	Smoke Aroma	Smoke Flavour	Significantly different from all three controls[ǂ]?	Significantly different from one control[†]?	Notes
2018-40 (Clean Control)	1.36	1.96	-	No	Several comments for cooked vegetables (reduction) and vinegar (oxidation)
2018-41 (Clean Control)	1.31	1.18	-	No	
2018-42 (Clean Control)	1.22	1.45	-	-	
2018-43	1.09	1.88	No	No	
2018-44	1.76	1.99	No	No	
2018-45	1.79	2.07	No	No	Slightly elevated smoke flavour rating, but also rated highest in overall fruit aroma.
2018-46	2.32	3.27	No	Yes, Flavour Only	
2018-47	2.88	3.84	Yes, Flavour only	Yes, Aroma and Flavour	
2018-49	2.79	3.77	Yes, Flavour only	Yes, Aroma and Flavour	
2018-50	4.79	5.36	Yes, Aroma and Flavour	Yes, Aroma and Flavour	
2018-51	4.83	5.02	Yes, Aroma and Flavour	Yes, Aroma and Flavour	Several comments for mint aroma and flavour
2018-52	5.70	6.62	Yes, Aroma and Flavour	Yes, Aroma and Flavour	

ǂDunnett's (left-sided) means comparison test, using the higher of the three control wines as the 'control'.
[†]Fishers's least significant difference (LSD) means comparison test.

Table 3. Smoke mean scores, rated on a 0 to 10 scale, for the Shiraz wines

Wines	Smoke Aroma	Smoke Flavour	Significantly different from the two controls‡?	Significantly different from one control†?	Notes
2018-20 (Clean Control)	0.98	2.46	NA*	NA*	Lots of comments for green and reductive type aromas
2018-21 (Clean Control)	0.17	0.85	-	-	Several comments for mint and cooked vegetables (reduction)
2018-22 (Clean Control)	0.35	1.44	-	No	Comments for green terms on palate
2018-23	0.00	1.05	No	No	Many comments for cooked vegetables (reduction)
2018-24	1.69	2.51	Yes, Aroma only	Yes, Aroma and Flavour	Many comments for cooked vegetables (reduction)
2018-25	0.58	1.61	No	No	
2018-26	0.52	1.34	No	No	
2018-27	0.71	1.21	No	No	
2018-28	0.69	1.69	No	No	Several comments for earthy and dirty drains (reduction)
2018-29	1.24	2.80	No	Yes, Aroma and Flavour	Comments for green terms on aroma and palate
2018-30	0.21	1.00	No	No	
2018-31	3.09	4.02	Yes, Aroma and Flavour	Yes, Aroma and Flavour	
2018-32	6.72	7.42	Yes, Aroma and Flavour	Yes, Aroma and Flavour	
2018-33	3.52	5.37	Yes, Aroma and Flavour	Yes, Aroma and Flavour	
2018-34	1.41	2.31	No	Yes, Aroma and Flavour	Comments for rubber, cooked vegetable and mint
2018-35	0.37	1.51	No	No	

‡Dunnett's (left-sided) means comparison test, using the higher of the three control wines as the 'control'. †Fishers's least significant difference (LSD) means comparison test.
*Wine 2018-20 was removed from further analysis due to a higher than expected mean value, which some of the assessors commented as being 'green/reductive', which may have been causing confusion during the assessments.

Appendix 1a. F-values†, degrees of freedom (df) and mean square error from analysis of variance for the Chardonnay wines

Attribute	Judge	Wine	PreRep	Judge* Wine	Judge* PreRep	Wine* PreRep	MSE
Overall Fruit A	13.92***	1.80ǂ	9.84**	0.82	1.50	1.18	1.655
Smoke A	4.61***	9.04***	0.29	1.46*	1.32	0.77	2.453
Other A	24.00***	1.78ǂ	3.55ǂ	1.11	3.00**	0.78	2.545
Overall Fruit F	33.15***	2.57**	7.52**	1.43*	1.09	1.20	1.310
Smoke F	3.98***	12.48***	0.03	1.13	0.66	0.85	3.535
Other F	36.29***	2.09*	11.03**	1.41*	3.10**	0.94	1.983
Df	8	12	1	96	8	12	96

A: aroma, F: Flavour. †Significance levels are as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ǂ $P < 0.10$.
PreRep = Presentation Replicate, df = degrees of freedom, MSE = Adjusted Mean Square Error.

Appendix 1b. F-values†, degrees of freedom (df) and mean square error from analysis of variance for the Pinot Noir wines

Attribute	Judge	Wine	PreRep	Judge* Wine	Judge* PreRep	Wine* PreRep	MSE
Overall Fruit A	21.66***	2.88**	3.19ǂ	1.05	2.82**	0.34	1.635
Smoke A	10.68***	17.12***	2.05	1.22	1.37	0.46	3.606
Other A	9.11***	3.40***	0.75	1.60**	1.70ǂ	0.47	1.744
Overall Fruit F	52.56***	5.57***	1.07	1.04	1.21	0.67	1.203
Smoke F	3.20***	16.14***	2.85ǂ	0.94	1.59	0.22	4.539
Other F	26.59***	2.81**	1.36	3.04***	2.01*	2.41*	1.218
Df	11	11	1	121	11	11	121

A: aroma, F: Flavour. †Significance levels are as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ǂ $P < 0.10$.
PreRep = Presentation Replicate, df = degrees of freedom, MSE = Adjusted Mean Square Error.

Appendix 1c. F-values†, degrees of freedom (df) and mean square error from analysis of variance for the Shiraz wines

Attribute	Judge	Wine	PreRep	Judge* Wine	Judge* PreRep	Wine* PreRep	MSE
Overall Fruit A	39.58***	7.50***	0.40	1.36*	0.99	0.86	1.076
Smoke A	5.62***	27.68***	0.97	1.16	1.83ǂ	1.01	2.184
Other A	15.11***	6.48***	0.01	1.27ǂ	2.38*	0.65	2.494
Overall Fruit F	40.11***	7.91***	8.10**	1.30ǂ	1.04	0.56	1.295
Smoke F	5.32***	15.49***	12.64**	1.08	1.43	1.08	4.167
Other F	29.31***	5.59***	0.38	1.58**	2.82**	0.96	1.840
Df	9	15	1	135	9	15	135

A: aroma, F: Flavour. †Significance levels are as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ǂ $P < 0.10$.
PreRep = Presentation Replicate, df = degrees of freedom, MSE = Adjusted Mean Square Error.

Notes
Sensory analysis does not provide an absolute measurement, with sensory rating being a comparative technique, requiring evaluation of statistically significant differences between samples. Even with a highly trained and experienced sensory panel, 'false positives' can occur, with non-zero scores given by assessors for unaffected wines, due in part to an expectation effect on the assessors. To minimise this effect, and to allow an assessment of the magnitude of a smoke rating that can be considered significant, an unaffected control wine is always included in the sample set. It should be noted that smoke scores of between 0.5 to 1.5 have been commonly obtained in unaffected control wines using the AWRI sensory methodology.

Current knowledge does not allow a strict assessment of acceptable or unacceptable level of smoke flavour or aroma. For some taints such as cork taint, consumer preference data and knowledge of the unpleasant character means the presence of any degree of taint can be considered unacceptable, but for other off-flavours a low intensity as determined by a trained sensory panel can potentially have no negative effects on consumer acceptance.

A recent consumer acceptance study at the AWRI showed that consumers respond negatively to the flavour of bushfire smoke in wine. Smoke intensity scores above 2.0 using the AWRI sensory methodology and rating system were required to negatively affect consumer preference in this study, involving only one wine type.

Each producer should consider the question of their tolerance to risk when making commercial decisions regarding acceptability ie potentially releasing a wine to the market with smoke intensity at a level that reduces consumer opinion, compared to not releasing a wine that may have a negligible effect on consumer opinion.

Disclaimer
This sensory analysis and interpretation of results has been conducted on samples of wine submitted to the AWRI. The results apply only to the samples submitted and their characteristics at the time of tasting and cannot be generalised to other related samples. You should not rely solely on the information presented in this document, your individual circumstances and company practices and policies, among other things, should also be taken into account when interpreting these results. The AWRI shall not be liable (whether in contract, tort, under any statute or otherwise) for loss or damage of any kind (including direct, indirect and consequential loss and damage of business revenue, loss of profits, failure to realise expected profits or savings or other commercial or economic loss of any kind) arising out of your reliance on results, information or commentary presented in this document.

Appendix 7. Masking of smoke aroma by common wine odorants

Table 1. Compounds spiked into smoky wine for aroma assessment

Compound	Added concentration (µg/L)	Notes on aroma compared to wine without addition
1,8-cineole	5	Red fruit, slight green, slightly minty, cherry, slight char, spice
1,8-cineole	30	Very green menthol mint, eucalyptus, slight red fruit
3-isobutyl-2-methoxypyrazine	0.010	Dry earthy, slight chalky, low charry note
3-isobutyl-2-methoxypyrazine	0.030	Green capsicum, very low burnt note
vanillin	200	Toasty vanilla oak
vanillin	500	Marshmallows and raspberry, strong vanilla
oak lactone	100	Slight wet grass, smoked bacon
oak lactone	500	Slightly oxidised, coconut and wet earth
wine lactone	0.2	Five spice, less smoke almost none
wine lactone	0.3	Pinot Noir varietal, strawberry, fragrant, earthy and cedar
3-sulfanylhexan-1-ol	100	Red fruit, dried strawberry, raspberry, plum
3-sulfanylhexan-1-ol	250	Soapy and detergent like, spicy, medium intensity red fruit
ethyl 3-methylbutanoate	5	Woody, slight spice note, low intensity fruit
ethyl 3-methylbutanoate	25	Slightly more intense fruit note, vague aroma, red fruit
β-Ionone	0.1	Clean red fruit, berry, strawberry
β-Ionone	15	Raspberries, very high intensity
Control wine with no additions	-	Smoky campfire moderate intensity

Appendix 8. Decision tool for grapes suspected of smoke exposure, to guide the assessment of risk management options by wine growers

Decision tool for grapes suspected of smoke exposure, to guide the assessment of risk management options by wine growers

Authors: M. Parker, I.L. Francis and M.J. Herderich



Who should use this tool?

This decision tool has been designed to help grapegrowers and wine producers with vineyards suspected of exposure to smoke from nearby fires. The tool is based on data obtained from wines produced from grapes with varied smoke exposure from the 2019-2020 Australian wildfires. It has been developed to minimise risk of producing wine with smoke flavour, while avoiding unnecessary crop losses.

I smell smoke. What should I do?

If you can smell smoke or see smoke haze in the vineyard, or if there have been active fires nearby, the grapes may be at risk. A slight smell of smoke may not always lead to smoke-affected grapes and/or noticeable quality defects in wine. If a fire is near the vineyard, consider the smoke duration, wind direction and intensity of smoke rather than simply proximity to fire. Review end-use plans, options for alternative supply, acceptance criteria and risk appetite. Consider preparing for some chemical testing, small-lot ferments and sensory evaluation prior to finalising harvesting and winemaking plans.

Establish whether grapes have been exposed to smoke

Testing is available for smoke exposure markers. Data from suspect grapes can be compared to known concentrations in non-smoke-exposed samples (Coulter et al. 2022). Concentrations of all smoke exposure markers should be used to determine smoke exposure. Assessment of grapes sampled approximately two weeks prior to harvest allows the most accurate interpretation. If

grapes are tested earlier, do not assume that increase in berry size will necessarily reduce estimated risk at harvest.

Samples with a concentration of markers consistent with those found in non-smoke-exposed grapes have negligible risk of producing smoke-tainted wine. Provided no further smoke exposure occurs between sampling and harvest, the assessment is not expected to change and grapes classified as non-smoke-exposed can be managed as normal.

Testing indicates smoke exposure. Now what?

Grapes with elevated smoke markers may produce wine with undesirable smoky flavours. Data from suspect samples can be compared to concentrations found after the 2020 Australian bushfires in grapes that produced smoky wines. This aids the assessment of the risk for producing wine with smoky flavour.

The following graphs summarise the lowest concentrations of smoke exposure markers in grapes that produced smoky wines, for the cultivars Chardonnay, Pinot Noir and Shiraz. Note the concentrations are different for the different varieties.

The red zone is high risk: all wines produced under controlled conditions at 50 kg scale from grapes with these, and higher, concentrations of smoke markers had obvious smoke flavour.

The dark grey zone describes grapes at moderate risk: some wines, but not all, produced from such grapes without further winemaking mitigation, had obvious smoke flavour.

Wine produced from grapes in the green (no smoke exposure) and light grey zones (low degree of smoke exposure) did not have obvious smoke flavour.

Special management during harvesting, crushing and winemaking may be required for grapes with moderate and high risk of smoke taint (dark grey and red zones). For information on options, including evaluation of taint minimisation or remediation techniques, refer to the AWRI's smoke taint webpage and relevant fact sheets linked from that page.

For cultivars not represented in the following figures, choose the data set closest to the wine style. For example, if making wine from white varieties or rosé wines with minimal skin contact, use the Chardonnay results for guidance. If making lighter red wine styles, use the Pinot Noir data, and if making full-bodied red styles, use the Shiraz data for guidance.

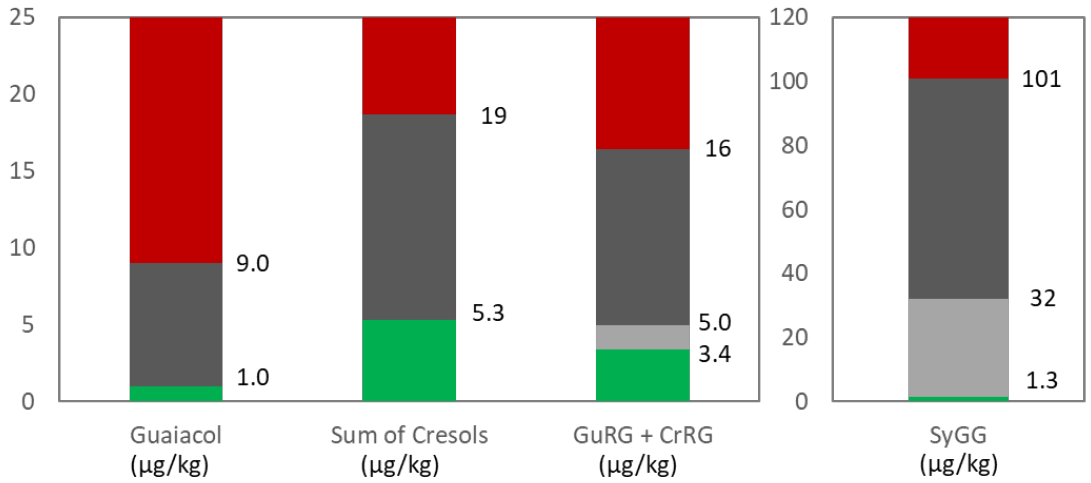


Figure 1. Risk zones for key smoke exposure markers in Chardonnay grapes. The red zone is high risk: all wines produced under controlled conditions at 50 kg scale from grapes with these concentrations of markers had obvious smoke flavour. The dark grey zone describes grapes at moderate risk: some wines, but not all wines, produced from such grapes without further winemaking mitigation, had obvious smoke flavour. Wine produced from grapes in the green (no smoke exposure) and light grey zones (low degree of smoke exposure) did not have obvious smoke flavour.

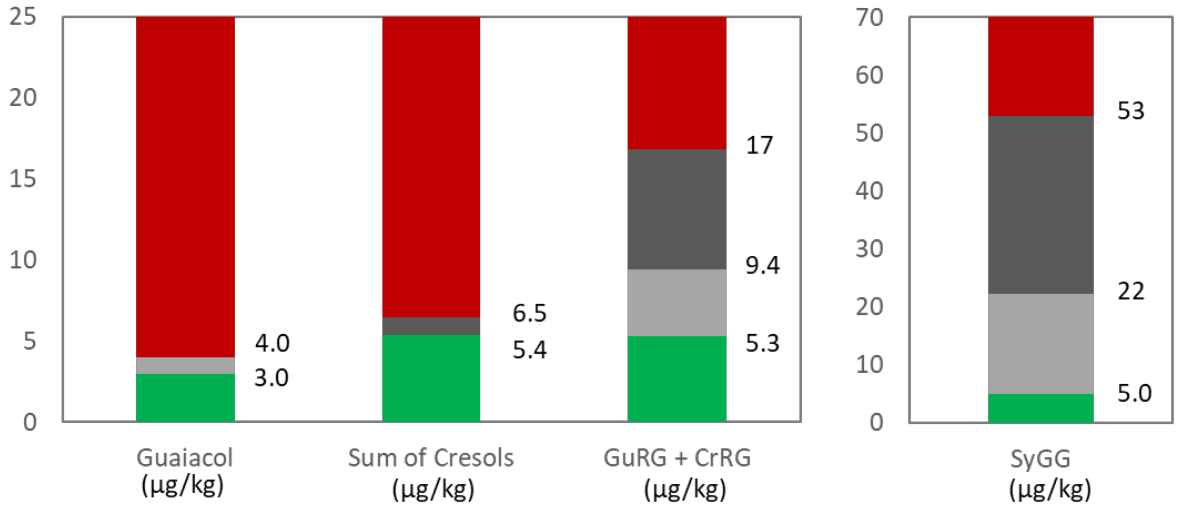


Figure 2. Risk zones for key smoke markers in Pinot Noir grapes. The red zone is high risk: all wines produced under controlled conditions at 50 kg scale from grapes with these concentrations of markers had obvious smoke flavour. The dark grey zone describes grapes at moderate risk: some wines, but not all wines, produced from such grapes without further winemaking mitigation, had obvious smoke flavour. Wine produced from grapes in the green (no smoke exposure) and light grey zones (low degree of smoke exposure) did not have obvious smoke flavour.

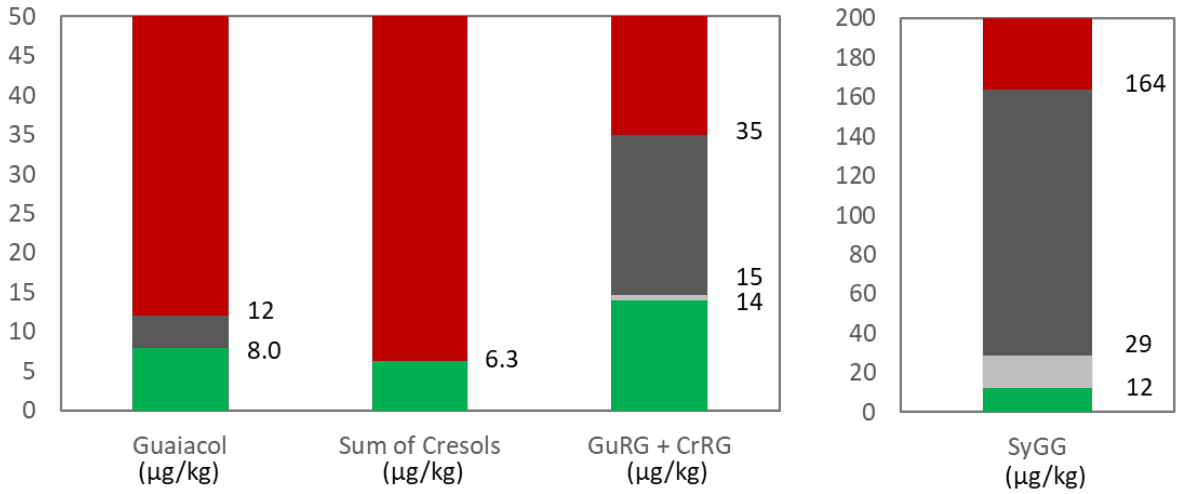


Figure 3. Risk zones for key smoke markers in Shiraz grapes. The red zone is high risk: all wines produced under controlled conditions at 50 kg scale from grapes with these concentrations of markers had obvious smoke flavour. The dark grey zone describes grapes at moderate risk: some wines, but not all wines, produced from such grapes without further winemaking mitigation, had obvious smoke flavour. Wine produced from grapes in the green (no smoke exposure) and light grey zones (low degree of smoke exposure) did not have obvious smoke flavour.

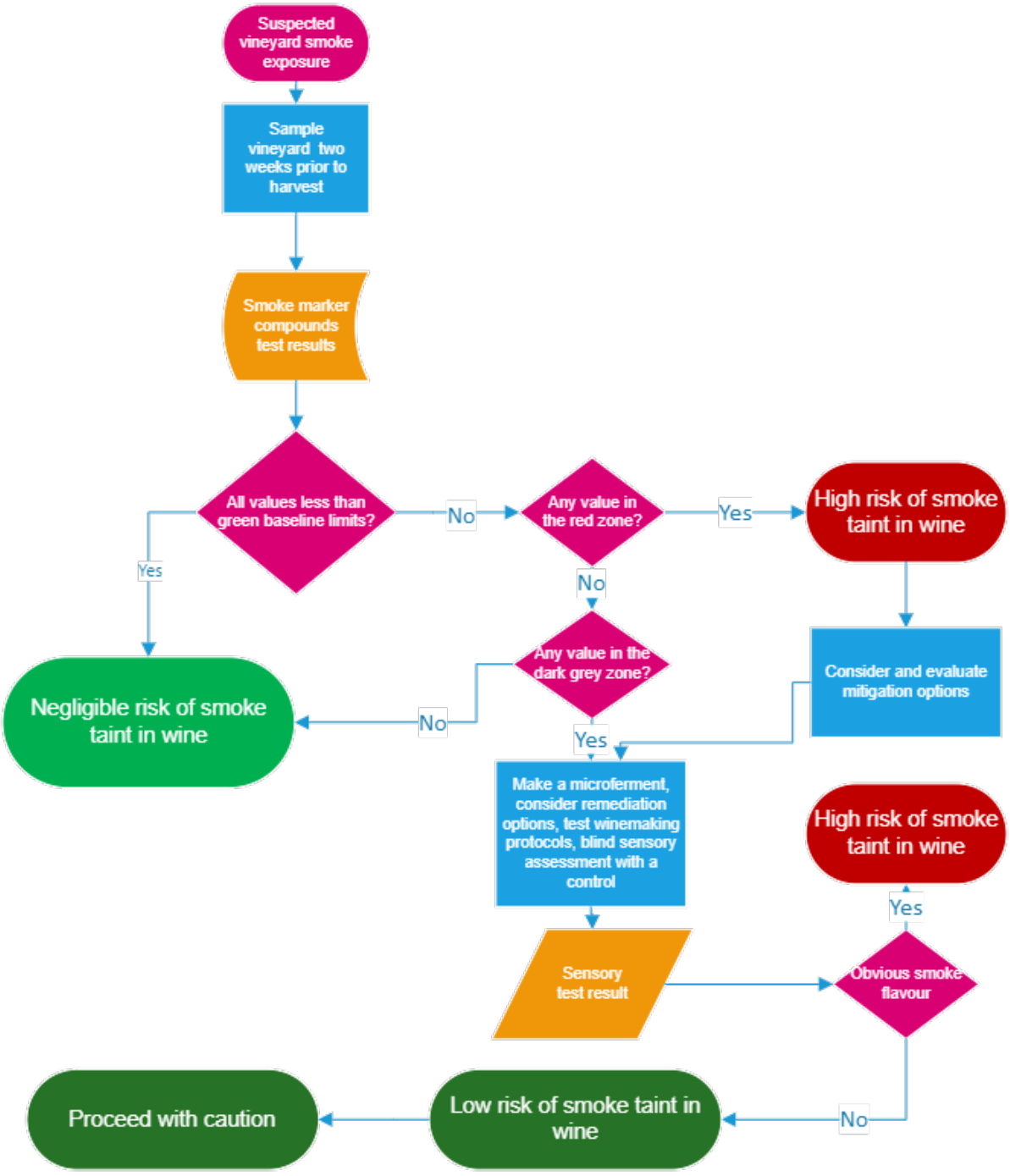


Figure 4. Decision tree for grape and wine producers to use when confronted with grapes suspected of smoke exposure

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Reference and further reading
Coulter, A., Baldock, G. A., Parker, M., Hayasaka, Y., Francis, I. L., Herderich, M. 2022. The concentration of smoke marker compounds in non-smoke-exposed grapes and wine in Australia. *Aust. J. Grape Wine Res.* 28(3): 459-474. Available from: https://doi.org/10.1111/ajgw.12543

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