Impact of Smoke Exposure on Grape and Wine Chemistry of *Vitis vinifera cv.* Cabernet Sauvignon Under Mechanical Leaf removal and Deficit Irrigation in the San Joaquin Valley of California

By

SONG YAN

THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Viticulture & Enology

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

Approved:

Anita Oberholster, Chair

Andrew Waterhouse

David Block

Committee in Charge

2022

Impact of Smoke Exposure on Grape and Wine Chemistry of *Vitis vinifera cv.* Cabernet Sauvignon Under Mechanical Leaf removal and Deficit Irrigation in the San Joaquin Valley of California

Song Yan[1], George Zhuang[2], Qun Sun[3], Anita Oberholster*[1]

[1]Department of Viticulture and Enology, University of California at Davis, 595 Hilgard Lane, Davis, CA, 95616

[2]University of California Cooperative Extension at Fresno County, 550 E. Shaw Ave, Suite 210-B, Fresno, CA 93710

[3]Department of Viticulture and Enology, California State University at Fresno, CA 93711

Abstract

Smoke-derived volatile phenols can absorb into grapes, resulting in smoke-impacted grapes, decreasing the quality of the resulting wine by developing 'smoke taint' flavor. This study explored the interactive impact of deficit irrigation and mechanical fruit zone leaf removal on grape smoke exposure and the resulting wine composition. Our results indicate limited smoke impact only on the grapes, which did not result in smoke-tainted wines. This is mainly because the smoke that the vineyard was exposed to originated from 150 km away, resulting in lower density and aged (from 5 to 10 days after the fires started) smoke exposure

only. The different irrigation treatments showed some effects on grapes' volatile phenols but had little to no impact on wine composition. On the contrary, leaf removal showed larger effects on wines' volatile phenols than the grapes' volatile phenols, indicating that larger leaf area could provide some protections to grapes during smoke exposure.

1. Introduction

1.1 Origin of volatile phenols in grapes and wines

In the United States, wildfires have impacted about 7.5 million acres (~3 million hectares) of land annually since 2011, with 10.3 million acres (~4 million hectares) burned in 2020. Forty percent of burns was in California (Mirabelli-Montan et al. 2021).

In California, the annual wildfire burned area increased fivefold during 1972–2018, and the wildfires are becoming more frequent and severe (Williams et al. 2019). The 2019/2020 bushfires in eastern Australia have been estimated to have cost the wine industry AUD 40 million due to grape smoke impact, burnt vineyards, and lost sales (Summerson et al. 2021).

Volatile phenols are released into the air when wood burns due to the thermal degradation of lignin. The released volatile phenol compounds can absorb into grapes (Krstic et al. 2015), which can result in smoke-impacted grapes, resulting in wines where the quality is adversely impacted by developing 'smoke taint' flavor (Krstic et al. 2015; Mirabelli-Montan et al. 2021). Smoke taint flavors are described as undesirable smoky, dirty, ashy, medicinal and more (Krstic et al. 2015; Pardo-Garcia et al. 2017; Ristic et al. 2017).

Different fuels have different volatile compound compositions (Kelly et al. 2012) and can lead to different physiological responses in grapevines (Bell et al. 2013). However, the lignin component of vegetation fuels' pyrolysis is thought to be the main origin of the compounds that are considered responsible for grapes or wines' smoke impact or taint (Kelly et al. 2012). Smoke caused by wildfires contains large amounts of gaseous pollutants (e.g., CO_2, NO_2), polycyclic aromatic hydrocarbon, and volatile organic compounds, including volatile phenols

(Oberholster et al. 2022; Ward et al. 2005; Wentworth et al. 2018), which are believed to be the major origin of the smoke taint flavor in wines (Krstic et al. 2015; Liu et al. 2020), which include guaiacol, 4-methylguaiacol (creosol), phenol, syringol, *o*-, *m*-, and *p*-cresol. (Kelly et al. 2012, Caffrey et al. 2019). Among these compounds, guaiacol and 4-methylguaiacol were used as the main smoke taint markers between 2003 and 2009 (Krstic et al. 2015), but they have been found to represent only about 20% of lignin derived smoke taint compounds in wines (Kelly et al. 2012). So, other than guaiacol and 4-methylguaiacol, the volatile phenols above should also be considered while exploring grape smoke exposure impact. The combination of free volatile phenols also needs to be noticed, because De Vries et al. (2016) found that although the measured levels of free volatile phenols were below or close to odor threshold levels for individual phenols, the combination of these phenols led to a perception of a 'burnt rubber' taint in some South African red wines.

Except for smoke taint, there are other sources of volatile phenols. *Brettanomyces* yeast can also synthesize volatile compounds, but the management of *Brettanomyces* is relatively easy and well researched (Agnolucci et al. 2017; Wedral et al. 2010). Oak can also introduce a variable amount of volatile phenols to wines (Garde-Cerdán and Ancín-Azpilicueta 2006). Oak contact such as barrel aging is a wine maturation technique used by winemakers to increase the complexity of wine by adding spicy, oaky aromas (Maga 1989).

Volatile phenols are also naturally present in grapes in low amounts. Thus, the mere presence of guaiacol (or other volatile phenols) in grapes does not necessarily indicate the occurrence of smoke taint, as guaiacol has been identified as a natural component of several cultivars of *V. vinifera,* including Merlot, Shiraz, Tempranillo, and Grenache (Krstic et al. 2015).

These low amounts of volatile phenols naturally present in grapes will not develop 'smoke taint' during the lifetime of the wine. That problem occurs when additional volatile phenols are absorbed from the environment.

The absorbed volatile phenols are glycosylated within hours in the grape berry. In nature, the transfer of a glycosyl moiety from an activated sugar donor to an acceptor molecule is generally catalyzed by glycosyltransferases (GTs), a resveratrol GT (*UGT72B27*) gene. This gene was found to be highly expressed in grapevine leaves and berries and was determined to be responsible for the production of the phenolic glycosides (Härtl et al. 2017). These volatile phenols are glycosylated in the vine to facilitate their storage, transportation, and detoxification (Günata et al., 1985; Krstic et al. 2015; Pardo-Garcia et al. 2017). Guaiacol glycoconjugates are almost exclusively located and evenly distributed between skin and pulp of grape berries, however, skins contained a higher proportion of total glycoconjugates by mass, than pulp; 6.7-fold and 4.5-fold higher concentrations for Merlot and Viognier respectively (Hayasaka et al. 2010; Dungey et al. 2011). Pardo-Garcia et al. (2017) observed different glycosylation patterns for berries and leaves, suggesting berries and leaves may have different glycosyltransferase enzymes activated while facing smoke. Wilkinson and Ristic (2020) found 1–7 μg/L guaiacol in six samples (including Gewurztraminer, Pinot Noir and Shiraz) and detectable amounts of volatile phenol glycosides in grapes (including Cabernet Sauvignon, Chardonnay, Gewurztraminer, Pinot Noir, Semillon, Shiraz and Tempranillo) that were not exposed to smoke.

After glycosylation, these volatile phenols are nonvolatile but can be released in the free form by hydrolysis during fermentation, aging, or inside the mouth while tasting wine, (Pardo-Garcia et al. 2017; Ristic et al. 2017; Liu et al. 2020; Mirabelli-Montan et al. 2021). Caffrey

et al. (2019) found that the most hydrolytic activities of volatile-phenol glycosides (approximately 28% of the volatile-phenol glycosides were hydrolyzed) occurred during the first half of primary fermentation, which is in accordance with the glycosidase activities of *Saccharomyces* yeast. As for the hydrolysis during ageing, Ristic et al. (2017) found mall increases (up to 4 μg/L) in guaiacol or 4-methylguaiacol concentrations following bottle aging of smoke-affected wines, and large increases in syringol levels were observed (22 μg/L increases for smoke-affected Cabernet Sauvignon wines, interestingly, bottle aging resulted in decreased concentrations of cresols (1 μg/L) in Cabernet Sauvignon wines, additionally, acid hydrolysis of smoke-affected wines (post-bottle aging) released additional quantities of volatile phenols, which demonstrated the relative stability of glycoconjugate precursors to the mildly acidic conditions of wine.

If there are considerable amounts of volatile phenols in the wine, these compounds will release in the mouth as enzymes present in human saliva are able to release the volatile aglycones from their glycoconjugates. In-mouth breakdown of monosaccharide and disaccharide glycosides is an important mechanism for smoke flavor from smoke affected wines (Mayr et al. 2014).

Wines made from relatively low smoke impacted fruit can seem fine and only become smoky after aging because of acid hydrolysis of conjugate forms of both naturally occurring and smoke-derived volatile phenols (Ristic et al. 2017).

There are different pathways by which smoke-derived volatile phenols can enter into the vine, via the berry cuticle and epidermis by passive diffusion or via the stomates in the leaves and then transferred to the grape berries by the vascular system (Krstic et al. 2015). However,

the translocation of volatile compounds from leaves to berries are believed to limited (Hayasaka et al. 2010; Summerson et al. 2021). Another potential pathway for smoke-derived volatile phenols is uptake by the root system because rainfall can wash the smoke compounds into soil and then these water-soluble compounds could be absorbed by the roots. However, this is less likely as wildfires mostly happen when there is no rain and the Casparian strip in roots can block the absorption of these compounds (Krstic et al. 2015; Summerson et al. 2021).

Kennison et al. (2009; 2011) found that the timing of smoke exposure has significant influences on the resulting wine chemical and sensory characters. They defined three key periods of smoke exposure risk by concentrations of volatile phenols in resulting wines during smoke exposure: from '10-cm shoots' to 'full bloom' is the period that led to relatively low concentrations of volatile phenols in wine (average guaiacol and 4-methylguaiacol concentrations in wine were 1.0 and 0.5 mg/L, respectively); from 'berries of pea size' to the 'onset of veraison' is the period that led to moderate but variable concentrations of volatile phenols in wine (average guaiacol and 4-methylguaiacol concentrations in wine were 21.4 and 5.0 mg/L, respectively); and from '7 days post-veraison' to 'harvest' is the period that led to the highest concentrations of volatile phenols in wine (average guaiacol and 4-methylguaiacol concentrations in wine were 48.9 and 8.9 mg/L, respectively) (Kennison et al., 2011). That said, the consensus is that although risk increase up to about veraison, there is smoke exposure risk so long as there are berries on the vine even if it is at pea-size.

1.2 Mitigation of Grape Smoke Exposure Impact

Since the absorption of smoke-derived volatile phenols can decrease the quality of wine grapes and the resulting wines, it is important to investigate potential mitigation in both the

vineyard and winery. There are some strategies that are considered as potential mitigation strategies to negate the negative influences caused by smoke exposure.

In the vineyard, hand-harvesting was regarded as a method that can mitigate smoke exposure as it can remove leaves that adsorb smoke-derived volatile compounds and limits skin damage (where volatile phenols are preferentially sequestered) and thus extraction into the juice (Favell et al. 2019; Mirabelli-Montan et al. 2021).

Kennison (2009) found that the wax bloom on the grape surface can protect grapes from the penetration of smoke compounds. In that study, the wax bloom was removed with chloroform, and grapes were then exposed to smoke. Grapes where the wax were removed, absorbed more guaiacol compared to waxed berries. So, it is logical to investigate the application of materials on the surface of grape berries to prevent or limit volatile phenols absorption. The application of biofilm, an artificial phospholipid cuticle was proved to have some protective effects for vines to decrease the negative impacts from smoke exposure when applied one week before smoke exposure (Favell et al. 2019). However, in a follow up study, Favell et al. (2021) applied the protective biofilm at three different vineyards to grapes 1, 7 or 14 days prior to smoke exposure, and they found that in all cases, the biofilm treatments led to increased concentrations of both free and total volatile phenols in smoke-exposed grapes compared to untreated controls, with earlier applications elevating concentrations of some volatile phenols more than the later time points. Van der Hulst et al. (2019) found that the use of kaolin (a particulate film usually used to mitigate light and heat stress in grapevines) can significantly reduce volatile phenol glycoconjugate levels in Merlot (with reductions of 58–92% for most of the volatile phenol glycoconjugates measured at maturity) depending on the rate of

application and extent of coverage, but kaolin was not successful in decreasing volatile phenol absorption when Sauvignon Blanc and Chardonnay were treated. These applications' concentration and timing, target cultivar (clone), other cultural practices, seasonal and site conditions will all have influences on the strategies' efficiency (Rogiers et al. 2020).

The impact of canopy management is another important variable to investigate during grape smoke exposure risk. Kelly et al. (2012) concluded that there is a negative correlation between vine leaf area/ leaf area per bunch and the concentrations of volatile phenols in wines because the leaves can block particulate phase emissions. Thus, the contact between smoke marker compounds and the surface of berries would be reduced. Ristic et al. (2013) obtained similar findings in their research: defoliation before smoke exposure increased the concentration of free volatile phenols and guaiacol glycoconjugates compared to vines that were not defoliated. On the contrary, defoliation after smoke exposure reduced the intensity of undesirable flavors (ashy, burnt rubber) and enhanced the fruity aromas in the resulting wines. Although translocation of guaiacol conjugates between leaves and grape berries have been shown previously, the researchers concluded that the extent thereof was limited (Hayasaka et al. 2010). However, in the instance of Ristic et al. (2013) defoliation after smoke exposure resulted in an observed difference in smoke exposure impact in the resulting wines. Thus, defoliation could help mitigate grape smoke exposure impact in the vineyard, however, this will increase the risk for any future smoke exposures as well as increase the risk of sun burn. Currently no defoliation recommendations are made due to the potential negative impacts of defoliation and additional research is needed to quantify the potential role of defoliation in a real-life situation. Compared with other mitigating strategies, defoliation will be easy to

implement in the vineyard.

In the winery, reducing the extraction from skin, using cold maceration, choosing suitable yeast, and adding oak chips or tannins are considered methods that can reduce the expression of smoke taint flavor by reducing the extraction of smoke-derived volatile compounds or masking the smoke taint (Ristic et al. 2011). Glycoconjugates are mostly located in the skin and pulp components of grapes (Dungey et al. 2011), thus, reducing the extraction from skin can reduce the smoke-derived volatile compounds' concentration in wines. Oberholster et al. (2022) found that fermentation temperature had little impact, potentially due to the ease of extraction of volatile phenols from grape skins, therefore, the skin contact time will be a more important variable than fermentation temperature. Because cold maceration typically reduces the extraction of aromatic and phenolic compounds, it may reduce the intensity of smoke-related characteristics of smoke-affected grapes during fermentation. The reason to add oak chips or tannins is that oak volatiles derived from the oak chips or tannin additions can mask the sensory contribution of smoke constituents, such as guaiacol and 4-methylguaiacol (Ristic et al. 2011). But the effectiveness of these methods is relatively low, and the wine styles would be limited (Mirabelli-Montan et al. 2021).

After wine production, the addition of activated carbon or cyclodextrin polymers can remove smoke-derived volatile phenols from wine, but the efficacy still needs more research, and activated carbon can also remove the color and desirable volatile compounds from wine (Fudge et al. 2012). Dilution or blending the wine that has smoke taint can decrease the intensity of the defect, but the result mostly depends on the initial concentration of smoke-derived volatile compounds in the wine and the volume of base wine used for blending

(Kennison et al. 2007; Mirabelli-Montan et al. 2021). Using reverse osmosis and solid phase adsorption can also reduce the concentration of smoked-derived volatile phenols and improve the sensory attributes of smoke-tainted wines (Fudge et al. 2011). The limitations of this method are that some inherent desirable wine volatiles would also be removed and smoke taint could slowly return with time. This is likely due to hydrolysis of glycoconjugate precursors, which were not removed during the treatment process (Fudge et al. 2011).

However, there is no single method that can fix the smoke taint problem directly and completely, and the efficacies of these methods are different while treating different varieties' wines.

The main objective of this study was to determine the interactive impact of deficit irrigation and mechanical fruit zone leaf removal on grape smoke exposure and the resulting wine composition. By understanding the effect of deficit irrigation and leaf removal on grape smoke exposure, we can adjust irrigation and canopy management strategies to potentially mitigate grape smoke exposure.

2. Materials and Methods

2.1 Vineyard Site

The field experiment was conducted at a commercial vineyard of Cabernet Sauvignon (clone 08) on Freedom rootstock planted on Pachappa fine sandy loam soil in 2013. The vine spacing is 10 cm × 25 cm (vine × row), and the vines were planted on a Northeast-Southwest orientation. The location of the vineyard is Madera County, CA (37°02'01.9"N 120°25'37.8"W).

The vines were quadrilateral cordon trained with a 56 cm cross-arm; the height of the vines is 1.2 m with a pair of catch wires at 1.5m. Drip-irrigated with pressure-compensating emitters spaced at 76 cm delivering 1.6 L/hr was used for the vineyard. All other cultural practices followed the commercial industry standards for that area.

2.2 Experimental Design

This is a two (deficit irrigation) × three (leaf removal) factorial design experiment conducted in five replicated blocks (10 rows). Two adjacent vine rows comprised one block with the same deficit irrigation applied as the main plot. One block was split into three sub-plots for three different leaf removal treatments, and each experimental unit included six data vines (Table 1).

Table 1. Vineyard map. R= Leaf removal at bloom, Y= Leaf removal at fruit set, W= No leaf removal; SDI= sustained deficit irrigation, RDI= regulated deficit irrigation

Row number	**Treatments**		
Row1	SDIR	SDIY	SDIW
Row2	RDIR	RDIY	RDIW
Row3	RDIR	RDIY	RDIW
Row4	SDIR	SDIY	SDIW
Row5	SDIR	SDIY	SDIW
Row6	RDIR	RDIY	RDIW
Row7	RDIR	RDIY	RDIW
Row8	SDIR	SDIY	SDIW
Row9	RDIR	RDIY	RDIW
Row10	SDIR	SDIY	SDIW

2.3 Deficit Irrigation Treatments

When the midday leaf water potential (Ψ) reached -1.0 MPa, the vineyard the vineyard started to be irrigated and follow up irrigation was maintained at 80% of weekly crop evapotranspiration (ETc), which was calculated by using the equation of ETc = ETo × Kc (Williams 2010). The reference evapotranspiration (ET_o) was collected from the nearby California Irrigation Management Information System (CIMIS) station (station #56) of Los Banos, Merced County, CA (approximately 48 km away from the vineyard). The crop coefficient (Kc) was calculated by measuring the shade on the vineyard floor beneath the canopy of non-water stressed vines. After berry set, sustained deficit irrigation (SDI) and regulated deficit irrigation (RDI) were applied at corresponding main plots. SDI maintained the 80% of weekly ETc from berry set to harvest with the targeted midday leaf water potential threshold of -1.2 MPa, and RDI maintained 50% of weekly ETc from berry set to veraison with the targeted midday leaf water potential threshold of -1.4 MPa, after veraison, the weekly ETc of RDI was back to 80% until harvest. SDI and RDI were set up by adjusting different emitters per vine. RDI was applied through drip irrigation with pressure-compensating emitters spaced at 76 cm delivering 1.5 L/hr and SDI was applied through drip irrigation with pressure-compensating emitters spaced at 61 cm delivering 1.9 L/hr.

2.4 Leaf Removal Treatment

There were three leaf removal treatments applied at subplots: leaf removal at bloom, leaf removal at berry pea size, and no leaf removal. Bloom leaf removal was applied to both sides of the canopy at around 400 GDD (EL Stage 19, approximately five to seven days before full

bloom, 05/19/2020) with a roll-over leaf plucker that has a sickle-bar sprawl clipper adapted for a sprawling-type canopy (Model EL-50, Clemens Vineyard Equipment, Woodland, CA). The leaf plucker defoliated a 50 cm window in the fruiting zone of the canopy to ensure at least a 50% transmission of photosynthetically active radiation (PAR). The berry pea size leaf removal was applied at around 630 GDD (EL Stage 31, approximately seven to fourteen days after full bloom, 06/15/2020) to both sides of the canopy, with the same goal of defoliating a 60 cm window to ensure a similar amount of PAR infiltration into the fruiting zone. In addition, two more samples were set for establishing the baseline analysis: 1) baseline of "completely shaded clusters" (control): clusters from vines irrigated under 120% ETc without leaf removal; 2) baseline of "completely exposed clusters" (exposure): clusters from vines which were half-defoliated and irrigated under SDI.

2.5 Harvest and winemaking

When the berry total soluble solids (TSS) approached 24 Brix (10/20/2020), approximately 20 clusters per experimental unit were sampled and stored in a two-gallon Ziploc bag, there were 30 samples (2 irrigation treatments × 3 leaf removal treatments × 5 replicates) plus the control and exposure samples that were used for general comparison. All samples were shipped to the UC Davis Research and Teaching Winery for storage (-20˚C) and analysis.

At the same time (berry TSS approximately 24 Brix), grapes for 18 wine lots were harvested from three blocks (2 irrigation treatments × 3 leaf removal treatments × 3 blocks) and each wine lot contained approximate 45 kg of fruits from each experimental unit and was

shipped to the Research Winery at California State University at Fresno for winemaking. In addition, grapes for two wine lots were harvested separately to establish the baseline analysis: 1) the baseline of "completely shaded clusters": clusters from vines irrigated under 120% ETc with no leaf removal; 2) the baseline of "completely exposed clusters": clusters from vines which were half-defoliated. These two additional samples had no bio replicates. Fruit was destemmed, crushed, and 3.32 g potassium metabisulfite (KMBS) (Enartis USA, Inc.,Windsor, CA, USA) was added to 45 liters of must in each 57-liter fermentor to reach 50 mg/L sulfur dioxide. After crushing, must (pH 4) were cold soaked at 4 °C for 2 days before fermentation was started. Before fermentation, 90.7 g tartaric acid (ATPGroup, Paso Robles, CA, USA) was added to adjust the pH to 3.7, 33 g SuperFood (BSG CraftBrewing, Shakopee, MN, USA) and 16.5 g diammonium phosphate (DAP) (ATPGroup, Paso Robles, CA, USA) were added to provide 250 mg/L yeast assimilable nitrogen. The musts were brought to 20°C and inoculated with 11g yeast EC 1118 (Scott Lab, Petaluma, CA, USA) for a concentration of 0.25 g/L. Fermentation temperature was maintained at approximately 20°C in a temperature control room. Cap management was performed twice per day by manual punch down during fermentation. Fermentation was considered complete when the residual sugar was less than 2 g/L. At the end of fermentation, the wine was racked, and the skins were pressed by a basket press. Both free run and press fractions were racked into glass carboys and inoculated with 0.26 g (0.01 g/L) LACTOENOS® B7 Direct (LAFFORT®, Petaluma, CA, USA) to start malolactic fermentation. LEUCOFOOD (BSG CraftBrewing, Shakopee, MN, USA) was added at 1.06 g (0.04 g/L) as nutrient. Upon completion of malolactic fermentation, KMBS was added to maintain 30 mg L^{-1} free sulfur dioxide. Wines were stabilized at 2°C and screened by

WineScanTM (FOSS, Hilleroed, Denmark) prior to bottling, Statistical analysis was run by Tukey HSD test in JMP. The wines' basic chemical composition is shown in Table 2.

Table 2. Wine basic chemical composition at bottling (n=3). No significant difference at P<0.05.

Treatments	**Free SO2 (ppm)**	**Total SO2 (ppm)**	**EtOH (%)**	**TA (g/L)**	**pH**	**VA (g/L)**	**Malic Acid (g/L)**	**Lactic Acid (g/L)**
RDI+No leaf removal	28.5±1.3	69±3	13.9±0.1	5.56±0.25	3.68±0.04	0.31±0.01	0	1.45±0.05
RDI+Bloom leaf removal	32.0±1.9	70±3	14.1±0.2	6.43±0.20	3.67±0.03	0.35±0.03	0	1.40±0.08
RDI+leaf removal	27.6±1.5	73±3	14.2±0.3	6.22±0.16	3.65±0.03	0.35±0.02	0	1.30±0.05
SDI+ No leaf removal	27.1±1.6	69±4	14.2±0.3	5.91±0.12	3.65±0.03	0.30±0.02	0	1.40±0.03
SDI+ Bloom leaf removal	26.7±1.2	70±6	13.7±0.5	6.04±0.16	3.64±0.04	0.31±0.05	0	1.50±0.03
SDI+ Berry set leaf removal	27.5±1.8	68±5	14.2±0.3	6.00±0.15	3.68±0.06	0.32±0.03	0	1.50±0.04
Control (120% ETc+ No leaf removal)	29.6±1.8	72±5	13.9±0.5	6.35±0.18	3.66±0.03	0.32±0.02	0	1.48±0.06
Exposure (SDI+Half-defoliated)	30.2±1.6	75±5.5	14.0±0.3	6.32±0.20	3.65±0.03	0.40±0.02	0	1.46±0.08

2.6 Measurement of Volatile Phenols

Eleven compounds were analyzed in this study: guaiacol, creosol (4-methylguaiacol), *m*-cresol, *o*-cresol, *p*-cresol, phenol, 4-ethylguaiacol, 2,3-dimethoxyphenol, 4-ethylphenol, syringol and 4-methylsyringol (4-MS). The limit of detection (LOD) and the limit of

quantification (LOQ) were calculated by using the instrumental signal to noise ratio 3:1 for LOD and 10:1 for LOQ (Table 3).

Stock solutions for volatile phenols and deuterated internal standards were prepared in HPLC-grade ethanol (assay: ≥99.8%) from Sigma-Aldrich (St. Louis, MO, USA). Calibration solutions were freshly prepared before analysis by adding known amounts of volatile phenols into a model wine (16% vol ethanol, 5 g/L potassium bitartrate, pH 3.75). The internal standard solution was composed of 5 mg/L of guaiacol-d3, 4-methylguaiacol-d3 (OD), *o*-cresol-d7, *p*-cresol-d7, *m*-cresol-d7, 4-ethylguaiacol-d5, 4-ethylphenol-d4 (OD) and syringol-d6. The gas chromatography–mass spectrometry (GC-MS) system used is an Agilent 7890B GC system equipped with the Agilent 5988B high-efficiency source (HES) mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The GC-MS setup was similar to that described in Oberholster et al. (2022): The column was A J&W DB-WAXetr capillary column (30 m × 0.25 mm i.d. × 0.25 μm thickness, Agilent, Santa Clara, CA, USA). The injection port temperature was set at 200 ℃. The oven temperature started at 40 ℃ and held for five minutes, then raised to 220 ℃ at 6 ℃ per minute, then finally increased to 250 ℃ at 50 ℃ per minute and held at this temperature for seven minutes. The carrier gas helium was at a constant flow of 1 mL/min. The temperature of the ion source and transfer line were maintained at 230 ℃ and 250 ℃, respectively. The mass spectra were collected in both scan and selective ion monitoring (SIM) modes with electron ionization. Both free and total volatile phenols were quantified using the stable isotopic dilution analysis (SIDA) method as described in Pollnitz et al. (2004).

Table 3. LOD and LOQ of the measured volatile phenols by GC-MS.

Compound	**LOD (ppb)**	**LOQ (ppb)**
guaiacol	0.14	0.43
creosol	0.06	0.17
o-cresol	0.23	0.69
phenol	0.17	0.52
4-ethylguaiacol	0.03	0.09
p-cresol	0.09	0.27
m-cresol	0.05	0.15
2,3-dimethoxyphenol	0.37	1.12
4-ethylphenol	0.07	0.22
syringol	0.20	0.60
4-methylsyringol	0.13	0.40

Seventy grams of grapes were firstly homogenized (2 min at speed 5 and then 3 min at speed 10) by an IKA digital ULTRA-TURRAX® (T18) disperser (IKA® Works, Inc., Wilmington, NC, USA). For the measurement of free volatile phenols, 5 g of homogenized grapes were transferred into a glass tube containing 3g $CaCl_2$ (Sigma-Aldrich, St. Louis, MO, USA) and 2 mL Milli-Q water produced by a Milli-Q Element system (Millipore, Rockville, MD, USA). Each analysis was performed in triplicate. Twenty μL internal standard mixture (5 mg/L) were added into the glass tube, followed by adding two mL of the extraction solvent, which is a 1:1 v/v mixture of pentane (Sigma-Aldrich, St. Louis, MO, USA) /ethyl acetate (Millipore Corporation, Darmstadt, Germany). After all additions, the glass tubes were vortexed for 30 seconds and then allowed to extract for 10 min. Next, the samples were centrifuged at 2800 rpm, at 4 °C for 5 min using an Eppendorf centrifuge 5810R (Eppendorf AG, Hamburg, Germany). Subsequently, the maximum amount of supernatant (from 1.0 mL to 1.5 mL) in the glass tube was transferred into a 2 mL HPLC vial and loaded on to the GC-MS.

As for the measurement of total volatile phenols, the pH of homogenized grapes (40 g)

was adjusted dropwise with 37% HCl (Sigma-Aldrich, St. Louis, MO, USA) until 1.0, then 10 g of the homogenized grapes were transferred into a PTFE tube (each grape sample was analyzed in triplicate), and 40 μL of internal standard mixture (5 mg/L) was added into the glass tube. After being sealed with Teflon tape and covered with aluminum foil, PTFE tubes were put into the 100 °C water bath for 1 hour to hydrolyze the samples. After 1 hour of hydrolysis, the tubes were cooled down to room temperature (about 23 °C) by using ice water, after which 5 g of the hydrolyzed samples were transferred into a 2 mL glass tube. The rest of the steps are the same as those for the measurement of free volatile phenols. Two mL of the extraction solvent (pentane/ethyl acetate, 1:1 v/v) was added into the same glass tube and the glass tubes were vortexed for 30 seconds and then put on the bench for 10 min for extraction. Again, the samples were centrifuged at 2800 rpm, at 4 °C for 5 min, after which the supernatant was transferred into a 2 mL HPLC vial. Finally, all samples were loaded on the GC-MS.

2.7 Statistical methods

The collection and calculation of data were done by Microsoft® Excel® 2019. One-way analysis of variance (ANOVA), two-way (deficit irrigation x leaf removal) ANOVA, Tukey's HSD test and Pearson Correlation were done by IBM® SPSS® Statistics 25. All analyses used an α of 0.05 for determining statistical significance.

3. Results and Discussion

3.1 Smoke exposure

There were two wildfires related to this project, the River Fire and the Creek Fire. This determination was made by comparing the dates of these fires and the changes in the air quality index (AQI) of the Madera-City air monitor site, which is about 30 km from the vineyard.

According to the information provided by U.S Environmental Protection Agency (https://www.epa.gov/pmcourse/patient-exposure-and-air-quality-index), when AQI/PM2.5 (particulate matter 2.5) values are above 100 μg/m^3, air quality is considered to be unhealthy, thus, 100 μg/m^3 was used as the value that can indicate considerable smoke plumes have arrived. Figure 1 showed the daily average PM2.5 concentrations at the Madera-City air monitor site from August 1st to October 31st. Figure 1 indicates that PM2.5 concentrations at the Madera-City air monitor site went above 100 μg/m^3 from August 21st, 2020, and then again from September 14th, 2020. The River Fire started on August 16th and was controlled on September 4th. The Creek Fire started on September 4th and was controlled by December 24th. This means all leaf removal treatments happened before the fires; thus, we do not expect any impacts caused by different times of leaf removal in this study.

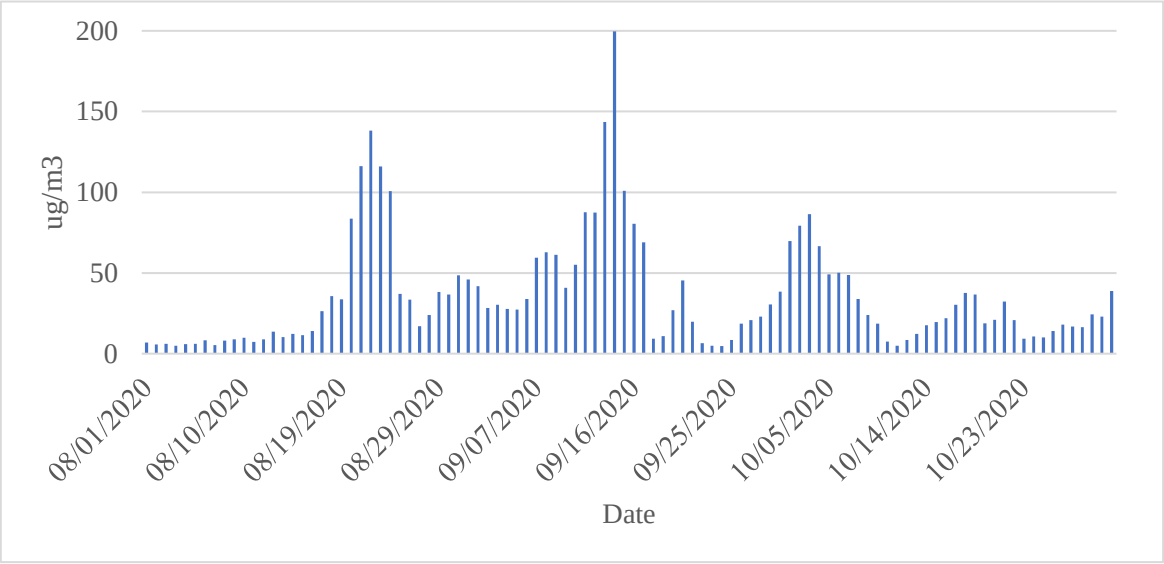

Figure 1. Daily average PM2.5 concentrations at the Madera-City air monitor site (Source: https://www.epa.gov/outdoor-air-quality-data/download-daily-data)

In the troposphere, volatile organic compounds are transformed by photolysis, as well as chemical reactions with the hydroxyl (OH) radical during daylight hours, reactions with the nitrate (NO_3) radical during evening and nighttime hours, reactions with O_3, and reactions with Cl atoms during daylight hours in coastal and marine areas (Atkinson and Arey 2003). Due to these chemical reactions, most volatile phenols can break down within several hours in the atmosphere (Atkinson and Arey 2003; Krstic et al. 2015). The River Fire started on August 16th, 2020, Figure 1 indicates that PM2.5 concentrations close to the vineyard were above 100 on August 21st 2020, which is 5 days after the River Fire started, whereas the Creek Fire started on September 4th with PM2.5 values (Figure 1) indicating that PM2.5 concentrations close to the vineyard went above 100 on September 14th 2020, which is ten days after the Creek Fire started. Thus, smoke arrived at the vineyard prior to harvest, but the smoke was not fresh and volatile phenols could have broken down significantly during those five to ten days that it took to reach the vineyard from the wildfire's location.

3.2 Volatile Phenols in the Grapes

Table 4 shows the concentrations of free guaiacol, creosol (4-methylguaiacol), cresols (*m*-cresol, *o*-cresol, and *p*-cresol) and syringol in grapes from grapevines managed using different leaf removal and irrigation treatments. Different leaf removal treatments did not show any significant effect on free volatile phenols concentrations in grapes. But irrigation had significant effects on all of the free volatile phenols measured. Grapes from grapevines treated with SDI irrigation had significantly higher concentrations of free guaiacol, creosol and cresols, while grapes from grapevines treated with RDI irrigation had significantly lower concentration

of syringol. There was an interaction of leaf removal and irrigation on free cresols' concentration, the grapes grown with RDI irrigation and treated with leaf removal at fruit set had significantly lower cresol concentrations than all other treatments except RDI without leaf removal.

Table 4. GC-MS measurement of free volatile phenols in grapes from different irrigation and leaf removal treatments. Means separated by a letter are significantly different according to Tukey's HSD test at *Pr* > *F* 0.05; error bars present the standard deviation; n=15.

	guaiacol	**creosol**	**cresols**	**syringol**
Treatment	**Average concentration (µg/kg)**			
Leaf removal				
At bloom	0.71±0.17	0.26±0.07	2.97±0.24	0.20±0.06
At fruit set	0.67±0.21	0.25±0.07	2.88±0.62	0.21±0.06
No leaf removal	0.65±0.18	0.25±0.07	3.05±0.51	0.22±0.06
Pr > *F*	0.521	0.819	0.109	0.421
Irrigation				
SDI	0.73±0.17a	0.27±0.07a	3.19±0.37a	0.18±0.06b
RDI	0.62±0.19b	0.24±0.06b	2.68±0.46b	0.24±0.05a
Pr > *F*	**0.014**	**0.029**	**<0.0001**	**<0.0001**
Interactive effects				
Pr > *F*	0.452	0.986	**0.020**	0.971

Free 4-MS concentration is lower than LOD and no shown.

Table 5 shows the concentrations of total guaiacol, creosol, cresols and syringol in grapes treated with different leaf removal and irrigation treatments measured by GC-MS. Similar to the free volatile phenols, leaf removal had no significant effect on any of the total volatile phenols measured. Irrigation only had a significant effect on total syringol, with the RDI irrigation treatment having a significantly higher concentration of total syringol than the SDI irrigation treatment. There was an interaction of leaf removal and irrigation on total cresols concentration. Grapes from RDI irrigation treated grapevines without leaf removal had a

significantly lower total cresols concentrations than grapes from grapevines treated with RDI irrigation with leaf removal at bloom and grapes from grapevines treated with SDI irrigation without leaf removal.

Firstly, total volatile phenol concentrations are higher than free volatile phenol concentrations due to being the sum of both free and bound volatile phenols. Previous studies have also shown that bound volatile phenol concentrations are higher in grapes than the free volatile phenols (Allen et al. 2013; Liu et al. 2020). Furthermore, different leaf removal treatments did not have a significant impact on either free or total grape volatile phenol composition and thus atmospheric absorption. This is not surprising as both leaf removal treatments took place (leaf removal at bloom and leaf removal at fruit set) prior to smoke exposure.

Table 5. GC-MS measurement of total volatile phenols in grapes from different irrigation and leaf removal treatments. Means separated by a letter are significantly different according to Tukey's HSD test at *Pr* > *F* 0.05; error bars present the standard deviation; n=15.

Treatment	**guaiacol**	**creosol**	**cresols**	**syringol**
	Average concentration (μg/kg)			
Leaf removal				
At bloom	8.00±1.34	0.87±0.21	9.13±1.23	5.50±1.25
At fruit set	7.92±0.79	0.86±0.18	8.64±0.84	5.54±0.73
No leaf removal	7.98±1.03	0.90±0.30	8.64±1.86	5.99±0.92
Pr > *F*	0.924	0.7510	0.324	0.100
Irrigation				
SDI	8.02±1.11	0.92±0.27	9.02±1.75a	5.40±0.88b
RDI	7.92±1.03	0.83±0.19	8.60±0.92b	5.93±1.04a
Pr > *F*	0.665	0.070	0.141	**0.010**
Interactive effects				
Pr > *F*	0.125	0.074	**0.007**	0.368

Total 4-MS concentration is lower than LOQ and not shown.

Table 6 shows the concentrations of both free and total volatile phenols in control and exposure grapevine treated grapes measured. Grapes from control grapevine had a significantly lower concentration of free guaiacol and a significantly higher concentration of free cresols compared to grapes from the exposure grapevine. As for the total volatile phenols, grapes from the exposed grapevine had significantly higher concentrations of all measured volatile phenols compared to grapes from the control grapevine. This indicated that a larger leaf area can provide some protection from atmospheric volatile phenols during smoke exposure.

Table 6. GC-MS measurement of grape volatile phenols in control and exposed grapevines. Means separated by a letter are significantly different according to Tukey's HSD test at *Pr* > *F* 0.05; error bars present the standard deviation; n=3.

	Free			**Total**			
Group	**Guaiacol**	**Creosol**	**Cresols**	**Guaiacol**	**Creosol**	**Cresols**	**Syringol**
Control	0.62±0.02 b	0.14±0.0 1	3.56±0.78 a	5.48±0.12 b	0.68±0.0b 5	5.74±0.1b 3	4.13±0.0b 6
Exposed	0.70±0.01 a	0.14±0.0 1	2.79±0.03 b	7.13±0.20 a	0.89±0.05 a	7.27±0.05 a	5.13±0.15 a
Pr > *F*	**0.001**	0.725	**<0.0001**	**<0.001**	**0.007**	**<0.0001**	**<0.001**

Free syringol and 4-MS concentrations are < LOD and total 4-MS concentration is < LOQ and not shown.

The average values of total volatile phenols in grapes from all treatments were 7.6 μg/kg for total guaiacol, 0.85 μg/kg for total creosol, 9.2 μg/kg for total cresols, and 5.4 μg/kg for total syringol. Grapes in the current study contained higher levels of smoke marker compounds compared to non-smoke-exposed Cabernet Sauvignon grapes which contained 0.5 μg/kg total guaiacol, 0.7 μg/kg total creosol, 1.3 μg/kg total cresols, and 1.7 μg/kg total syringol (in median) (Coulter et al. 2022). However, when compared to a previous study by Szeto et al. 2020, our

smoke marker compound concentrations are even much lower than the low-density (burning approximately 1.5 kg barley straw in a 6.0×2.5×2.0 m tent) smoke exposure grapes. The concentrations of these compounds in low-density smoke exposure grapes were 76.1 μg/kg for total guaiacol, 41.6 μg/kg for total creosol, 159.0 μg/kg for total cresols, and 88.0 μg/kg for total syringol (Szeto et al. 2020). Thus, the smoke impact of grapes in this study was potentially very low.

3.3 Leaf Area

Table 7 shows the leaf area per vine of vines treated with different irrigation and leaf removal strategies.

Table 7 indicates that there was no significant difference in leaf area among vines treated with different irrigation and leaf removal strategies. This can explain why there was no large or consistent difference in grapes' volatile phenol concentrations while comparing different treatments.

Table 7. Leaf area of grapevines treated with different irrigation and leaf removal strategies. Error bars present the standard deviation; n=5.

Treatment	**Leaf area/vine (m^2)**
Leaf removal	
At bloom	7.09±0.82
At fruit set	6.28±0.89
No leaf removal	7.22±1.85
Pr > F	0.210
Irrigation	
SDI	6.78±1.05
RDI	6.95±1.55
Pr > F	0.705
Interactive effects	
Pr > F	0.133

3.4 Wine Volatile Phenol Composition

Table 8 shows the concentrations of free guaiacol, cresols and syringol in wines as measured by GC-MS. Leaf removal had significant effects on all these compounds. Wines made from grapes from grapevines with leaf removal at fruit set had the highest concentrations of free guaiacol, cresols and syringol, with no significant difference between leaf removal at bloom and no leaf removal. Although there was no significant difference among the three leaf removal treatments' leaf areas, the vines with leaf removal at fruit set had the smallest leaf area (6.28 m^2), and the leaf areas of vines treated by leaf removal at bloom (7.09 m^2) and no leaf removal (7.22 m^2) were similar (Table 7). Irrigation had significant effects on both free cresols and free syringol in the wines. Wines made from grapevines treated with SDI irrigation had significantly higher concentrations of free cresols, and wines made from grapevines treated with RDI irrigation had significantly higher concentrations of free syringol. There was no interaction of leaf removal and irrigation on concentrations of free volatile phenols in the wines.

Table 9 shows the concentrations of total guaiacol, creosol, cresols, syringol and 4-MS in the wines made from different grapevine treatments. Leaf removal had significant effects on most volatile phenols (total guaiacol, creosol, cresols and syringol). Wines made from grapevines with leaf removal at fruit set contained the highest concentrations of total guaiacol, cresols and syringol, while wines made from grapevines without leaf removal contained the highest concentrations of total creosol. Irrigation had significant effects on both total guaiacol and syringol in the wines. Wines made from grapevines treated with SDI irrigation had significantly lower concentrations of total guaiacol and syringol. There were interactions of leaf removal and irrigation on both total creosol and cresols. The wines made from grapeviness

treated with SDI irrigation and without leaf removal had significantly higher concentrations of creosol than wines made from grapevines with bloom leaf removal and wines made from grapevines treated with SDI irrigationwith leaf removal at fruit set.

Table 8. GC-MS measurement of free volatile phenols in wines from different irrigation and leaf removal treatments. Means separated by a letter are significantly different according to Tukey's HSD test at *Pr* > *F* 0.05; error bars present the standard deviation; n=18.

	Guaiacol	Cresols	Syringol
Treatment	**Average concentration (µg/kg)**		
Leaf removal			
At bloom	1.31±0.14b	4.87±0.57b	13.87±2.53a
At fruit set	1.51±0.08a	5.52±0.52a	15.05±1.68a
No leaf removal	1.35±0.06b	5.09±0.60b	14.01±2.62ab
Pr > *F*	**<0.0001**	**<0.0001**	**<0.0001**
Irrigation			
SDI	1.39±0.11	5.48±0.59a	13.17±2.05b
RDI	1.38±0.15	4.84±0.46b	15.45±2.08a
Pr > *F*	0.6497	**<0.0001**	**0.027**
Interactive effects			
Pr > *F*	0.730	0.201	0.286

Free creosol concentration is lower than LOQ. Free 4-MS concentration is lower than LOD.

Table 10 shows the concentrations of free volatile phenols in wines made from control and exposed grapevines. The control wines had a significantly higher concentration of free cresols and significantly lower concentrations of both free guaiacol and syringol compared to the exposed wines. As for the total volatile phenols, the wines made from exposed fruit had significantly higher concentrations of total guaiacol, creosol, cresols and syringol compared to the control wines. The control wines only had a significantly higher concentration of total

4-MS compared to the exposed wines (Table 11).

Table 9. GC-MS measurement of total volatile phenols in wines from different irrigation and leaf removal treatments. Means separated by a letter are significantly different according to Tukey's HSD test at *Pr* > *F* 0.05; error bars present the standard deviation; n=18.

	Guaiacol	Creosol	Cresols	Syringol	4-MS
Treatment	**Average concentration (µg/kg)**				
Leaf removal					
At bloom	3.98±0.32ab	0.91±0.06b	10.90±0.71b	19.05±2.95b	0.50±0.03
At fruit set	4.14±0.37a	0.95±0.10b	11.45±0.81a	20.54±2.17a	0.50±0.02
No leaf removal	3.93±0.35b	0.99±0.06a	11.34±0.88a	20.00±3.35ab	0.50±0.03
Pr > *F*	**0.031**	**<0.0001**	**0.010**	**0.041**	0.882
Irrigation					
SDI	3.91±0.35b	0.94±0.08	11.11±0.80	18.23±2.36b	0.50±0.03
RDI	4.12±0.33a	0.96±0.08	11.34±0.85	21.48±2.50a	0.50±0.02
Pr > *F*	**0.002**	0.360	0.130	**<0.0001**	0.318
Interactive effects					
Pr > *F*	0.123	**0.022**	**0.002**	0.141	0.350

In the control grapevines no leaf removal took place, whereas in the exposed grapevines were half-defoliated. This means the main difference between these two treatments was the leaf area. These results indicated that leaves could provide some protection for grapes during smoke exposure.

Table 10. GC-MS measurement of free volatile phenols in wines made from control and exposed grapevines. Means separated by a letter are significantly different according to Tukey's HSD test at *Pr* > *F* 0.05; error bars present the standard deviation; n=6.

	Guaiacol	Cresols	Syringol
Group	**Average concentration (µg/kg)**		
Control	1.55±0.25b	7.06±0.16a	11.45±0.28b
Exposed	3.93±0.15a	5.61±0.18b	17.14±0.71a
Pr > *F*	**<0.0001**	**<0.0001**	**<0.0001**

Volatile phenol concentrations no shown were < LOQ.

Table 11. GC-MS measurement of total volatile phenols in wines made from control and exposed grapevines. Means separated by a letter are significantly different according to Tukey's HSD test at *Pr* > *F* 0.05; error bars present the standard deviation; n=6.

	Guaiacol	Creosol	Cresols	Syringol	4-MS
Group	**Average concentration (μg/kg)**				
Control	3.60±0.17b	0.89±0.04b	11.32±0.25b	15.99±0.59b	0.48±0.03a
Exposure	7.09±0.30a	1.22±0.12a	12.71±1.17a	24.16±0.58a	0.57±0.02b
Pr > *F*	**<0.0001**	**<0.0001**	**0.017**	**<0.0001**	**<0.001**

In the wines, leaf removal showed more influences on volatile phenol concentrations. The wines made from grapevines with leaf removal at fruit set had significantly higher concentrations of most volatile phenols (both free and total guaiacol, cresols and syringol). This is potentially because this treatment had the smallest leaf area, which would provide less protection for grapes from smoke than vines with larger leaf area, although there was no significant difference among different leaf area treatments (Table 7). More of the total volatile phenol compound concentrations are above LOQ in the wines than the grapes. The increase of volatile phenol concentrations in the wines could be attributed to the extraction from skin tissues during fermentation as well as the enzymatic release of free volatile phenols from their glycosides during fermentation (Kennison et al. 2008).

Table 12 and Table 13 show the Pearson correlation analysis of volatile phenol concentrations between the wines and leaf area per vine. Although there was no significant relationship between most volatile phenols in the wines and leaf area per vine, except for syringol. All volatile phenols in the wines were negatively correlated with the leaf area although there were no significantly differences between treatments. This indicates that a larger leaf area can potentially provide more protection for grapes during smoke exposure, and that in this case it seems that this protection was more important than the translocation of volatile phenols

between leaves and grape berries. Hayasaka et al. (2010) concluded that the translocation of volatile phenols between leaf and grape cluster occurred to a very limited extent. Another research study (Kelly et al. 2012) indicated that a dense canopy may provide a shielding effect, which could reduce contact between smoke with the surface of berries and also reduce absorption of atmospheric volatile phenols into the grape berries. Ristic et al. (2013) found that leaf removal pre-smoke exposure led to higher concentrations of volatile phenols and guaiacol glycoconjugates in wines compared to those treated by defoliation post-smoke exposure and no defoliation. These findings can explain why the exposed treatment exhibited higher concentrations of most total volatile phenols in the wines than the control treatment as well as. the higher total volatile phenol concentration in wines made from grapevines with leaf removal at fruit set compared to the other two treatments.

Table 12. Pearson correlation between leaf area per vine and free volatile phenol concentration in the wines (n=6).

	guaiacol	cresols	syringol
R	-0.663	-0.586	0.039
P-value(1-tailed)	0.076	0.111	0.471

Table 13. Pearson correlation between leaf area per vine and total volatile phenols concentration in the wines (n=6).

	guaiacol	creosol	cresols	syringol	4-MS
R	-0.592	-0.234	-0.718	0.161	-0.177
P-value (1-tailed)	0.108	0.327	0.054	0.380	0.368

Compared to leaf removal, irrigation had less influence on wines' volatile phenol

concentrations. There is no research focused on the relationship between irrigation and smoke-derived volatile phenols in grapes, but Noestheden et al. (2018) found that overhead irrigation after a smoke-exposure event is unlikely to decrease the smoke-derived volatile phenols that are concentrated in the berry. Szeto et al. (2020) came to a similar conclusion although they found that spraying grapevines with water during smoke exposure appeared to partially mitigate the uptake of volatile phenols by grapes during smoke exposure. However, it did not significantly decrease the concentration of volatile phenols or the sensory perception of smoke taint in wine.

The average values of free volatile phenols in all wines were 1.4 μg/L for free guaiacol, 5.2 μg/L for free cresols and 14.3μg/L for free syringol. Compared to wines made from non-smoke-exposed Cabernet Sauvignon grapes that had 0.9 μg/L free guaiacol, 1.3 μg/L free cresols, and 2.5 μg/L free syringol (in median) (Coulter et al. 2022), wines in our study had higher concentrations of these smoke marker compounds. But when compared to a previous study by Ristic et al. (2017), these compounds' concentrations are similar to the non-smoke-affected wines following bottling (except for free syringol), and much lower than smoke-affected wines following bottling (except for free syringol). The concentrations of these compounds in non-smoke-affected following bottling were 2 μg/L for free guaiacol, 5 μg/L for free cresols, and 7 μg/L for free syringol. The concentrations of these compounds in smoke-affected wines following bottling were 20 μg/L for free guaiacol, 17 μg/L for free cresols, and 10μg/L for free syringol.

Based on published baseline data (non-smoked impacted grapes and wines) and data from smoke impacted grapes and wines, grapes in this study were smoke impacted at a low level.

GC-MS analysis showed limited impact due to smoke exposure and we do not foresee any impact sensorially. In fact, bench tasting of the wines by trained panelists indicated no discernable smoke impact and for this reason, a descriptive analysis of the wines was not performed.

4. Conclusions

Our results indicate that there was some smoke exposure and impact on the grapes and the wines in this study, but it was not enough to result in smoke-tainted wines since the smoke was not fresh or dense.

The different irrigation treatments showed some effects on grapes' volatile phenols but had little to no effect on the wines. On the contrary, leaf removal showed more effects on wines' volatile phenols than the grapes' volatile phenols. The half-defoliated (Exposed) treatment wines exhibited significantly higher concentrations of most volatile phenols than the control wines. These indicated that a larger leaf area can provide protection to grapes during smoke exposure. In this study, the protection provided by the canopy was of more importance than any potential translocation of volatile phenols from the leaves to the grape bunches.

For most smoke-derived volatile phenols, although the correlations were not significant, there were negative correlations between most volatile phenols in wines and leaf area per vine supporting previous research.

5. Acknowledgements

The authors would like to acknowledge the help from Dr Anita Oberholster's lab for daily lab work; the suggestions from Dr Andrew L. Waterhouse and Dr. Kaan Kurtural while writing this thesis.

Thanks to the financial support from Horace O. Lanza Scholarship, Margrit Mondavi Scholarship, Mario P. Tribuno Memorial Research Fellowship, Peter J. Shields and Henry A. Jastro Research Award, Rossi Graduate Research Scholarship and The Wine Group (TWG).

6. References

Agnolucci M, Tirelli A, Cocolin L, Toffanin A. 2017. Brettanomyces bruxellensis yeasts: impact on wine and winemaking. World J Microbiol Biotechnol 33:180.

Allen D, Bui AD, Cain N, Rose G, Downey M. 2013. Analysis of free and bound phenolics in wine and grapes by GC–MS after automated SPE. Anal Bioanal Chem 405:9869–9877.

Atkinson R, Arey J. 2003. Atmospheric Degradation of Volatile Organic Compounds. Chem Rev 103:4605–4638.

Bell TL, Stephens SL, Moritz MA. 2013. Short-term physiological effects of smoke on grapevine leaves. Int J Wildland Fire 22:933.

Caffrey A, Lerno L, Rumbaugh A, Girardello R, Zweigenbaum J, Oberholster A, Ebeler SE. 2019. Changes in Smoke-Taint Volatile-Phenol Glycosides in Wildfire Smoke-Exposed Cabernet Sauvignon Grapes throughout Winemaking. Am J Enol Vitic 70:373–381.

Coulter A, Baldock G, Parker M, Hayasaka Y, Francis IL, Herderich M. 2022. Concentration of smoke marker compounds in non‐smoke‐exposed grapes and wine in Australia. Aust J Grape and Wine Res 28:459–474.

Dungey KA, Hayasaka Y, Wilkinson KL. 2011. Quantitative analysis of glycoconjugate precursors of guaiacol in smoke-affected grapes using liquid chromatography–tandem mass spectrometry based stable isotope dilution analysis. Food Chemistry 126:801–806.

de Vries CJ, Buica A, Brand J, McKay M. 2016. The Impact of Smoke From Vegetation Fires on Sensory Characteristics of Cabernet Sauvignon Wines Made From Affected Grapes. SAJEV 37.

Favell JW, Noestheden M, Lyons SM, Zandberg WF. 2019. Development and Evaluation of a Vineyard-Based Strategy to Mitigate Smoke-Taint in Wine Grapes. J Agric Food Chem 67:14137–14142.

Favell JW, Fordwour OB, Morgan SC, Zigg I, Zandberg WF. 2021. Large-Scale Reassessment of In-Vineyard Smoke-Taint Grapevine Protection Strategies and the Development of

Predictive Off-Vine Models. Molecules 26:4311.

Fudge AL, Ristic R, Wollan D, Wilkinson KL. 2011. Amelioration of smoke taint in wine by reverse osmosis and solid phase adsorption: Amelioration of smoke taint in wine. Australian Journal of Grape and Wine Research 17:S41–S48.

Fudge AL, Schiettecatte M, Ristic R, Hayasaka Y, Wilkinson KL. 2012. Amelioration of smoke taint in wine by treatment with commercial fining agents: Amelioration of smoke taint in wine. Australian Journal of Grape and Wine Research 18:302–307.

Garde-Cerdán T, Ancín-Azpilicueta C. 2006. Review of quality factors on wine ageing in oak barrels. Trends in Food Science & Technology 17:438–447.

Günata YZ, Bayonove CL, Baumes RL, Cordonnier RE. 1985. The aroma of grapes I. Extraction and determination of free and glycosidically bound fractions of some grape aroma components. Journal of Chromatography A 331: 83–90.

Hayasaka Y, Baldock GA, Pardon KH, Jeffery DW, Herderich MJ. 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. J Agric Food Chem 58:2076–2081.

Härtl K, Huang F-C, Giri AP, Franz-Oberdorf K, Frotscher J, Shao Y, Hoffmann T, Schwab W. 2017. Glucosylation of Smoke-Derived Volatiles in Grapevine (Vitis vinifera) is Catalyzed by a Promiscuous Resveratrol/Guaiacol Glucosyltransferase. J Agric Food Chem 65:5681–5689.

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

Kennison KR, Wilkinson KL, Williams HG, Smith JH, Gibberd MR. 2007. Smoke-derived Taint in Wine: Effect of Postharvest Smoke Exposure of Grapes on the Chemical Composition and Sensory Characteristics of Wine. J Agric Food Chem 55:10897–10901.

Kennison KR, Gibberd MR, Pollnitz AP, Wilkinson KL. 2008. Smoke-Derived Taint in Wine: The Release of Smoke-Derived Volatile Phenols during Fermentation of Merlot Juice following Grapevine Exposure to Smoke. J Agric Food Chem 56:7379–7383.

Kennison KR. 2009. Bushfire Generated Smoke Taint in Grapes and Wine. Final Report to Grape and Wine Research and Development Corporation; RD 05/02-3; Department of Agriculture and Food Western Australia: Perth, WA, Australia.

Kennison KR, Wilkinson KL, Pollnitz AP, Williams HG, Gibberd MR. 2009. Effect of timing and duration of grapevine exposure to smoke on the composition and sensory properties of wine. Australian Journal of Grape and Wine Research 15:228–237.

Kennison KR, Wilkinson KL, Pollnitz AP, Williams HG, Gibberd MR. 2011. Effect of smoke application to field-grown Merlot grapevines at key phenological growth stages on wine sensory and chemical properties: Effect of smoke on grapevine phenology. Australian Journal of Grape and Wine Research 17:S5–S12.

Krstic MP, Johnson DL, Herderich MJ. 2015. Review of smoke taint in wine: smoke-derived volatile phenols and their glycosidic metabolites in grapes and vines as biomarkers for smoke exposure and their role in the sensory perception of smoke taint: Review of smoke taint in wine. Australian Journal of Grape and Wine Research 21:537–553.

Liu Z, Ezernieks V, Reddy P, Elkins A, Krill C, Murphy K, Rochfort S, Spangenberg G. 2020. A Simple GC-MS/MS Method for Determination of Smoke Taint-Related Volatile Phenols in Grapes. Metabolites 10:294.

Maga JA. 1989. The contribution of wood to the flavor of alcoholic beverages. Food Reviews International 5:39–99.

Mayr CM, Parker M, Baldock GA, Black CA, Pardon KH, Williamson PO, Herderich MJ,

Francis IL. 2014. Determination of the Importance of In-Mouth Release of Volatile Phenol Glycoconjugates to the Flavor of Smoke-Tainted Wines. J Agric Food Chem 62:2327–2336.

Mirabelli-Montan YA, Marangon M, Graça A, Mayr Marangon CM, Wilkinson KL. 2021. Techniques for Mitigating the Effects of Smoke Taint While Maintaining Quality in Wine Production: A Review. Molecules 26:1672.

Noestheden M, Dennis EG, Romero-Montalvo E, DiLabio GA, Zandberg WF. 2018. Detailed characterization of glycosylated sensory-active volatile phenols in smoke-exposed grapes and wine. Food Chemistry 259:147–156.

Noestheden M, Dennis EG, Zandberg WF. 2018. Quantitating Volatile Phenols in Cabernet Franc Berries and Wine after On-Vine Exposure to Smoke from a Simulated Forest Fire. J Agric Food Chem 66:695–703.

Oberholster A, Wen Y, Dominguez Suarez S, Erdmann J, Cauduro Girardello R, Rumbaugh A, Neupane B, Brenneman C, Cantu A, Heymann H. 2022. Investigation of Different Winemaking Protocols to Mitigate Smoke Taint Character in Wine. Molecules 27:1732.

Pardo-Garcia AI, Wilkinson KL, Culbert JA, Lloyd NDR, Alonso GL, Salinas MR. 2017. Accumulation of guaiacol glycoconjugates in fruit, leaves and shoots of Vitis vinifera cv.

Monastrell following foliar applications of guaiacol or oak extract to grapevines. Food Chemistry 217:782–789.

Parker M, Osidacz P, Baldock GA, Hayasaka Y, Black CA, Pardon KH, Jeffery DW, Geue JP, Herderich MJ, Francis IL. 2012. Contribution of Several Volatile Phenols and Their Glycoconjugates to Smoke-Related Sensory Properties of Red Wine. J Agric Food Chem 60:2629–2637.

Pollnitz AP, Pardon KH, Sykes M, Sefton MA. 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 52:3244–3252.

Rogiers SY, Fahey DJ, Holzapfel BP. 2020. Mitigating sunburn, dehydration and smoke taint in the vineyard: is there a role for sunscreens, antitranspirants and film forming barriers? Acta Hortic:71–78.

Ristic R, Osidacz P, Pinchbeck KA, Hayasaka Y, Fudge AL, Wilkinson KL. 2011. The effect of winemaking techniques on the intensity of smoke taint in wine: The effect of winemaking on smoke taint. Australian Journal of Grape and Wine Research 17: S29–S40.

Ristic R, Pinchbeck KA, Fudge AL, Hayasaka Y, Wilkinson KL. 2013. Effect of leaf removal

and grapevine smoke exposure on colour, chemical composition and sensory properties of Chardonnay wines: Defoliation and smoke taint. Australian Journal of Grape and Wine Research 19:230–237.

Ristic R, van der Hulst L, Capone DL, Wilkinson KL. 2017. Impact of Bottle Aging on Smoke-Tainted Wines from Different Grape Cultivars. J Agric Food Chem 65:4146–4152.

Summerson V, Gonzalez Viejo C, Pang A, Torrico DD, Fuentes S. 2021. Review of the Effects of Grapevine Smoke Exposure and Technologies to Assess Smoke Contamination and Taint in Grapes and Wine. Beverages 7:7.

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 25:3720.

van der Hulst L, Munguia P, Culbert JA, Ford CM, Burton RA, Wilkinson KL. 2019. Accumulation of volatile phenol glycoconjugates in grapes following grapevine exposure to smoke and potential mitigation of smoke taint by foliar application of kaolin. Planta 249:941–952.

Ward TJ, Hamilton RF, Smith GC. 2005. The Missoula Valley Semivolatile and Volatile

Organic Compound Study: Seasonal Average Concentrations. Journal of the Air & Waste Management Association 55:1007–1013.

Wedral D, Shewfelt R, Frank J. 2010. The challenge of Brettanomyces in wine. LWT - Food Science and Technology 43:1474–1479.

Wentworth GR, Aklilu Y, Landis MS, Hsu Y-M. 2018. Impacts of a large boreal wildfire on ground level atmospheric concentrations of PAHs, VOCs and ozone. Atmospheric Environment 178:19–30.

Wilkinson KL, Ristic R, Pinchbeck KA, Fudge AL, Singh DP, Pitt KM, Downey MO, Baldock GA, Hayasaka Y, Parker M, et al. 2011. Comparison of methods for the analysis of smoke related phenols and their conjugates in grapes and wine: Analysis of smoke related phenols. Australian Journal of Grape and Wine Research 17:S22–S28.

Wilkinson K, Ristic R. 2020. Comparing the chemical and sensory consequences of grapevine smoke exposure in grapes and wine from different cultivars and different wine regions in Australia. In Proceedings of the 13th International Terroir Congress, Adelaide, Australia.

Williams AP, Abatzoglou JT, Gershunov A, Guzman-Morales J, Bishop DA, Balch JK, Lettenmaier DP. 2019. Observed Impacts of Anthropogenic Climate Change on Wildfire in California. Earth's Future 7:892–910.

Williams LE. 2010. Interaction of rootstock and applied water amounts at various fractions of estimated evapotranspiration (ETc) on productivity of Cabernet Sauvignon: Effects of rootstock and water amounts on yield. Australian Journal of Grape and Wine Research 16:434–444.