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Research Article

The Effect of Pre-Veraison Smoke Exposure of Grapes on Phenolic Compounds and Smoky Flavour in Wine

W. W. Jiang,[1] **E. Bilogrevic**,[1] **M. Parker**,[1] **I. L. Francis**,[1] **P. Leske**,[2] **Y. Hayasaka**,[1] **S. Barter**,[1] **and M. Herderich**[1]

[1]*The Australian Wine Research Institute, Glen Osmond (Adelaide), SA 5064, Australia*
[2]*Revenir Winemaking Pty Ltd, Lenswood, SA 5240, Australia*

Correspondence should be addressed to M. Herderich; markus.herderich@awri.com.au

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Background and Aims. Smoke exposure occurred in the Adelaide Hills region in December 2019 due to a wildfire, when wine grapes were peppercorn-size green berries. Previously, pre-veraison smoke exposure had been identified through model experiments as unlikely to affect grape composition, whereas smoke exposure after veraison can have a major effect on wine flavour. Hence the effects of pre-veraison smoke on grape and wine composition, and smoky sensory properties of wine were investigated. *Methods and Results*. Chardonnay, Pinot Noir and Shiraz were investigated and eight blocks with varied smoke exposure were selected for each cultivar. Berries were sampled initially four weeks after the fire and at harvest, and mature grapes were made into unoaked wines. Established smoke exposure markers, phenolic glycosides, were found in berries at pre-veraison and at harvest from the high smoke exposure sites, with concentrations well above those found in non-smoke exposed fruit. Volatile phenols were also elevated in grapes at harvest. The resulting red wines from some exposure vineyards were high in volatile phenols, glycosides and smoky flavours. However, most of the Chardonnay wines expressed much less smoky flavours, despite similar levels of smoke exposure of grapes. *Conclusions*. Pre-veraison smoke exposure can result in elevated concentrations of volatile phenols and their glycosidic metabolites in grape berries and wine and cause strong smoky flavour in wine. *Significance*. The wine sector and land management agencies responsible for controlled burns need to consider the effect of smoke from fires near vineyards even very early in the growing season.

1. Introduction

Smoke, from bushfires and prescribed burns, has caused significant losses to the Australian wine sector since first reported in 2003 [1]. Major fire events occurred in 2004, 2007, 2009, 2013, 2015, 2020 and 2021, with losses to the Australian wine sector estimated at $1.4 billion [2]. Looking beyond Australia, wildfires have affected wine production in many countries including Canada, Chile, South Africa, the United States and Southern Europe [3]. Climate modelling predicts that the incidence and intensity of wildfires will increase in many wine regions, in the south-eastern parts of Australia, which contain most of Australia's wine producing vineyards, and particularly early in spring [4].

The effects of smoke exposure post-veraison on ripening and mature wine grapes through the uptake of volatile phenols, followed by metabolism to form phenolic glycosides, have been well documented [5, 6]. Wine made from smoke-exposed berries can be high in volatile phenols and phenolic glycosides which contribute to smoky aroma and flavours [7–9]. Though phenolic glycosides do not contribute directly to the aroma as they are non-volatile grape metabolites, they are important to the flavour and aftertaste of smoke-affected wines, through release of volatile phenols during wine storage and tasting [8, 9].

To assess grapes and wine suspected of smoke exposure a range of marker compounds including volatile phenols and glycosides can be used [7]. Volatile phenols associated with smoke exposure include guaiacol, 4-methylguaiacol, *o*-cresol, *m*-cresol, *p*-cresol, syringol and 4-methylsyringol; glycosides include 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) [7]. The concentration of these seven volatile phenols and six glycosides has been determined in over 1,000 samples of non-smoke exposed grapes and wine of twelve major cultivars produced in Australia and can be used to identify samples suspected of smoke exposure [10]. The concentrations of all seven volatile phenols and six glycosides need to be considered when assessing smoke exposed grapes, as they can be present in varying proportions: typically SyGG is the most abundant marker for smoke exposure of grapes and volatile phenols are present in low concentrations, but patterns such as elevated volatile phenols and low concentrations of glycosides have also been observed in some samples [11].

Grape berries were previously shown in model experiments to vary in their susceptibility to smoke uptake during the growing season [12, 13]. In this early research Merlot grape berries were deliberately exposed to smoke at various phenological stages. These studies were based on assessing the volatile smoke exposure markers available at the time, guaiacol and 4-methylguaiacol, and did not include the phenolic glycosides. The data identified the highest risk from one-week post-veraison until harvest and smoke exposure during the pre-veraison period was identified as low to variable risk [13]. Furthermore, sensory assessment of the wine made for this early study was based on aroma only, so did not capture the smoky/ashy flavour and undesirable aftertaste characteristics of smoke-affected wines. Guided by this early work, further model experiments have focused on smoke exposure of ripening grapes close to harvest [14–19], and did not address the impact of smoke exposure at earlier stages of pre-veraison grape development.

This knowledge gap became particularly problematic over the last decade due to the changing pattern of wildfires: Historically, wildfires have generally occurred in summer and autumn in southern Australia, which normally coincides with the stage when grapes are advanced in ripening and close to harvest. However, in 2019, smoke from wildfires affected viticultural regions much earlier in the season. Smoke was noted in the Hunter Valley in New South Wales continuously from October 2019 to January 2020 [20], arising from several fire events, resulting in some exposure of small green berries between Eichhorn-Lorenz (E-L) stage 27–38 [21] during much of the early grape ripening period. Phenolic glycosides were observed as a consequence of smoke exposure in both berry and leaf samples collected pre-veraison as early as E-L 29 [20]. Unfortunately, the continuous exposure to smoke occurring in the Hunter Valley during the ripening period meant the timing of smoke exposure could not be isolated and linked specifically to compositional changes, and with no wines produced in the study the link between pre-veraison smoke exposure, occurrence of smoke exposure markers in grapes and wine composition and sensory properties could not be confirmed.

In the Adelaide Hills winemaking region of South Australia, a wildfire burned out of control from early on 20 December 2019. The “Cudlee Creek bushfire” was likely started by a tree falling on powerlines shortly after 9 am [22]. Fanned by strong northerly winds, it burned rapidly in a south-easterly direction from Cudlee Creek, and by midday was threatening several towns including Lobethal and Woodside. A westerly wind change pushed the fire towards the east in the afternoon before a southerly wind change turned the fire front towards the town of Mount Torrens in the north at ca. 7 pm [23]. By the time the fire was declared contained on 3 January 2020, the fire had burnt 23,253 hectares of land in total, including 1,100 hectares of vineyards [22]. In addition to the damage sustained by burnt vineyards, a large amount of smoke-affected many more vineyards throughout the Adelaide Hills grape growing region, where Chardonnay, Pinot Noir and Shiraz are common cultivars.

The smoke exposure pattern caused by the “Cudlee Creek bushfire” differed markedly from that of the Hunter Valley, described above. The change in the wind direction meant that in the Adelaide Hills, the majority of the smoke drifted to the north and west and cleared relatively quickly away from the viticultural area. Unlike the prolonged period of repeated smoke exposure observed in the Hunter Valley, in most cases smoke exposure of Adelaide Hills’ vineyards was brief, intense and unevenly distributed, and was observed to arise from adjacent burnt forest and pastures, but also from combustion of mid-row grasses, and vineyard infrastructure including posts, irrigation dripline, and vines themselves. In addition, some pockets of smoke also settled in some valleys for 48 to 72 hours, causing localised effects as far as 4.5 km from the fire scar. After the fire was declared safe on 3 January 2020, there was negligible additional smoke exposure evident until the grapes reached commercial maturity and were harvested in March [22]. Still, witnesses noticed that the smell of burnt vegetation persisted for some weeks in badly burnt areas.

The smoke from the Cudlee Creek fire occurred when grape berries were small, hard and green, at approximately average development stage of E-L 29. The smoke-affected vineyards in the Adelaide Hills presented an opportunity to investigate the effects of a single wildfire smoke event prior to veraison on grapes and wine. In addition to verifying uptake of volatile phenols by green, unripe berries and the occurrence of their glycosides as observed earlier for the grape samples from the Hunter Valley, several additional questions arose that could be tested. In this context, it was of particular interest to assess if berry size increases, with grape berries approximately doubling in weight from E-L 29 to harvest, would result in decreases in the concentration of glycosides? Or with no further smoke exposure, would glycoside content per berry remain constant? And finally, would elevated concentrations of glycosides remain in the berries at harvest, potentially leading to significant smoky aromas and flavours in the resulting wine?

In summary, this study was designed to assess the impact of the early season smoke on three cultivars, Chardonnay, Pinot Noir and Shiraz, with grape berries sampled pre-veraison (E-L 33) four weeks after the fire, and again at harvest in March 2020 (between 10.5 and 12.5 weeks after the start of the fire). For each cultivar, eight blocks chosen from 12 vineyards were sampled (not all vineyards had all three cultivars grown in nearby blocks, hence the larger number of vineyards), representing a range of smoke exposure including control samples with no known smoke exposure and sites likely exposed to some smoke. Amongst the vineyards selected some sites had mild fire damage evident within the vineyard, such as burnt grass in the swathe, but still were able to successfully ripen the grapes. Here we present and discuss the chemical composition of grapes at pre-veraison and at harvest, the chemical composition of the resultant wine, and the smoky sensory properties in the wines. The study aimed to improve our understanding of whether smoke early in the growing season can be taken up by pre-veraison berries, whether volatile phenols will be subsequently metabolised to form glycosides as is seen in post-veraison berries, and ultimately whether such early smoke exposure of unripe grapes can affect wine composition and sensory attributes.

2. Materials and Methods

2.1. Chemicals. Water used in this study was purified via a MilliQ water purification system (Millipore, North Ryde, NSW, Australia). High performance liquid chromatography (HPLC) grade methanol and acetonitrile (Lichrosolv) were purchased from Merck (Bayswater, Vic, Australia). Glacial acetic acid was purchased from Rowe Scientific (Lonsdale, SA, Australia). Deuterium-labelled standards (d_3-syringol gentiobioside, d_3-guaiacol, d_3-4-methylguaiacol, d_3-syringol, and d_7-*p*-cresol) had been synthesized in house as reported previously [24, 25].

2.2. Winemaking Additives. Rohavin® L pectinase enzyme, AB Mauri PDM yeast, Activator and Pinnacle MaloSafe malolactic acid (MLF) bacteria were sourced from AB Biotek (Sydney, NSW, Australia). Diammonium phosphate (DAP), potassium metabisulfite (PMS) and tartaric acid (H_2T) were sourced from EE Muir and Sons (Lenswood, SA, Australia).

2.3. Vineyard Sites. Eight blocks each of Chardonnay, Shiraz and Pinot Noir grapevines were selected from a total of 12 vineyards (Figure 1). The 12 vineyards were classified into three categories based on the degree of smoke exposure, which was in turn assessed based on the proximity of the sites to the fire scar and the degree to which fire damage was evident adjacent or within the vineyards: "no exposure;" "smoke suspected;" and "fire and smoke apparent" (Table 1). Two control vineyards (A and B) containing all three cultivars were selected from Adelaide Hills areas where there was no record of smoke or haze in the 2019 to 2020 grape growing season. Vineyard A was near Kuitpo and located 16 km to the west of vineyard B (at Macclesfield) and 44 km south of the southern-most tip of the Cudlee Creek fire scar. The cultivar blocks within the control vineyards, were approximately 0.5 km apart. The "smoke suspected" sites category included four vineyards (C–F) on the edge of the fire zone. Vineyards C and D located in Woodside, vineyard E in Forreston and vineyard F in Kenton Valley were moderately exposed to smoke for a short time in the afternoon of 20 December 2019. Vineyards C and D contained all the three target cultivars, and the blocks were within 0.5 km of one another. Vineyard C was technically within the fire scar; the Shiraz vines were downhill closest to the fire whilst the adjacent Chardonnay and Pinot Noir blocks were further up the hill. Vineyard E, approximately 4.5 km north of the fire scar, contained adjacent Chardonnay and Pinot Noir blocks and the sampling points were within 0.6 km. The fire scar surrounded vineyard F which contained only Shiraz.

Six vineyards (G-L) within the fire scar were categorised as "fire and smoke apparent". Vineyard G in Lobethal contained all three cultivars adjacent to one another from west to east. The fire came within 300 m of the Pinot Noir and Chardonnay blocks, whereas the Shiraz block in vineyard G was slightly further to the east, away from the fire. Vineyard H in Charleston consisted of Pinot Noir, vineyard I in Woodside contained only Shiraz, and vineyard J in Kenton Valley contained Chardonnay vines. Vineyard K in Woodside was planted with Chardonnay and Shiraz adjacent to one other (maximum of 0.6 km between the two sampling points). Vineyard L had some active fire within, and was located 2 km to the east of vineyard K.

On the day of the wildfire on 20 December 2019, the grapevines were at or beyond E-L stage 29 with berries at peppercorn size and bunches tending downwards. At the first sampling point in January 2020, grapes were between E-L stage 32 to 33 with hard and green berries [21].

2.4. Grape Sampling and Processing. Chardonnay, Shiraz and Pinot Noir were sampled as described above. Three adjacent rows were selected and tagged for sampling per cultivar and vineyard. Five bunches of grapes were sampled randomly from each of the three rows, with each row being treated as one replicate. Sampling was conducted at two time points after the smoke exposure during the growing season. The first time point (marked as pre-veraison) on 14 January 2020 was four weeks after the smoke exposure, and all cultivars across the 24 blocks were sampled on the same day. The second grape sampling time point was at maturity and was decided based on the average maturity level of each cultivar. To keep time between exposure and sampling a constant within a cultivar, and to simplify the logistics for winemaking, all blocks of the same cultivar were sampled on the same day regardless of the varying soluble solids level across a cultivar, resulting in three different sampling dates (Table S1): Pinot Noir grapes were sampled on 3 March 2020, 10.5 weeks after the start of the wildfire, followed by Chardonnay grapes on 13 March 2020, 12 weeks after the start of the wildfire, and Shiraz grapes on 18 March 2020, 12.5 weeks after the start of the wildfire. Grape bunches were sampled using the same sampling protocol as for the January sampling. Samples were placed in plastic zip lock bags and stored at −20°C prior to processing.

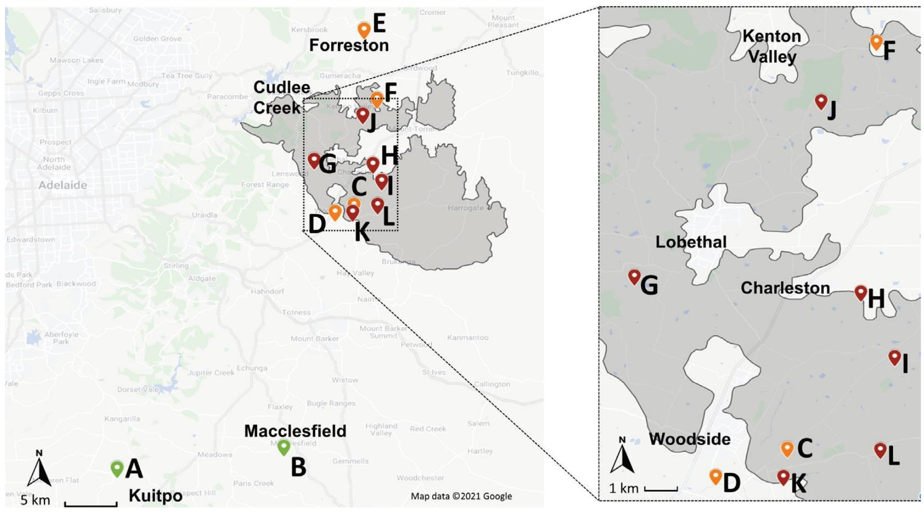

Figure 1: Locations of the vineyards included in the project. The fire scar is indicated in grey shading, and the location pin colours relate to extent of smoke exposure: green-"no exposure" control sites; orange-"smoke suspected" sites; red-"fire and smoke apparent" sites. Details about cultivars are in Table 1.

Table 1: Overview of vineyard locations, cultivars and fire or smoke impact.

Fire/smoke impact	Location	Vineyard	Cultivar
No exposure	Kuitpo	A	Chardonnay
		A	Shiraz
		A	Pinot Noir
	Macclesfield	B	Chardonnay
		B	Shiraz
		B	Pinot Noir
Smoke suspected	Woodside	C	Chardonnay
		C	Shiraz
		C	Pinot Noir
	Woodside	D	Chardonnay
		D	Shiraz
		D	Pinot Noir
	Forreston	E	Chardonnay
		E	Shiraz
	Kenton Valley	F	Pinot Noir
Fire and smoke apparent	Lobethal	G	Chardonnay
		G	Shiraz
		G	Pinot Noir
	Charleston	H	Pinot Noir
	Woodside	I	Shiraz
	Lobethal	J	Chardonnay
	Woodside	K	Chardonnay
		K	Shiraz
	Woodside	L	Pinot Noir

The grape bunches were thawed at 4°C overnight then de-stemmed by hand. Depending on the berry size, between 100 and 180 berries were counted and weighed prior to homogenization using an IKA T18 Basic Ultra Turrax® (Staufen, Germany) at ambient temperature. The grape berry homogenate was subsequently used for the analysis of volatile phenols and glycosides.

2.5. Winemaking. At the harvest timepoint, an additional 50–90 kg of grapes was hand-picked from each block for small-scale winemaking, involving a single fermentation replicate. The Pinot Noir from vineyard H had been previously harvested by the landowner, and no wine could be made from vineyard H. The grapes were delivered to the WIC winemaking services at the Hickinbotham Roseworthy Wine Science Laboratory on the day of picking for winemaking using the standard small-scale winemaking protocol below.

2.5.1. Chardonnay. The Chardonnay grapes were stored at 0°C for three days before being de-stemmed and crushed. At crush, 50 mg/L of SO_2 and 6.7 mL/hL of Rohavin® L enzyme was added. Grapes were then pressed off skin immediately after crush with an extraction rate of 560–600 L/T. The free run and pressing Chardonnay juice were combined in a 50 L stainless steel keg and cold settled for one week at 0°C. Juice from each vineyard was then racked off the gross lees into individual clean 50 L stainless steel kegs in preparation for ferment. Juices were warmed to 15°C then inoculated with 250 mg/L of AB Mauri PDM yeast and 250 mg/L Activator. DAP was added to fermented juice at 200 mg/L on day 7 after inoculation. The ferments were kept in a 15°C temperature-controlled room and alcoholic fermentation was complete within 30 days for six of the eight ferments. Once sugar-dry (residual sugar <2 g/L), SO_2 was added at 80 mg/L. The Chardonnay ferments from vineyards A and J did not complete alcoholic fermentation within 45 days of inoculation, so SO_2 was added at this point. The wines were cold settled at 0°C for 10 days, then racked off lees into 20 or 30 L stainless steel kegs with no ullage and stored at 0°C prior to bottling. No oak treatment was applied.

2.5.2. Pinot Noir and Shiraz. Grapes were stored at 0°C overnight prior to being de-stemmed and crushed into 100 L stainless steel open top fermenters. Similarly to Chardonnay, 50 mg/L of SO_2 and 6.7 mL/hL of Rohavin® L enzyme was added into each parcel of grapes at the crusher. The following day, 250 mg/L of AB Mauri PDM yeast and 250 mg/L activator were added to the musts. The ferments were inoculated with 10 mg/L of 5% MLF bacteria rehydrated in 20 times its weight of RO water for 15 minutes, 48 hours after yeast inoculation. The Shiraz grapes from vineyards I and K had high soluble solids levels (Table S1), so water was added after crushing at 10% of the must weight. DAP was added at 250 mg/L to the Pinot Noir from vineyard B must to assist the fermentation. The red musts were fermented on skins for seven days in a 20°C temperature-controlled room with twice daily hand plunging for cap management. They were then pressed off skins into individual pallet tanks to complete the primary and malolactic fermentation at the same temperature. Once the glucose and fructose concentrations were less than 2 g/L total and malic acid below 0.2 g/L, 80 mg/L of SO_2 was added to each wine. Tartaric acid (50% w/w in water) was added to the wines to reduce the pH of the Pinot Noir wines to below 3.5 and the Shiraz wines to below 3.6. No oak was added to the wines. The wines were racked off gross lees into 30 L stainless steel kegs with no ullage and stored at 0°C until bottling.

By the end of June 2020, all wines were subjected to crossflow filtration before being bottled into 375 mL OI 30157 AG Punted Claret BVS bottles with screwcap closures (Vinpac International, Angaston, SA, Australia). Wines were stored at 15°C for six weeks before conducting chemical and sensory analysis.

2.6. Volatile Phenols Analysis. The concentrations of guaiacol, 4-methylguaiacol, *m*-, *o*-, and *p*-cresols, syringol, and 4-methylsyringol in grape homogenates and wine samples were quantified by a stable isotope dilution GC-MS method. Samples were extracted by liquid-liquid extraction with 2 mL of pentane-ethyl acetate (1 : 1) and analysed using an Agilent 6890 gas chromatograph coupled to an Agilent 5973 mass selective detector as reported previously [25], noting that for grape analysis, deuterated standards were accurately added to a weighed subsample of homogenate to enable the concentration to take into account extraction and other matrix effects, and to allow the result to be expressed per kg of grapes. The analysis was performed by the Australian Wine Research Institute's (AWRI's) Commercial Services Laboratory (Urrbrae, SA, Australia). The limit of quantification for volatile phenols was 1-2 μg/kg depending on the analyte.

2.7. Grape and Wine Phenolic Glycosides Analysis. Grape homogenates were extracted and the grape extracts and wines were analysed as outlined in previous studies [7, 26]. For grape analysis, deuterated standards were accurately added to a weighed subsample (5 g) of each grape homogenate to take into account extraction and other matrix effects, and to allow the result to be expressed per kg of grapes. Briefly, the homogenates were thoroughly mixed by vortex and centrifuged at 2850 g for 5 min (Allegra® X-12R centrifuge, Beckman Coulter, California, USA) and the supernatant was purified and concentrated by solid phase extraction (Extract Clean C18–HF SPE 500 mg/4 mL cartridges (S * Pure, Singapore) as per Hayasaka et al. [7]. Deuterated standards were accurately added to the wine samples (1 mL) followed by vortex mixing and filtration 0.45 μm GHP (Pall, Melbourne, Victoria). The phenolic glycosides in grape extracts and wine samples were analysed using an Exion UHPLC coupled to a 6500 QTrap+ (Sciex, Mulgrave, VIC, Australia) under the conditions reported previously [7]. The limit of quantification for phenolic glycosides was 1 μg/kg.

To assess potential matrix effects during analysis of homogenates prepared from grapes at varying maturity, separate calibration curves with SyGG ranging from 0–200 μg/kg were established using non-smoke exposed pre-veraison and mature grape berries respectively. Linearity was excellent independent of grape maturity as demonstrated by R^2 better than 0.999 for each calibration curve and matrix. In addition, two concentrations (25 μg/kg and 50 μg/kg) of SyGG were spiked separately into pre-veraison grape homogenates from either control vineyards or vineyards with smoke exposure. The recovery was between 99% and 113%, confirming the accuracy of the LC-MS analysis independent of grape maturity. Together, the data demonstrate that the routine method for analysis of phenolic glycosides in mature grapes is also suitable for the analysis of grapes from pre-veraison samples.

2.8. Basic Chemical Composition. Standard chemical analyses including pH, volatile acidity (VA) as acetic acid, free and total SO_2, α-amino nitrogen, ammonia, yeast assimilable nitrogen, total soluble solids (TSS), malic acid, titratable acid (TA), alcohol, glucose + fructose, and specific gravity were performed by the AWRI's Commercial Services Laboratory.

2.9. Sensory Analysis. Smoke sensory rating of each wine cultivar was evaluated separately, with testing carried out on separate days. Panels of ten qualified and highly experienced AWRI judges were convened to assess the wines. Judges were selected for their ability to perceive smoke flavour from phenol glycosides, and on the basis of previous experience and performance in smoke sensory panels.

The judges rated 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 Band-Aid and barnyard) and smoke flavour (as also including bacon, smoked meat and ashy aftertaste), together with overall fruit aroma (defined as any type of fruit character, including citrus fruit, stone fruit, and tropical fruits including pineapple for the Chardonnay wines, and red fruit, dark fruit, red berry, strawberry, raspberry and cherry for the Pinot Noir and Shiraz wines) and overall fruit flavour. Judges also had the freedom to use an "other" term for both aroma and palate to capture any additional noteworthy characteristics in the wines.

Samples were presented in 30 mL aliquots in 3-digit-coded, covered, ISO XL5 standard wine glasses at 22–24°C, in isolated booths under colour-masking lighting, with randomised presentation order using a modified Williams

Latin Square design generated by Compusense20 sensory evaluation software (Compusense Inc., Guelph, Canada). All samples were assessed in duplicate in a complete block design. The judges were forced to have a 2-minute rest between each sample and a 10-minute rest between sets of two and three samples, to minimise carryover [27, 28]. Water was provided for palate cleansing. The intensity of each attribute was rated using an unstructured 15 cm line scale (scoring from 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.

2.10. Statistical Analysis. The berry weight, volatile phenols and their glycosides in grapes and wines were analysed by one-way analysis of variance (ANOVA) using XLSTAT (version 19.4.45342, Addinsoft, New York, NY, USA). For the sensory data ANOVA, fixed effects of wine, presentation replicate, the random effect of judge, and their two-way interactions were conducted, followed by a Dunnett's means comparison test to determine whether the wines were rated significantly higher than a control. It was decided that the control that received the higher smoke flavour score would be the wine used for all Dunnett's calculations for that cultivar to account for variation commonly observed in grapes without smoke exposure. This was followed for the comparisons of the chemical data also.

3. Results and Discussion

3.1. Composition of Pre-veraison Grapes. To determine the effect of smoke exposure on grape berries early in the growing season, three cultivars (Chardonnay, Pinot Noir and Shiraz) were investigated, with grape bunches sampled four weeks after the start of the 2019 Cudlee Creek wildfire event from eight vineyards per cultivar with varied exposure across vineyards (Table 1). At sampling in January 2020 grapes were at a development stage of approximately four weeks before 50% veraison and the grape berry weights ranged from 0.33 to 0.55 g depending on the cultivar and location (Table 2).

The concentrations in pre-veraison grape berries of volatile phenols from smoke and a range of their glycosidic metabolites which are typically used as biomarkers for smoke exposure are listed in Table 2 [7]. All six phenolic glycosides which are commonly used as markers for smoke exposure of ripe grapes were detected above the limit of quantification in all the "fire and smoke apparent" vineyards (G-L), providing compelling evidence that volatile phenols can be taken up and glycosylated in pre-veraison berries. Amongst the glycoside markers, SyGG was the most abundant glycoside in all 'fire and smoke apparent' samples, with the highest concentration of 160 μg/kg observed in vineyard J (Chardonnay, Table 2), far above the other glycosides which had maximum concentrations ranging from 22 μg/kg (GuRG, Pinot Noir G) and 21 μg/kg (MSyGG, Shiraz G) to 13 μg/kg (PhRG, Pinot Noir G). Despite its proximity to the fire zone, Chardonnay from vineyard K had low concentrations of phenolic glycosides (SyGG 21 μg/kg), with no significant difference compared to control Chardonnay grapes. This was somewhat different for Shiraz grapes from vineyard K (SyGG significantly elevated at 51 μg/kg; Table 2), though the two Shiraz and Chardonnay blocks in vineyard K were only 600 m apart. Of the vineyards that had no fire, but were suspected of smoke exposure, vineyard C had similar concentrations of SyGG compared to the vineyards where fire and smoke were apparent. While vineyard C itself was not burnt, it was located within the fire scar, and presumably was exposed to substantial quantities of fresh smoke.

The concentration of SyGG and other glycosides varied according to the vineyard location and could be linked to the extent of smoke exposure as reported by eye-witness observers. Unfortunately, no quantitative measurement of smoke intensity was available for the specific locations and times relevant to this study. While vineyard D was classified as a vineyard suspected of smoke exposure, the glycoside concentration of each of the three cultivars was close to the limit of quantification and similar to the concentrations observed in grapes from two control vineyards, indicating that the uptake of smoke at this site was negligible and probably reflected the wind direction: anecdotal observations regarding vineyard *D* indicated that despite the vineyard being located only approximately 600 m from the fire scar, the prevailing wind direction at the time blew the majority of the smoke away from the vineyard, resulting in little smoke exposure as evident from the low concentrations of glycosidic smoke exposure markers. On the other hand, two cultivars (Chardonnay and Pinot Noir) from vineyard *E* and Shiraz from vineyard F had SyGG concentration above 10 μg/kg, plus traces of other phenolic glycosides (elevated yet not significantly higher than the control grapes, Table 2), indicating some smoke exposure of these vineyards despite being further from the fire scar (i.e. vineyard E was approximately 4.5 km away from fire scar), consistent with observations that the smoke plume drifted to the north in the evening of the day of the wildfire.

In contrast to the glycosides, the volatile phenols were rarely detected in the grape samples, and only in relatively low concentrations (below 5 μg/kg). Guaiacol and *o*-cresol were generally elevated in grapes sampled from the "fire and smoke apparent" vineyards (G-L), and *m*-cresol was only detected in some "fire and smoke apparent" vineyards at low levels (below 2 μg/kg). These results are consistent with previous observations that xenobiotic volatile phenols are rapidly metabolised by grapes, and glycosides of these phenols can be detected within hours following exposure and remain present in grapes thereafter [18,19,29]. Similar results for unripe grapes had also been reported recently after repeated exposure to smoke for months during the ripening period [20]. Notably *p*-cresol, syringol, 4-methylguaiacol and 4-methylsyringol were below the limit of quantitation in all of the pre-veraison samples.

For each of the three cultivars, the volatile phenols and phenolic glycosides from vineyards A and B which had no noticeable exposure to smoke were all below or at the limit of quantification (1 μg/kg for syringol and or 2 μg/kg for 4-

Table 2: Phenolic analytes concentration (μg/kg) in grapes sampled at pre-veraison.

Cultivar	Vineyard†	Bwt (g)	SyGG	MSyGG	PhRG	GuRG	CrRG	MGuRG	Gu	*o*-cresol	*m*-cresol
Chardonnay	A	0.47 ± 0.03	1.9 ± 0.30	<LoQ	<LoQ	<LoQ	1.3 ± 0.20	<LoQ	<LoQ	<LoQ	<LoQ
	B‡	0.39 ± 0.01	2.3 ± 0.10	<LoQ	<LoQ	<LoQ	1.1 ± 0.10	<LoQ	<LoQ	1.0 ± 1.0	<LoQ
	C	0.43 ± 0.02	75 ± 2.7*	11 ± 1.5*	1.7 ± 0.7	2.9 ± 0.10	3.4 ± 0.20	5.2 ± 1.3*	<LoQ	2.0 ± 2.0	<LoQ
	D	0.45 ± 0.13	2.3 ± 0.30	<LoQ	<LoQ	<LoQ	1.6 ± 0.60	<LoQ	<LoQ	1.0 ± 1.0	<LoQ
	E	0.55 ± 0.02*	13 ± 2.5	1.2 ± 0.10	<LoQ	1.0 ± 0.20	1.5 ± 0.50	1.4 ± 0.6	<LoQ	1.0 ± 1.0	<LoQ
	G	0.40 ± 0.01	120 ± 43*	15 ± 3.7*	4.4 ± 3.6*	7.3 ± 2.8*	6.4 ± 3.3*	11 ± 3.8*	1.7 ± 1.5	3.0 ± 2.6	1.7 ± 1.5*
	J	0.41 ± 0.01	160 ± 43*	14 ± 4.2*	3.5 ± 1.4*	5.2 ± 1.4*	6.9 ± 0.80*	7.4 ± 1.6*	2.0 ± 1.0*	4.0 ± 2.0	2.0 ± 1.0*
	K	0.48 ± 0.01	21 ± 5.0	1.9 ± 0.20	1.3 ± 0.6	1.6 ± 0.20	2.1 ± 0.20	1.8 ± 0.2	<LoQ	1.3 ± 1.2	<LoQ
Pinot noir	A	0.41 ± 0.02	1.3 ± 0.20	<LoQ	<LoQ	1.2 ± 0.10	1.7 ± 0.20	<LoQ	<LoQ	<LoQ	<LoQ
	B‡	0.39 ± 0.02	1.0 ± 0.10	<LoQ	<LoQ	<LoQ	1.1 ± 0.20	<LoQ	<LoQ	<LoQ	<LoQ
	C	0.39 ± 0.05	29 ± 6.0*	2.4 ± 0.60*	3.0 ± 2.0	5.0 ± 1.4*	4.0 ± 1.0	3.4 ± 1.5	<LoQ	1.3 ± 1.2	<LoQ
	D	0.37 ± 0.02	1.9 ± 0.10	<LoQ	1.1 ± 0.6	<LoQ	1.6 ± 0.20	<LoQ	<LoQ	<LoQ	<LoQ
	E	0.55 ± 0.04*	12 ± 4.2	<LoQ	1.5 ± 0.9	1.8 ± 0.60	2.6 ± 0.70	1.3 ± 0.6	<LoQ	<LoQ	<LoQ
	G	0.38 ± 0.04	96 ± 22*	6.9 ± 1.3*	13 ± 5.2*	22 ± 3.0*	19 ± 2.1*	16 ± 1.8*	1.0 ± 1.0	2.3 ± 1.5	1.0 ± 1.0
	H	0.45 ± 0.04	81 ± 18*	4.8 ± 0.90*	14 ± 10*	14 ± 1.4*	19 ± 4.7*	11 ± 5.0*	1.3 ± 1.2	3.7 ± 2.3*	1.3 ± 1.2*
	L	0.43 ± 0.04	55 ± 4.7*	5.1 ± 1.4*	10 ± 6.6	13 ± 1.0*	13 ± 2.1*	8.0 ± 3.4*	1.0 ± 1.0	2.7 ± 1.5*	<LoQ
Shiraz	A‡	0.43 ± 0.04	1.8 ± 0.05	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ
	B	0.38 ± 0.02	1.8 ± 0.28	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ
	C	0.41 ± 0.00	51 ± 6.1*	8.7 ± 0.98*	1.0 ± 0.30	2.6 ± 0.10	2.4 ± 0.40	4.4 ± 0.90	2.0 ± 2.0	1.7 ± 1.5	<LoQ
	D	0.44 ± 0.05	2.9 ± 0.44	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ
	F	0.48 ± 0.04	37 ± 7.1	5.8 ± 1.9	<LoQ	2.1 ± 0.2	1.6 ± 0.50	3.0 ± 1.4	1.7 ± 1.5	1.0 ± 1.0	<LoQ
	G	0.36 ± 0.07	120 ± 53*	21 ± 8.6*	2.5 ± 1.6*	7.5 ± 5.1*	5.9 ± 3.7*	14 ± 8.1*	2.0 ± 1.0	1.7 ± 1.5	<LoQ
	I	0.33 ± 0.05	94 ± 11*	16 ± 2.2*	2.0 ± 0.70*	4.8 ± 0.30*	4.6 ± 0.4*	7.6 ± 2.0*	2.7 ± 0.58*	1.3 ± 1.2	<LoQ
	K	0.35 ± 0.04	51 ± 3.1*	9.6 ± 1.3*	1.2 ± 0.40	3.5 ± 0.40	3.0 ± 0.50	5.0 ± 1.6	2.3 ± 0.60	1.7 ± 1.2	<LoQ

Mean values ± standard deviation ($n = 3$). All phenolic glycoside values are expressed as syringol gentiobioside equivalents. Bwt, berry weight; Ph, phenol; Gu, guaiacol; Cr, cresol; Sy, syringol; MGu, 4-methylguaiacol; MSy, 4-methylsyringol; GG, gentiobiosides; RG, rutinoside; LoQ, limit of quantitation = 1 μg/kg. Values for the analytes, 4-methylguaiacol, p-cresol, syringol and 4-methylsyringol were below LoQ for all samples. †: for details of vineyard code, see Table 1. ‡: control block selected for comparison; *: significantly higher than selected control using Dunnett's means comparison test ($P = 0.05$).

methylsyringol). All the volatile phenols were also below or close to the limit of quantification (1 μg/kg) for all grapes sampled from "smoke suspected" vineyards (C–F).

The higher concentration of phenolic glycosides, in particular SyGG and MSyGG in grapes from "fire and smoke apparent" vineyards G to L compared to the volatile phenols clearly demonstrates that grape berries can transform volatile phenols almost completely into glycosides and potentially other yet to be identified metabolites at the early growing stage (pre-veraison) after smoke exposure. Overall, the concentrations of phenolic glycosides quantified in this study were comparable to the results found by Jiang et al. [20] for unripe grapes sampled at a similar E-L stage after continuous exposure to smoke.

3.2. Composition of Grapes at Harvest. Berry weight almost doubled in all the cultivars from pre-veraison to harvest, reaching approximately 1 g per berry (Table 3). Previous studies have found that smoke exposure has no effect on berry weight development, so the statistically significant differences in berry weight within cultivar in this paper are presumed to be independent of smoke exposure and reflect site location effects, grape ripeness and different vineyard management practices such as different irrigation regimes [16, 17]. The vineyards in this study continued to be managed as commercial vineyards during the trial, with varying management techniques. The basic composition of the grapes at harvest is provided in Table S1.

In mature grapes at harvest from the smoke- and fire-affected vineyards, volatile phenols were found despite their absence in the earlier pre-veraison samples (Table 3). Guaiacol was the most abundant volatile phenol, with a maximum concentration of 32 μg/kg detected in Pinot Noir vineyard H (Table 3). In contrast to pre-veraison results, *p*-cresol was found in Chardonnay and Pinot Noir grapes, at higher concentration in the "fire and smoke apparent" samples (maximum 8 μg/kg), but only detected in one of the Shiraz samples (vineyard I). Apart from *p*-cresol, all volatile phenols in the three cultivars from vineyards C and G-L were significantly higher than the control grapes. For grape samples from vineyards suspected of smoke exposure, all three cultivars from vineyard D also had low concentrations of volatile phenols consistent with non-smoke exposed samples. Although the cresol isomers in Chardonnay grapes from this vineyard were significantly elevated compared to the control, the concentration was similar to that observed typically in other non-smoke exposed grape berries. Similar to the results observed in pre-veraison grapes, syringol and 4-methylsyringol were below the limit of quantitation in all of the harvest samples.

The presence of elevated volatile phenols at harvest, more than ten weeks after the single smoke exposure, was an unexpected result, especially given only low concentrations of volatile phenols were observed in the pre-veraison samples. The conversion of volatile phenols to glycosides is quite rapid, with generally most volatile phenols glycosylated within the first week of smoke exposure [14, 18, 19, 29]. The result is also in contrast to the smoke exposed grape berries from the Hunter Valley in 2020, where no volatile phenols were detected above baseline levels [10, 20]. However, similar results were previously observed when guaiacol solutions or oak solutions were applied to vines one week after veraison (Monastrell cultivar) [30], where free guaiacol was not detected 10 days after the guaiacol application, but was detected 36 days after the application, at harvest. The presence of volatile phenols could be due to glycosidase activity late in the ripening, a common fruit ripening mechanism whereby volatiles are released from glycosides [31, 32]. Alternatively, it could potentially be attributed to release of volatile phenols from other unknown storage forms in grapes and/or potentially other plant tissues.

"Fire and smoke apparent" vineyards (G-L) samples that had high SyGG in January also had high SyGG at harvest in March, with concentrations up to 140 μg/kg (Chardonnay, vineyard G), well above those seen in non-smoke exposed survey samples which rarely exceed 10 μg/kg [10]. SyGG was the most abundant glycoside, and other glycosides had maximum concentrations ranging from 22 μg/kg (GuRG in Shiraz vineyard I) to 54 μg/kg (CrRG in Pinot Noir vineyard H). The "fire and smoke apparent" grapes were also found to be significantly higher than control grapes in most phenolic glycosides, apart from PhRG and CrRG in Shiraz from vineyard G and SyGG and MSyGG in Chardonnay sampled from vineyard K, due to variation between grape sample replicates. For the "smoke suspected" vineyards (C–F), there was a range in concentration in phenolic glycosides at harvest, similar to the pre-veraison results. All three cultivars from vineyard D were low in all volatile phenols and glycosides in grapes sampled pre-veraison and at harvest, whereas Chardonnay samples from vineyard C were higher in glycosides (for example, 71 μg/kg SyGG), and comparable to the lower concentrations seen in the fire impacted vineyards. The Pinot Noir grapes from vineyards C and E had elevated phenolic glycoside concentration (32 μg/kg and 22 μg/kg SyGG respectively) but were not significantly different to control grapes (Table 3).

Contrary to expectations, the phenolic glycoside concentration on a per kg basis did not decrease from January to harvest, despite the large increase in berry size. In fact, all the phenolic rutinosides in Chardonnay and Shiraz berries sampled from all blocks increased during the growing season, in line with recent observations on grapes continuously exposed to smoke during the ripening period [20]. While most rutinosides increased in the fire-affected Pinot Noir grapes, GuRG decreased during the season. The change in SyGG and MSyGG concentrations varied between cultivars. Grapes from those lightly smoke-affected vineyards, in particular *C* and E, had only a marginal increase of the SyGG concentrations at harvest, whereas SyGG increased by over 40% in Pinot Noir grapes between pre-veraison and harvest, for the "fire and smoke apparent" vineyards G, H and L. Both increases and decreases in SyGG concentration were observed in Chardonnay grapes (increases in grapes from vineyard G at harvest, but reductions in the grapes from vineyard J). SyGG decreased by 40 to 60% between January and harvest in Shiraz grapes sampled from most

Table 3: Phenolic analytes (μg/kg) in grapes sampled at harvest compared to published data from non-smoke exposed samples.

Cultivar	Vineyard†	Bwt (g)	SyGG	MSyGG	PhRG	GuRG	CrRG	MGuRG	MGu	Gu	*o*-cresol	*p*-cresol	*m*-cresol
	Non-smoke	—	1.3	1.7	1	1	2.4	1.5	1	1	3.3	1	1
	A	0.75 ± 0.06	2.2 ± 0.15	<LoQ	1.0 ± 0.07	1.6 ± 0.17	2.7 ± 0.35	2.3 ± 0.21	<LoQ	<LoQ	1 ± 0	<LoQ	<LoQ
	B‡	0.78 ± 0.01	2.5 ± 0.24	<LoQ	1.1 ± 0.10	1.7 ± 0.42	2.4 ± 0.16	3.0 ± 1.1	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ
	C	0.88 ± 0.11	77 ± 14*	14 ± 2.1*	4.9 ± 0.14*	7.5 ± 0.99*	8.9 ± 1.7*	18 ± 2.9*	3.3 ± 0.58*	8.3 ± 1.5*	6.3 ± 1.2*	4.3 ± 0.58*	6.7 ± 0.58*
Chardonnay	D	0.84 ± 0.06	2.3 ± 0.13	<LoQ	1.5 ± 0.07	1.0 ± 0.07	2.8 ± 0.24	3.0 ± 0.49	<LoQ	1.0 ± 0	2.3 ± 0.58*	1.3 ± 0.58*	1.7 ± 0.58*
	E	1.1 ± 0.06*	20 ± 0.98	3.1 ± 0.44	2.4 ± 0.24*	1.0 ± 0.07	4.3 ± 0.56	7.0 ± 0.41	<LoQ	2.7 ± 0.58*	3.3 ± 0.58*	1.3 ± 0.58*	3.0 ± 0*
	G	0.97 ± 0.07*	140 ± 44*	25 ± 11*	7.0 ± 1.4*	14 ± 3.0*	11 ± 1.8*	31 ± 5.1*	5.7 ± 1.2*	16 ± 1.0*	10 ± 1.5*	7.3 ± 0.58*	10 ± 1.0*
	J	0.97 ± 0.07*	100 ± 16*	16 ± 3.5*	5.0 ± 0.78*	9.2 ± 1.3*	11 ± 1.8*	25 ± 1.6*	5.3 ± 0.58*	14 ± 0.58*	11 ± 0.58*	8.0 ± 1.0*	11 ± 1.2*
	K	0.92 ± 0.11	34 ± 6.4	4.8 ± 1.1	5.0 ± 0.44*	5.6 ± 1.1*	6.4 ± 0.92*	11 ± 2.7*	2.7 ± 0.58*	9.0 ± 1.7*	7.0 ± 1.0*	4.7 ± 0.58*	7.0 ± 1.0*
	Non-smoke	—	5	1	1.1	1.2	4.1	2.6	1	3	3.4	1	1
	A	0.99 ± 0.06	2.8 ± 0.55	1.6 ± 0.78	2.1 ± 1.3	3.8 ± 1.3	3.5 ± 0.66	3.1 ± 1.1	<LoQ	<LoQ	1.7 ± 0.58	<LoQ	<LoQ
	B‡	0.96 ± 0.09	2.2 ± 0.14	<LoQ	3.1 ± 0.17	3.0 ± 0.52	2.3 ± 0.16	2.6 ± 0.83	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ
	C	1.0 ± 0.07	32 ± 12	4.1 ± 0.86	5.9 ± 1.6	4.3 ± 0.44	5.9 ± 2.2	6.7 ± 2.2	2.0 ± 0*	12 ± 0.58*	4.7 ± 0.58*	1.3 ± 0*	3.7 ± 0*
Pinot noir	D	1.1 ± 0.03	2.8 ± 0.15	1.0 ± 0.10	2.2 ± 0.14	2.9 ± 0.12	2.9 ± 0.46	3.0 ± 0.12	<LoQ	1.7 ± 0	1.7 ± 0	<LoQ	1.0 ± 0
	E	1.1 ± 0.05	22 ± 2.3	2.7 ± 0.29	4.4 ± 0.27	3.5 ± 0.51	6.9 ± 1.1	6.0 ± 0.79	<LoQ	4.0 ± 0.58	3.0 ± 0.58	1.0 ± 0.58*	2.0 ± 1.0*
	G	0.91 ± 0.06	140 ± 17*	12 ± 2.1*	20 ± 2.5*	8.5 ± 1.5*	23 ± 3.3*	30 ± 4.3*	4.3 ± 1.0*	26 ± 7.0*	12 ± 3.5*	3.3 ± 0.58*	8.0 ± 1.2*
	H	0.80 ± 0.21	150 ± 43*	16 ± 5.1*	27 ± 8.4*	11 ± 3.6*	54 ± 17*	49 ± 16*	5.0 ± 0.58*	32 ± 2.1*	16 ± 1.2*	4.7 ± 0*	12 ± 0.58*
	L	0.93 ± 0.06	86 ± 8.3*	12 ± 1.2*	13 ± 1.8*	6.4 ± 0.63*	24 ± 3.9*	23 ± 3.4*	2.7 ± 0*	19 ± 0.58*	9.7 ± 0*	3.0 ± 0*	6.3 ± 0*
	Non-smoke	—	12.3	4.5	2	8.8	5.2	4.7	1	8	4.3	1	1
	A‡	1.1 ± 0.04	3.4 ± 0.46	<LoQ	<LoQ	3.1 ± 0.21	2.7 ± 0.67	4.5 ± 1.1	<LoQ	1.7 ± 0.58	<LoQ	<LoQ	<LoQ
	B	0.90 ± 0.05	3.3 ± 0.67	<LoQ	<LoQ	3.6 ± 0.72	3.4 ± 0.35	4.8 ± 1.2	<LoQ	2.0 ± 0	<LoQ	<LoQ	<LoQ
	C	1.2 ± 0.08	32 ± 5.6*	5.4 ± 1.4*	2.2 ± 0.63*	12 ± 4.4*	6.2 ± 1.7	27 ± 7.3*	2.7 ± 0.58*	16 ± 2.5*	4.0 ± 0*	<LoQ	2.3 ± 0.58*
Shiraz	D	1.2 ± 0.06	4.4 ± 1.7	<LoQ	1.3 ± 0.4	4.3 ± 0.8	4.0 ± 1.4	6.0 ± 1.7	<LoQ	5.0 ± 1.0*	1.0 ± 0	<LoQ	<LoQ
	F	1.2 ± 0.07	32 ± 5.9*	5.7 ± 1.1*	2.1 ± 0.29*	11 ± 1.5*	7.6 ± 1.1*	25 ± 5.1*	1.0 ± 1.0	7.3 ± 2.1*	1.7 ± 0.58*	<LoQ	<LoQ
	G	1.1 ± 0.06	71 ± 11*	13 ± 2.4*	1.9 ± 0.24	12 ± 2.4*	5.4 ± 1.3	33 ± 5.3*	2.3 ± 0.58*	12 ± 2.0*	2.7 ± 0.58*	<LoQ	1.7 ± 0.58*
	I	0.89 ± 0.04	46 ± 8.8*	8.2 ± 2.0*	4.9 ± 1.22*	22 ± 5.3*	15 ± 3.8*	44 ± 14*	2.3 ± 0.58*	16 ± 1.2*	4.0 ± 1.0*	1.7 ± 0.58*	2.7 ± 0.58*
	K	0.96 ± 0.08	40 ± 5.5*	6.7 ± 1.4*	5.0 ± 0.64*	20 ± 0.96*	13 ± 0.16*	44 ± 2.8*	1.7 ± 0.58*	10 ± 0.58*	2.3 ± 0.58*	<LoQ	1.0 ± 0*

Apart from "non-smoke" values, mean values ± standard deviation ($n = 3$). "Non-smoke" exposed concentrations are the 99th percentile values of at least 50 samples per cultivar from multiple regions and vintages [10]. All phenolic glycosides values are expressed as syringol gentiobioside equivalents. Bwt, berry weight; Ph, phenol; Gu, guaiacol; Cr, cresol; Sy, syringol; MGu, 4-methylguaiacol; MSy, 4-methylsyringol; GG, gentiobiosides; RG, rutinoside; LoQ, limit of quantitation = (1 μg/kg). †: for details of vineyard code, see Table 1. ‡: control block selected for comparison; *: significantly higher than selected control using Dunnett's means comparison test ($P = 0.05$).

vineyards. For vineyard D, with minimal effect of smoke, all compounds remained low at harvest. While the halved concentration of SyGG in Shiraz berries on a per kg basis appears to reflect the doubled berry weight, calculating the content of SyGG on a per berry basis showed increases over time in all smoke-affected Shiraz berries instead of remaining constant as expected (Table S2).

The increase in grape glycosides measured from pre-veraison to harvest occurred despite there being no further noticeable smoke exposure. Increases in some phenolic glycosides in the weeks following smoke events have been previously observed in model smoke experiments when smoke was applied after veraison [18]. However, given that the smoke exposure occurred weeks before veraison in this study, and almost three months before harvest, the increase in berry size was significant, and as a result, the concentrations were expected to decrease from January to harvest in March. The presence of intermediates, or other volatile phenol metabolites that have not been identified as yet has been suggested as a possible explanation for such increases and potentially could explain the observations in our field experiments [18]. Another plausible explanation is that additional sources of volatile phenols exist that persist after the smoke has disappeared, such as volatile phenols bound on ash particles, perhaps also on other parts of the vine, or volatile phenols might be emitted by burnt material within or near to vineyards, e.g., from burnt grasses in swathe. Conceptually, these volatile phenols from other reservoirs and storage forms could continue to be taken up by berries after smoke has disappeared, forming phenolic glycosides and resulting in an ongoing increase, particularly for vineyards with fire activity inside or nearby. Overall, the changes in phenolic glycosides during the growing season, from pre-veraison to harvest, are not well understood at this time as this is the first field study of the effect of pre-veraison smoke exposure. Most importantly, the data obtained here demonstrate that a simple correction for berry weight changes cannot be applied to predict harvest concentrations of commonly used smoke exposure markers from that in pre-veraison berries, until the kinetics of formation and degradation of these glycosides in grapes are better understood.

Again, volatile phenols were largely not detected in the control samples at harvest, and in no instances did the volatile phenol concentration in grapes from control vineyards A and B exceed those found in a comprehensive survey of non-smoke exposed grape berries [10]. Glycosides in control samples at harvest were below or only slightly above concentrations reported in a comprehensive survey of non-smoke exposed grapes (Table 3) [10]. The low volatile phenol and glycoside concentrations in grapes from the control vineyards provide further compositional evidence that these sites indeed had negligible smoke exposure.

3.3. Basic Wine Composition. All the grapes of the same cultivar were hand harvested on the same day at all vineyard sites despite maturity difference between sites. This meant that time between exposure and sampling was a constant, yet the total soluble solids levels of the grapes varied from site to site (Table S1). After harvest, a single winemaking replicate for each vineyard was produced using a standardized protocol. Note that some water was added to Shiraz must from vineyard I and K to mitigate potential fermentation difficulties from high soluble solids. Because the cultivars were all harvested on the same day across all sites, which at that point were at slightly different stages of ripeness, some variation is evident in basic parameters such as alcohol, residual sugar and malic acid. These differences cannot be attributed to the smoke exposure, but are likely the result of variation in winemaking practices and the performance of micro-organisms such as yeast and malolactic bacteria during fermentation (Table S3). While smoke exposure cannot be completely ruled out as a factor in contributing to differences in basic wine composition, (particularly for the fire-affected vineyards where irrigation infrastructure may have been damaged by the fire and irrigation may have been disrupted for a short period until repairs were carried out) the results are consistent with previous studies which have shown that smoke exposure has no material effect on basic wine composition [16, 18, 26].

3.4. Wine Volatile Phenol and Phenolic Glycoside Concentrations. An elevated concentration of volatile phenols, particularly guaiacol, was found in the Shiraz and Pinot Noir wines made from grapes harvested from "fire and smoke apparent" vineyards (G, I, K and L, Table 4), with concentrations generally exceeding those found in non-smoke exposed wines [10]. Guaiacol was the most abundant volatile phenol measured, and the maximum concentration observed in the wines was 78 μg/L in Shiraz from vineyard I, well above the sensory best-estimate threshold in red wine of 23 μg/L [9]. Syringol concentrations were mainly elevated in the Pinot Noir wines (maximum concentration 65 μg/L in Pinot Noir vineyard L), despite not being detected in the grape samples. Overall, the syringol concentrations in Shiraz wines across all vineyards were at or close to baseline level. Cresol isomers were also detected in the red wines but at low concentration. The maximum concentration found in wine from Pinot Noir vineyard G was 17 μg/L *m*-cresol although individual concentrations did not exceed sensory thresholds (the sensory threshold for *m*-cresol, *o*-cresol and *p*-cresol, respectively are reported as 20 μg/L, 62 μg/L, and 64 μg/L in red wine) [9]. Generally, the concentrations of volatile phenols were several-fold higher in the wines compared to the grapes at harvest for both Shiraz and Pinot Noir, likely due to release of volatile phenols from glycosides during winemaking and storage [33]. When comparing between cultivars, the results are also consistent with previous studies which demonstrated that Shiraz wine made from smoke exposed grapes can be high in guaiacol [17].

As expected, relatively low volatile phenol concentrations were found in Pinot Noir and Shiraz wines from vineyards A, B and D which had no or low smoke exposure. The concentrations of most volatile phenols were close to or only slightly above those reported in a comprehensive survey of wines made from non-smoke exposed grapes [10].

Table 4: Phenolic analytes (μg/L) and smoke flavour ratings in wines made from Adelaide Hills vineyards after the 2019 Cudlee creek fire compared to published data from non-smoke exposed samples.

Cultivar	Vineyard†	SyGG	MSyGG	PhRG	GuRG	CrRG	MGuRG	MGu	Gu	*o*-cresol	*p*-cresol	*m*-cresol	Sy	MSy	Smoke flavour
Chardonnay	*Non-smoke*	3.0	1.0	1.0	1.0	1.9	1.5	1.0	1.0	1.4	1.0	1.0	3.0	1.0	—
	A	1.2	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	0.9
	B‡	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	1.7
	C	19.5	1.9	2.6	7.1	2.6	4.8	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	1.5
	D	2.8	<LoQ	1.1	1.6	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	2.2
	E	6.0	<LoQ	1.1	1.5	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	2.2
	G	40.7	<LoQ	1.9	3.0	1.4	4.2	<LoQ	1.0	<LoQ	1.0	<LoQ	1.0	<LoQ	2.7
	J	33.5	<LoQ	3.7	6.8	2.6	7.8	<LoQ	2.0	<LoQ	2.0	1.0	1.0	<LoQ	3.3*
	K	16.1	1.4	3.9	7.8	5.4	11.9	<LoQ	1.0	<LoQ	1.0	<LoQ	<LoQ	<LoQ	2.5
Pinot noir	*Non-smoke*	5.3	1.0	1.8	1.8	5.4	2.8	1.0	2.4	3.4	1.0	1.1	2.3	1.0	—
	A	1.0	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	<LoQ	2.0	2.0	2.0	<LoQ	3.0	<LoQ	1.5
	B‡	1.1	<LoQ	<LoQ	<LoQ	1.0	<LoQ	<LoQ	2.0	<LoQ	2.0	<LoQ	3.0	<LoQ	1.8
	C	12.8	<LoQ	1.6	4.6	3.3	5.5	7.0	26	4.0	6.0	8.0	12	3.0	6.0*
	D	2.1	<LoQ	<LoQ	1.7	3.3	2.4	2.0	5.0	2.0	3.0	3.0	5.0	<LoQ	2.7
	E	8.1	<LoQ	<LoQ	2.8	4.4	3.8	2.0	8.0	3.0	4.0	4.0	4.0	<LoQ	4.3*
	G	63	3.1	6.8	30	19	29	15	52	11	15	17	23	7.0	8.2*
	L	17	<LoQ	5.1	14	13	15	14	47	9.0	12	15	65	19	7.5*
Shiraz	*Non-smoke*	8.8	1.0	2.7	12.6	7.2	6.4	1.0	13.1	1.3	1.0	1.0	6.9	1.0	
	A‡	2.4	<LoQ	<LoQ	5.0	2.3	4.0	<LoQ	11	<LoQ	1.0	<LoQ	4.0	<LoQ	1.7
	B	2.9	<LoQ	<LoQ	11	4.5	7.1	<LoQ	16	1.0	1.0	<LoQ	8.0	<LoQ	0.5
	C	56	5.3	2.3	33	10	44	10	68	8.0	6.0	7.0	7.0	2.0	6.2*
	D	9.6	<LoQ	2.7	11	4.2	8.4	2.0	21	2.0	2.0	2.0	5.0	<LoQ	1.2
	F	30	2.4	2.8	18	5.4	23	5.0	37	4.0	3.0	3.0	6.0	<LoQ	4.5*
	G	71	3.9	1.1	24	5.9	41	13	62	6.0	4.0	5.0	7.0	2.0	6.2*
	I	46	4.0	3.1	53	15	55	7.0	78	7.0	5.0	6.0	9.0	2.0	2.5
	K	38	2.2	6.6	36	9.1	40	6.0	47	5.0	4.0	4.0	6.0	<LoQ	4.0*

Volatile phenols and phenolic in wine results are represented as one replicate ($n = 1$). Non-smoke exposed concentrations are 99th percentile values of at least 40 samples per cultivar from multiple regions and vintages [10]. The sensory smoke ratings of each wine are the mean of 8 assessors x 2 presentation replicates. All phenolic glycosides values are expressed as syringol gentiobioside equivalents. Ph, phenol; Gu, guaiacol; Cr, cresol; Sy, syringol; MGu, 4-methylguaiacol; MSy, 4-methylsyringol; GG, gentiobiosides; RG, rutinoside; LoQ, limit of quantitation = (1 μg/L apart from syringol and 4-methylsyringol which are 2 μg/L). †: for details of vineyardcode, see Table 1. ‡: control block selected for comparison; *: significantly higher than selected control using Dunnett's means comparison test (P = 0.05).

However, the guaiacol concentration of Shiraz wine D was 21 μg/L, which is above the 99th percentile concentration of 13 μg/L that is typically observed in non-smoke exposed Shiraz wines [10].

In contrast to the red cultivars, volatile phenols in Chardonnay wines were all at trace concentrations regardless of whether the vineyard was smoke-affected or not (Table 4), almost certainly related to minimal skin contact during typical white winemaking [1]. Such low concentrations of volatile phenols in Chardonnay wine from smoke exposed grapes were also seen before by Culbert et al. [26].

In all wines from 'fire and smoke-affected' vineyards the phenolic glycosides were present at high concentration, with a maximum of 71 μg/L SyGG in Shiraz from vineyard G (Table 4). Rutinosides GuRG and MGuRG were also found at elevated concentration in red wines in agreeance with observations from model smoke exposure trials [17, 18, 29]. Shiraz wine from vineyard I had a higher concentration of GuRG (53 μg/L) and MGuRG (55 μg/L) than SyGG (46 μg/L) and in wines of Pinot Noir vineyard G and Shiraz vineyard K, GuRG and MGuRG were at similar concentrations compared to its SyGG. The pattern of abundance in wine closely reflected that seen in the pre-veraison and harvest grape samples. The concentration of the glycosides in wine from the control vineyards (A and B) and vineyard D was below (or within method uncertainty of) the concentrations typically found in wine made from non-smoke exposed grapes [10]. In contrast to the volatile phenols, some phenolic glycosides were also seen in the Chardonnay wines (Table 4), likely because the glycosides are typically found located in both grape skin and pulp [24] and/or some skin contact and extraction occurred before and during pressing. The phenolic glycosides were found at moderate concentration in wine from all three cultivars sampled in "smoke suspected" vineyards and vineyards K (for Chardonnay only) and L. Wine made from the grapes from those "fire and smoke apparent" vineyards (G-J) were several-fold higher in glycosides compared to the concentrations found in wine made from non-smoke exposed grapes [10]. In summary, the phenolic glycosides in the smoke-affected grapes were present in samples of pre-veraison and mature grapes, were extracted during winemaking and persisted in the wine.

Comparing the concentration in wine to the grapes at harvest, previous studies have estimated that approximately three quarters of the glycosides in grapes or juice can be found in wine [26, 34]. Hayasaka et al. [5] noted that the sum of glycosides was approximately 78% in the wine compared to grapes for Chardonnay made with skin contact, or 67% for Cabernet Sauvignon. There are also examples of a smoke-affected Grenache and Shiraz, where individual guaiacol glycosides did not decrease during winemaking, and in some cases (GuRG) increased by 55% [35]. In the present study, SyGG in Chardonnay and Pinot Noir was 30–80% lower in wine compared to the grapes, but in the Shiraz corresponding wine and grape samples the SyGG concentrations were similar. Overall, no obvious relationship between the concentration of glycosides in grape and glycosides in the wine could be observed in this study.

3.5. Wine Sensory Smoke Flavour Ratings. Wine from each of the cultivars was assessed by a screened and trained smoke assessment panel [36]. Ratings of smoke aroma and smoke flavour for each cultivar (Table S4) were highly correlated (data not shown), so only the quantitative smoke flavour data is presented in Table 4.

Wines made from control vineyards (A and B) grapes were all rated below 2.0 for *smoke aroma* and *smoke flavour* across all the cultivars (Tables 4 and S4). It should be noted that panel mean values for controls that are non-zero are expected, due to expectation bias, and even for well-trained and screened panels assessing smoky flavour, it has been found that unaffected wines can receive mean scores between 0.5 and 2.5 [8]. Overall, most wines made from grapes harvested from "fire and smoke apparent" vineyards were rated significantly higher than the control wines in smoke aroma and flavour. Many of the wines from "smoke suspected" vineyards were also rated higher than the control wines, specifically those that had significantly elevated smoke marker compounds at pre-veraison and harvest. Conversely, several of the Chardonnay wines (made from grapes from vineyard C, D, E, G and K) had many of the smoke glycosides and volatile compounds significantly elevated from as early as pre-veraison through to harvest, thus were recognised as made from grapes with a degree of smoke exposure yet were not rated significantly higher in smoke sensory attributes. This observation provides further support that different grape cultivars are not necessarily uniform in their response to smoke exposure. It also reflects the differences in winemaking approaches, especially differences between white wines which are typically produced with no or limited skin contact, and red wines made from whole grapes which are crushed and where skin extraction is common.

For the Chardonnay wines, the smoke flavour was rated significantly higher than the control only for vineyard J (mean 3.3), with wines from vineyards D, E, G and K slightly elevated. For the Pinot Noir wines, those from vineyards C, E, G and L were rated significantly higher than the control (4.3–8.2). Vineyard D also had a relatively high smoke flavour score. The Shiraz wines from vineyards C, F, G and K were significantly higher than the control, with smoke ratings above 4.0. The wine from vineyard I had a slightly elevated rating.

However, it should be noted that due to the variation in ripeness between sites at the time of harvest, some wines in each set of cultivars were lower in alcohol and from initial sensory assessment were found to be also low in body, low in flavour and higher in acidity ratings, with others much higher in alcohol, with concomitant astringency, heat, acidity and fruit flavour differences. Some wines had "green" flavour characters, others eucalypt or overripe flavours which might contribute to suppressive or other masking effects. This means that establishing a direct relationship between smoke ratings and volatile phenol concentrations in wine is problematic, especially for a relatively small sample set with a number of potentially confounding factors. Further study of a wider range of wines, preferably made from grapes harvested at consistent ripeness, is required to assess associations of individual phenolic compounds with smoke aroma and flavour attributes. Still, it is noteworthy that even with these unavoidable differences, those red wines made from fruit from “fire and smoke apparent” vineyards generally were clearly and often strongly smoky flavoured.

4. Conclusion

Early season smoke exposure of grapes led to distinct increases in phenolic glycosides, but not volatile phenols, in pre-veraison grape berries. This means that uptake of volatile phenols by the unripe grapes would have occurred, together with complete metabolic transformation to their glycosides, and possibly other metabolites and bound storage forms. The concentration of phenolic glycosides in grapes was not diluted by increases in berry size during ripening, despite berries approximately doubling in size between the sampling time points in January and harvest in March. At harvest, the concentrations in grapes of phenolic glycosides and some volatile phenols were well above those found in non-smoke exposed samples, especially from sites that were considered to have been exposed to intense smoke pre-veraison. Smoky flavour in wines was linked to grape and wine phenol composition, with Chardonnay wines rated much lower in smoke flavour than the red wines, likely reflecting the differences in skin contact during the winemaking process. Work is underway to understand the impact of smoke exposure on consumer liking of the resulting wines.

As noted above, despite no smoke being recorded from January to harvest, both the concentrations of smoke exposure markers and the phenolic glycoside content of berries increased unexpectedly, and volatile phenols were present in the berries at harvest. These observations could not be adequately explained and may be due to potential presence of a significant pool of unknown smoke metabolites which would warrant further investigation.

In summary, this is the first study to show that exposure of commercial vineyards to wildfire smoke at the pre-veraison stage of berry development can lead to elevated concentration of multiple volatile phenols and glycosides in grapes and wine and may result in strong smoky flavours in the wine. This work has also indicated that wind direction as well as proximity to a fire are factors to take into account in assessing the likely effect of a fire on grapes and wine. For wine producers the study has shown that a significant risk exists for the development of undesirable smoky flavour in wine, even if fire events occur only close to a vineyard and pre-veraison. To avoid potential quality defects, the concentrations of volatile phenols and phenolic glycosides in grapes from such vineyards close to harvest should be assessed.

Data Availability

The data support the findings are available upon the author reasonable request.

Disclosure

The authors declare that they have no conflict of interest in performing and publishing this work.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

The authors acknowledge that all authors have contributed significantly and agree with the manuscript.

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Supplementary Materials

Table S1: Basic chemical composition for grapes sampled at harvest. Table S2: Phenolic glycosides (ng/berry) in grapes sampled at pre-veraison and harvest. Table S3: Basic composition of single-site, varietal wines. Table S4: Mean sensory ratings for single-site wines made from three cultivars. (*Supplementary Materials*)

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