agronomy

MDPI

Article

The Influence of UV on the Production of Free Terpenes in *Vitis vinifera* cv. Shiraz

Wen Miao, Jiaqiang Luo, Junda Liu, Kate Howell and Pangzhen Zhang *

School of Agriculture and Food, University of Melbourne, Parkville, VIC 3010, Australia; wmmi@student.unimelb.edu.au (W.M.); jiaqiangl@student.unimelb.edu.au (J.L.); leoliupro@outlook.com (J.L.); khowell@unimelb.edu.au (K.H.)

* Correspondence: pangzhen.zhang@unimelb.edu.au; Tel.: +61-3-8344-6890

Received: 13 August 2020; Accepted: 17 September 2020; Published: 20 September 2020

Abstract: Terpenes contribute to the desirable flavour and aroma of grapes and wine. The biosynthesis of these plant secondary metabolites is influenced by both physiological and environmental factors, such as grapevine phenological stage and sunlight exposure. In this study, we investigated the influence of ultraviolet (UV) at different grapevine phenological stages on free terpenes in grape at harvest. Two types of transparent polymer films were applied to grape bunches to eliminate both UV-A and UV-B or only eliminate UV-B, followed by the identification and quantification of terpenes using headspace solid-phase microextraction with gas chromatography–mass spectrometry (HS–SPME–GC–MS) analysis. In all, 27 free terpenes were identified, including eight monoterpenes/monoterpenoids, four norisoprenoids and fifteen sesquiterpenes. Higher concentrations of γ-terpinene, linalool and β-damascenone were observed in grapes with UV-B attenuation compared to the naturally exposed grape bunches. Elevated α-muurolene was observed in UV-attenuated grapes from pre-veraison to harvest, while higher concentrations of γ-cadinene were observed in naturally exposed grapes. The impacts of UV exclusion on grape terpenes at harvest were specific to phenological stages, where applying UV films from veraison to intermediate ripeness reduced the concentrations of key terpenes in grape harvest and UV attenuation from intermediate ripeness to harvest promoted the accumulation of α-muurolene and γ-cadinene. This study provides information for viticulturists to better manage grape terpene composition through UV shading.

Keywords: terpene; sesquiterpene; ultraviolet radiation; grape; HS–SPME–GC–MS

1. Introduction

Terpenes are secondary metabolites naturally produced by plants, and can be classified as monoterpenes, sesquiterpenes, diterpenes, triterpenes and carotenes, based on the isoprene structure [1]. Terpenes and their oxygenated derivatives, terpenoids, present in wine mostly originate from grape. Up to now, there are approximately 200 terpenes and terpenoids reported in grapes and wine [2,3]. Terpenes present either as glycosidic precursors or as unbound free volatiles in grapes, where bound terpenes are more abundant, especially toward harvest [4]. Bound terpenes from grape can be hydrolysed to release free terpenes during the winemaking process [3]. Terpenes, especially free terpenes, are responsible for attracting insect pollinators and acting as plant defence [5], and importantly contribute to the distinctive flavours and aroma of grapes and wine [6]. Moreover, many terpenes, such as D-limonene, β-caryophyllene and carnosol, have been reported to have anticancer, antioxidant and antimicrobial properties [7–9], which may enhance the health benefits of consumers.

The biosynthesis of terpenes in grapes varies at different grapevine phenological stages. Kalua and Boss [10] reported continuous reduction of monoterpenes and sesquiterpenes from fruit-set to harvest in Riesling and Cabernet Sauvignon grapes. On the contrary, Coelho et al. [11] reported the

maximum concentration of sesquiterpenes at harvest in Baga grapes. This finding was consistent with our previous study, where rotundone accumulation sped up toward the end of the ripening period [12]. Furthermore, the total amount of monoterpenes and C_{13}-norisoprenoids in Fernão-Pires grapes peaked at 20 days after veraison and declined until harvest [13]. Terpenes biosynthesis could be influenced by environmental factors, such as temperature, rainfall and solar exposure, and these factors normally interact with each other in field conditions. Solar ultraviolet radiation (UV), such as UV-A (wavelength 315–400 nm) and UV-B (280–315 nm), is a type of solar energy reaching the Earth's surface [14]. UV-B radiation could activate the plant defence system and promote the biosynthesis of secondary metabolites such as terpenes and phenolics [15–17], while UV-A and visible light (400–700 nm) may interact synergistically to promote the production of UV-B absorbing compounds thus enhancing plant resistance to UV-B [14]. Šuklje et al. [18] reported that the concentration of linalool in grape was increased by 60% under sunlight exposure, while Gil et al. [19] suggested that UV-B radiation could induce the production of monoterpenes to protect grape tissues from UV-B itself. In agreement with this finding, Sasaki et al. [20] confirmed through molecular study that shaded berries contained fewer linalool. On the other hand, Song, Smart, Wang, Dambergs, Sparrow and Qian [16] suggested that sunlight and UV exposure led to increases of nerol, geraniol and citronellol, while Jug and Rusjan [21] suggested that solar radiation could promote the biosynthesis of norisoprenoids through the elevated production of carotenoids. Research studies on peach have reported that UV radiation could alter the concentration of sesquiterpenes by modifying the transcript levels of relevant terpene synthase [22].

The accumulation of terpenes in grapes could be influenced by environmental factors at different grapevine phenological stages. So far, there is limited research on the impacts of UV on the production of free terpenes, especially sesquiterpenes in grapes, at different grapevine phenological stages. This study aimed to investigate the influences of UV attenuation at different phenological stages of grape ripening on the free terpenes produced in grapes. Most specifically, a field trial was conducted at a commercial vineyard for two consecutive years using UV attenuation films to shade grape bunches at different grapevine phenological stages, followed by headspace solid-phase microextraction with gas chromatography–mass spectrometry (HS–SPME–GC–MS) analysis of the grape berries' terpene content at harvest. This research could provide a practical suggestion for grape growers to manipulate grape free terpene content for enhanced sensory attributes.

2. Materials and Methods

2.1. Chemicals

Tartaric acid (TA), polyvinylpolypyrrolidone (PVPP), sodium sulphite, C7-C30 saturated alkane standard solution and authentic terpene standards, including α-terpineol, linalool, geraniol, geranylacetone and β-cedrene, were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). Purified water was supplied by a Milli-Q system (Millipore Australia, Bayswater, Australia).

2.2. Vineyard Site

The experiment was conducted at a commercial *Vitis vinifera* L. cv. Shiraz grapevine vineyard (Mount Langi Ghiran 37°31′ S, 143°14′ E) in a cool climate wine region of Victoria, Australia (the Grampians) in the 2016 and 2017 vintages. Grapevines were planted in a northeast–southwest orientation with a spacing of 3.0 m between rows and 1.8 m between vines. A vertical shooting-positioned (VSP) trellis was adapted to the grapevine with the standard management of irrigation, fertilisation and pest/disease control. No significant pest and disease stresses were observed during the experimental seasons. Weather information was collected from the nearest Australian Government Bureau of Meteorology weather station 15.5 km northwest of the vineyard (Ararat Prison Station, BoM No: 089085). The mean January maximum/minimum temperatures recorded in vintage 2016 were 30 °C and 11.1 °C, respectively, with a larger diurnal temperature variation than that in vintage 2017 (29 °C and 12.4 °C, respectively). The monthly mean rainfall in vintage 2016 (32.16 mm) was lower than that in vintage 2017

(39.12 mm). The intensities of solar radiation were similar between the two vintages. Solar radiation declined from 24.1 MJ m^{-2} in January to 16.7 MJ m^{-2} in March in vintage 2016, and changed from 24.4 MJ m^{-2} to 17.3 MJ m^{-2} in vintage 2017 (Table S1, Supplementary Materials).

2.3. Experimental Design and Sampling

Two transparent polymer screens were applied to grape bunches to attenuate UV. Polyethylene terephthalate glycol (PETG) film (Mulford Australia, Dandenong South, Australia) was used to exclude UV-B, but allow the transmission of UV-A, which has been proven in a previous study [17]. Polycarbonate (PC) films (Suntuf®, Palram Australia, Derrimut, Australia) were used to remove approximately 99.9% UV transmission, including both UV-A and UV-B. UV attenuation films were applied to grape bunches at the different phenological stages based on the E-L system [23]. Phenological stages E-L 31, E-L 35, E-L 36, E-L 38 were selected, representing berries in pea-size (at pre-veraison), at full veraison, intermediate ripening and harvest, respectively. Treatments were performed in the same way over two vintages: (i) control represented naturally exposed grape bunches throughout the whole stages; (ii) TW treatments (TW PETG/TW PC) were enclosed by PETG or PC film from pre-veraison to harvest; (iii) TV treatments (TV PETG/TV PC) were enclosed by PETG or PC film from pre-veraison to full veraison; (iv) TI treatments (TI PETG/TI PC) were enclosed by PETG or PC film from full veraison to intermediate ripeness; v) TH treatments (TH PETG/TH PC) were enclosed by PETG or PC from intermediate ripeness to harvest (Figure 1). Five field replicates were performed for each treatment. Each replicate was conducted on one panel of five grapevines with one panel of grapevines between every replicate as a buffer zone. Transparent UV materials were fixed on the stem of the vine by zip ties and covered the whole grape bunch. Grapes were harvested on 14 March 2016 and 18 April 2017. Ten grape bunches were picked from different grapevines of each experimental replicate and immediately frozen at −20 °C in the winery before being transferred on dry ice back to the laboratory at the University of Melbourne. Then, impurities, including stems, rachis, leaves, soil and insects, were removed from the grapes and the frozen grape berries were stored at −20 °C until analysis. Grape juice pH was measured by a pH meter (Model HI5221, Hanna Instruments, Inc., Keysborough, Australia), TA was measured by alkaline titration, and °Brix was measured by a visual portable refractometer (Model HI5221, Hanna Instruments, Inc.). A standard method was used to estimate grape total phenolic content by homogenising the frozen grape sample, followed by extraction with ethanol (50% *v*/*v*, pH = 2.0), then centrifuged with supernatant collected and transferred to a flat bottom microplate, which was measured at 280 nm using a spectrophotometer (Multiskan™ GO Microplate Spectrophotometer, Thermo Fisher Scientific Inc., Waltham, MA, USA) [24]. This method is widely used in the wine industry for a quick comparison of total phenolics in grape and wine. Technical duplicates were performed for all samples.

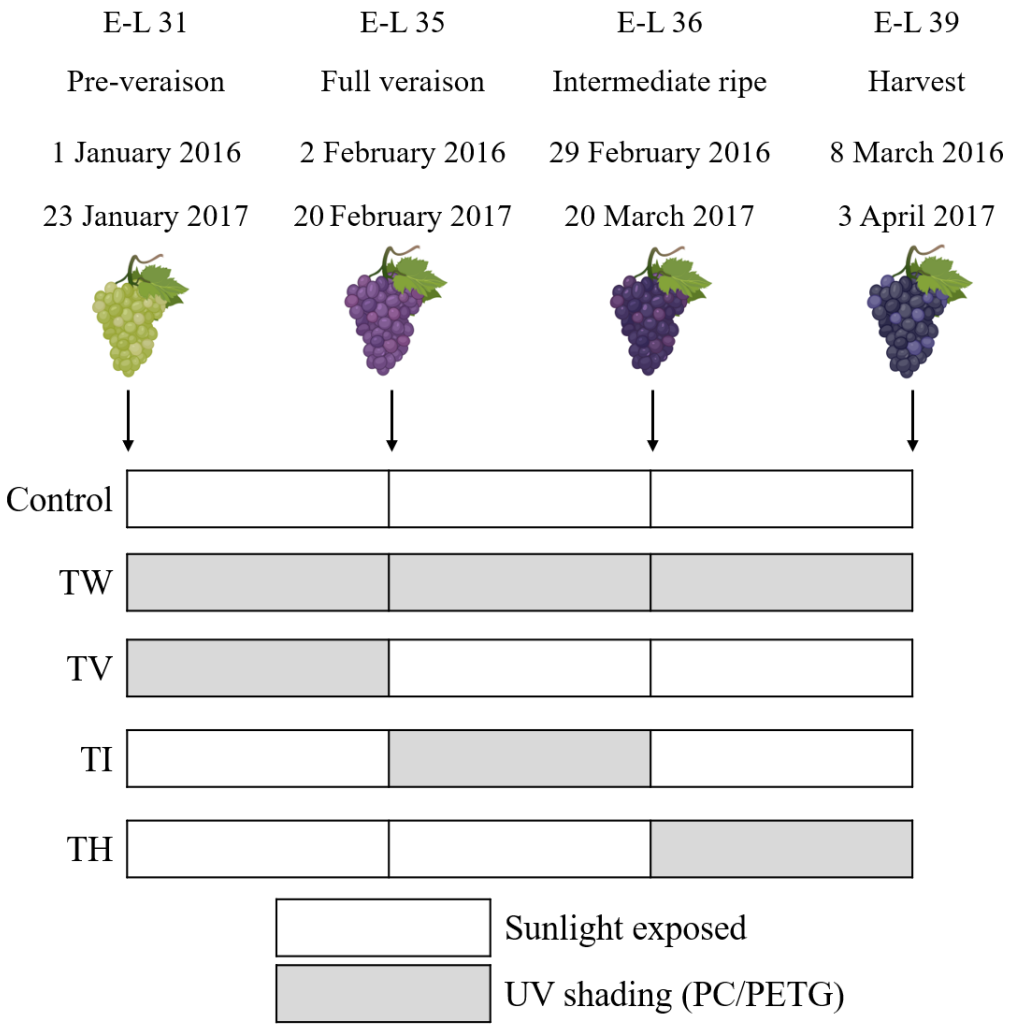

Figure 1. Experimental design for the ultraviolet (UV) attenuation trial at different phenological stages of grapevine development. The treatments were as follows: (i) control: grape bunches were naturally exposed to sunlight; (ii) grape bunches were enclosed by polyethylene terephthalate glycol (PETG) or polycarbonate (PC) film from pre-veraison to harvest (TW PETG/TW PC); (iii) grape bunches were enclosed by PETG or PC from pre-veraison to full veraison (TV PETG/TV PC); (iv) grape bunches were enclosed by PETG or PC from full veraison to intermediate ripe (TI PETG/TI PC); (v) grape bunches were enclosed by PETG or PC from intermediate ripe to harvest period (TH PETG/TH PC).

2.4. Headspace Solid Phase Microextraction Gas Chromatography–Mass Spectrometry (HS–SPME–GC–MS) Analysis

First, 50 g of grape sample were ground into powder in liquid nitrogen. Then, 5 g of the sample powder were mixed with 30 mL of extraction solution (5 g/L of TA, 5 g/L of PVPP, 0.5 g/L of sodium sulphite, pH 3.2) and shaken for 24 h in a temperature-controlled incubator (ZWYR-240, Labwit Scientific, Ashwood, VIC, Australia) at 100 rpm and 22 °C. After centrifugation at 9000 rpm for 15 min at 20 °C, the supernatant was filtered through a 0.45 µm nylon syringe filter (Thermo Scientific, Waltham, MA, USA). Then, 5 mL of the filtrate were transferred to a 20 mL gas chromatography (GC) sampling vail and mixed with 1 g of NaCl and 20 µL of internal standard β-cedrene (2 mg/L). A polydimethylsiloxane/divinylbenzene (PDMS/DVB) 65 µm solid-phase microextraction (SPME) fibre (Supelco, Bellefonte, PA, USA) was exposed to the headspace of the GC vial for 1 h at 45 °C with agitation before desorbing in a GC inlet.

An Agilent PAL multipurpose sampler connected to an Agilent 6890–5973 gas chromatography–mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) was used to carry out the analysis. The GC was equipped with a J&W DB-5ms capillary column (Agilent Technologies 30 m × 0.25 mm, 0.25 µm film). The carrier gas was helium (99.999% purity, BOC, Adelaide, Australia) at a constant flow rate at 1.0 mL/min. A resilanised borosilicate glass SPME inlet liner (Supelco, 6.5 mm o.d., 0.75 mm i.d., 78.5 mm long) was installed in the GC inlet and held at 220 °C [25]. The SPME fibre was desorbed for 10 min in the pulsed splitless mode at 25 psi for 30 s. The mass spectrometry was operating at scan/selected ion monitoring (SIM) mode at gain factor 15 to maximise the signal. Scan mode ranged from *m/z* 35 to 280. SIM mode recorded common sesquiterpene ions, namely *m/z* 105, 133, 147, 161 and 204, to facilitate the identification of sesquiterpenes at low concentrations. Quantification of all terpenes delivered to the GC via SPME sampling was based on the target ion response areas [26]. A mixed alkane standard with a range of C7-C30 was analysed to calculate the retention index (RI). Sesquiterpenes were tentatively identified using the MassFinder 4 software with its built-in terpenes library (Hochmuth Scientific Consulting, Hamburg, Germany) by comparing the RI and mass spectra. Quantification was conducted using standard calibration curves of terpene standards (α-terpineol, linalool, geraniol and geranylacetone). Identified terpenes without authentic chemical standard were semi-quantified by a known terpene standard. Sesquiterpenes and norisoprenoids were semi-quantified with the internal standard as described previously [27].

2.5. Statistical Analysis

One-way analysis of variance (ANOVA) tests were performed to compare grape TA, pH, total soluble solid (TSS), total phenolics and terpenes. Two-way ANOVA tests were performed to compare TV, TI and TH treatments over two vintages. Outlier exclusion based on the Grubbs test was performed before all statistical analysis using XLSTAT version 2020.1.3 (Addinsoft, Paris, France). All ANOVA tests were conducted using SPSS version 25.0 (IBM Corp., Armonk, NY, USA).

3. Results and Discussion

3.1. Basic Chemical Parameters of the Shiraz Grapes

Variations in grape TSS were observed among treatment groups, and no statistically significant difference could be established between individual treatment and control likely due to variations among field replicates (Table 1). Previous studies on Pinot noir grapes [16] and "Jingxiangyu" grapes [28] reported slight reductions in TSS in UV and light reduction treatments, which could not be confirmed in the current study. In the current study, UV-attenuated treatments, especially from intermediate ripeness to harvest, tended to have lower TA and higher pH (Table 1). This was different from previous studies where lower grape pH was observed in UV-reduced treatment [16], while no significant differences were observed among slight reduction treatments [28,29]. Total grape phenolics measured at 280 nm increased in UV-attenuated treatments, which was in accordance with previous research that the concentrations of hydroxycinnamic acids and hydroxybenzoic acids would be promoted by UV attenuation [30]. The same study also reported significant reduction in all types of grape anthocyanins, total flavonols and total flavanols in UV-attenuated treatment at harvest.

Table 1. Conventional chemical parameters of Shiraz grapes under different UV attenuation treatments in 2017 *.

Treatment	Total Soluble Solid (°Brix)	pH	Titratable Acid (g/L)	Total Phenolic (Absorbance Units Per Gram)
Control	21.7 ± 0.7 ^{abc}	3.93 ± 0.11 ^{ab}	4.7 ± 0.3 ^{cd}	1.20 ± 0.09 ^{a}
TH PC	21.4 ± 1.1 ^{ab}	4.03 ± 0.18 ^{ab}	3.7 ± 0.8 ^{a}	1.31 ± 0.16 ^{abc}
TH PETG	22.7 ± 1.0 ^{bc}	4.03 ± 0.11 ^{ab}	3.7 ± 0.3 ^{a}	1.32 ± 0.11 ^{abc}
TI PC	21.9 ± 0.8 ^{abc}	4.03 ± 0.09 ^{ab}	4.0 ± 0.4 ^{abc}	1.32 ± 0.16 ^{abc}
TI PETG	21.6 ± 0.3 ^{abc}	4.00 ± 0.18 ^{ab}	4.5 ± 0.8 ^{bcd}	1.48 ± 0.07 ^{c}
TV PC	22.0 ± 1.7 ^{abc}	3.97 ± 0.11 ^{ab}	4.6 ± 0.6 ^{bcd}	1.40 ± 0.16 ^{bc}

Table 1. *Cont.*

Treatment	Total Soluble Solid (°Brix)	pH	Titratable Acid (g/L)	Total Phenolic (Absorbance Units Per Gram)
TV PETG	20.9 ± 1.1 ^{a}	3.88 ± 0.13 ^{a}	4.9 ± 0.4 ^{d}	1.32 ± 0.11 ^{abc}
TW PC	22.9 ± 1.0 ^{c}	4.08 ± 0.09 ^{b}	4.2 ± 0.5 ^{abcd}	1.29 ± 0.09 ^{ab}
TW PETG	22.2 ± 0.7 ^{abc}	3.99 ± 0.13 ^{ab}	3.9 ± 0.4 ^{ab}	1.31 ± 0.09 ^{abc}

* Different letters in the column represent statistically significant ($p < 0.05$) different means ± SD ($n = 5$ field replicates).

3.2. Response of Terpene Compounds to UV-Attenuated Treatments

A total of twenty-seven terpene compounds was identified, including eight monoterpenes, four norisoprenoids and fifteen sesquiterpenes. Of the fifteen sesquiterpenes, five were only observed in vintage 2017, including *cis*-muurola-4(15),5-diene, δ-cadinene, zonarene, ω-cadinene and cubenol (Figure 2). Monoterpenes dominated the identified terpene compounds in terms of concentrations, accounting for approximately 95% of the total terpenes, followed by sesquiterpenes, with norisoprenoids being the least (Tables S2 and S3, Supplementary Materials). Higher total terpenes were observed in vintage 2016 compared to vintage 2017 in accordance with our previous study, which reflected the consistence between sugar ripening and flavour ripening in vintage 2016 [27]. This was likely due to the combined effects of environmental factors, such as growing season cumulative heat and harvest time selection, which has been detailed in our previous study [27].

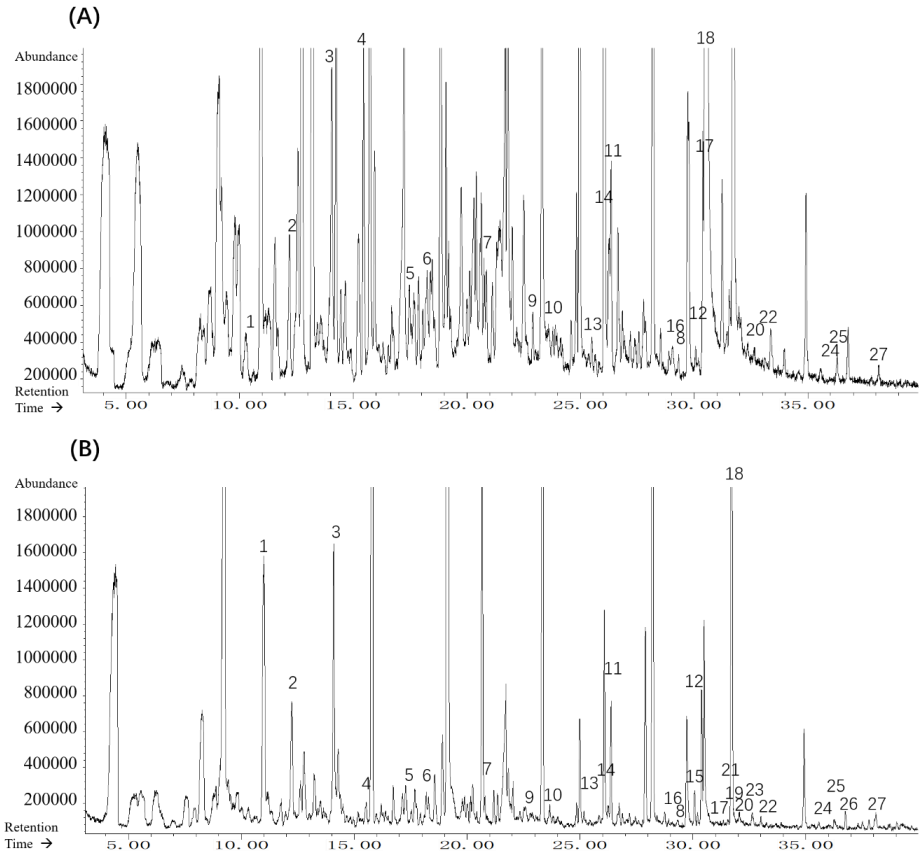

Figure 2. Gas chromatography–mass spectrometry (GC–MS) chromatograms showing the identified terpenes in (**A**) vintage 2016 and (**B**) vintage 2017. Peaks: (1) cymene(m- and p-); (2) γ-terpinene; (3) linalool; (4) citronellal; (5) menthol (+isomenthol); (6) α-terpineol; (7) geraniol; (8) geranylacetone; (9) theaspirane (isomer 1); (10) theaspirane (isomer 2); (11) (*E*)-β-damascenone; (12) β-ionone; (13) α-ylangene; (14) β-bourbonene; (15) *cis*-muurola-4(15),5-diene; (16) α-humulene; (17) α-muurolene; (18) γ-cadinene; (19) δ-cadinene; (20) calamenene(*cis* + trans); (21) zonarene; (22) α-calacorene; (23) ω-cadinene; (24) 1-epi-cubenol; (25) γ-eudesmol; (26) cubenol; (27) cadalene.

3.2.1. Effects on the Concentrations of Free Monoterpene Compounds

Overall, higher concentration of total free monoterpene was observed in control compared to TW PC and TW PETG treatments in vintage 2016, but not in 2017. Among all the detected monoterpenes, major differences were observed in γ-terpinene and linalool (Figure 3A). Significantly higher concentrations of γ-terpinene and linalool were observed in TW PETG treatment compared to other treatments in vintage 2016, and a similar trend was also observed in vintage 2017. The application stage of UV attenuation films influenced the concentration of γ-terpinene and linalool at harvest (Figure 4). UV attenuation from veraison to intermediate ripeness (TI) significantly reduced both monoterpenes at harvest compared to application before veraison, whereas the UV attenuation from intermediate ripeness to harvest (TH) increased γ-terpinene content in vintage 2016 but reduced that in vintage 2017. Compared to the pre-veraison (TV) treatment, UV attenuation post-veraison tended to reduce linalool concentration at harvest for both vintages. Application of the PC film at specific stages during ripening resulted in a lower concentration of γ-terpinene compared to the PETG film in vintage 2016, but no differences in vintage 2017.

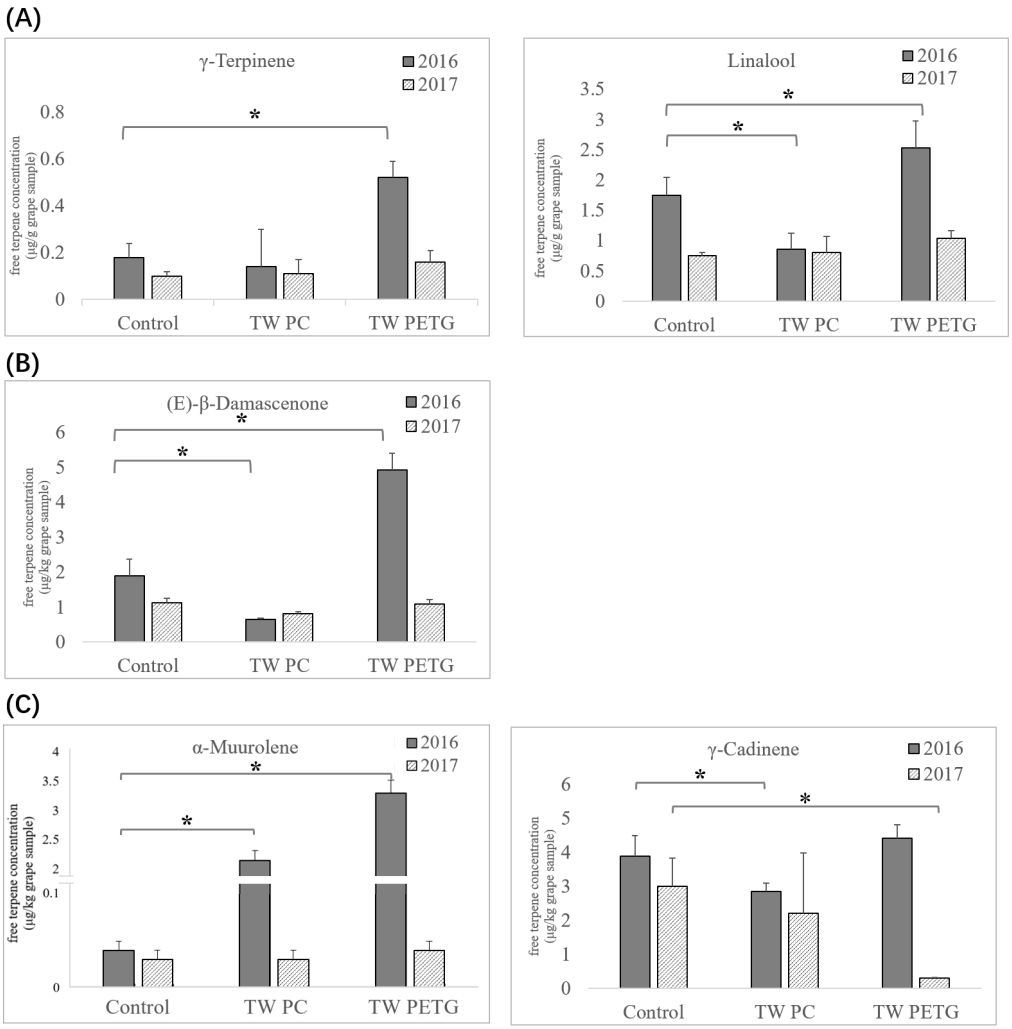

Figure 3. Comparison of key grape terpenes in vintages 2016 and 2017. One-way ANOVA was conducted to compare the concentration of (**A**) free monoterpenes, (**B**) free norisoprenoid and (**C**) sesquiterpene between control and TW treatments. * represents significant difference at $p < 0.05$.

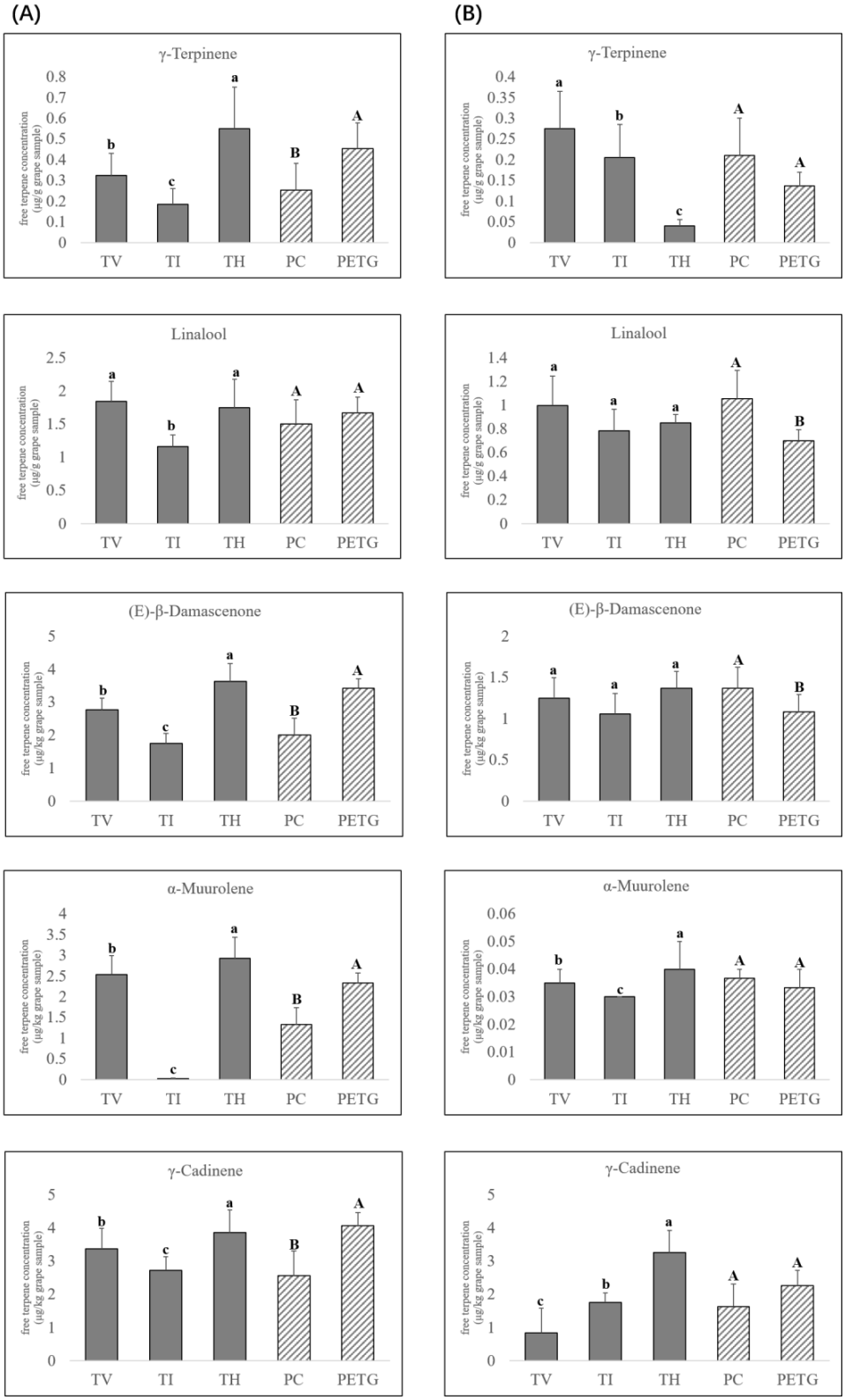

Figure 4. Comparison of key grape terpenes at harvest treated with UV attenuation at different phenological stages in (**A**) vintage 2016 and (**B**) vintage 2017. Two-way ANOVA was conducted to compare grape terpenes between different UV attenuation sheets (A, B were used to illustrate the statistically significant differences at $p < 0.05$) and applied stages (a, b, c were used to illustrate the statistically significant differences at $p < 0.05$).

A previous study reported elevated monoterpenes in grapes with enhanced UV radiation [16], which was consistent with the observation between control and TW PC treatment in the current study. Gil, Pontin, Berli, Bottini and Piccoli [15] found that the monoterpene synthase in the grapevine leaves could be promoted by UV radiation, and the monoterpene production in response to UV radiation follows a similar pattern between leaves and grape bunches [19]. The same study reported elevated monoterpenes at pre-harvest by moderate UV exposure compared to UV attenuation; however, this difference in monoterpenes disappeared at harvest. This was due to the fact that terpene synthase genes could express at later ripening stages after veraison and thus were upregulated by UV radiation during this period [15,31]. This could explain the reduced γ-terpinene and linalool in UV-attenuated grapes from veraison to intermediate harvest observed in this study. Additionally, the glycosylation of monoterpenes progresses toward harvest, where β-D-glucosyltransferases, as an upstream pathway enzyme, could intervene in the conversion of free terpenes to glycosidic forms [32]. As a result, the impacts of UV on free terpenes in grapes at harvest may not be significant as was shown in previous and current studies [19,33]. UV radiation may not always promote the biosynthesis of monoterpenes, but it may depend on the type of monoterpenes and the intensity of radiation. Zhang, Chai, Zhang, Li, Liang and Fan [29] reported that β-D-glucosyltransferase genes *VvGT7* and *VvGT14* in grapes (responsible for the production of glycosylated myrcene, nerol, geraniol and β-ocimene) could be upregulated close to harvest when light was completely excluded from the bunch at post-veraison to harvest period, whereas the gene *VvPNLinNer1* (responsible for free and bound linalool) was continuously downregulated by light exclusion.

However, another study on peach found that extra UV-B radiation could dramatically reduce the concentration of linalool through the downregulation of *PpTPS1* in the TPS-g terpene synthase subfamily [22]. This phenomenon was in accordance with the observation in grapes, where moderate UV radiation led to increased monoterpenes, but excessive radiation decreased the monoterpenes [19]. Thus, the actual impacts of UV attenuation on grape monoterpenes at harvest may vary from case to case under field conditions, depending on the type of monoterpene, attenuation time and UV radiation intensity at the field.

3.2.2. Effects on the Concentrations of Free Norisoprenoid Compounds

Overall, UV attenuation has limited impacts on the concentration of norisoprenoids, where most changes were quite small despite significance with clear vintage variations (Tables S2 and S3). A dramatical increase of β-damascenone was observed in the TW PETG treatment compared to control in vintage 2016, but a significant reduction was recorded in the TW PC treatment (Figure 3B). No significant differences were observed among three treatment groups in vintage 2017. Significant influences of UV attenuation time were observed, where the TI treatment from veraison to intermediate ripeness reduced the concentration of β-damascenone compared to treatments at other phenological stages (Figure 4).

A previous study suggested that UV radiation could increase the concentration of β-damascenone as a result of carotenoid degradation [34], whereas UV attenuation during grape ripening could reduce the concentration of both free and bound norisoprenoids at harvest [33,35]. However, other researchers reported no statistically significant differences in norisoprenoids among different UV treatments [16,36]. Similar to monoterpenes, the concentration of free norisoprenoids at harvest may rely on their biosynthesis and the conversion between the free and bound forms. β-Damascenone is biosynthesised via the apo-carotenoid pathway and influenced by the *VvCCD1* gene, which could stably express from veraison to harvest in Shiraz [37]. Therefore, this compound could be detected at most phenological stages during grape ripening [26,38]. However, the influences of light and UV radiation on the expression of *VvCCD1* and other carotenoid degradation genes remain inconclusive [36], thus the mechanism of UV impacts on norisoprenoid biosynthesis requires further investigation. Although some monoterpene-specific β-D-glucosyltransferases have been identified, it remains unclear which specific β-D-glucosyltransferases could be responsible for the conversion of free norisoprenoids

into bound forms [39]. Nevertheless, the reduced free β-damascenone in UV-shaded grape from veraison to intermediate ripeness observed in the current study indicates a possible downregulation of carotenoid degradation genes or upregulation of relevant β-D-glucosyltransferases during this period, which requires further investigation.

3.2.3. Effects on the Concentrations of Sesquiterpene Compounds

Fifteen sesquiterpenes identified in this study can be classified into four groups according to their biosynthesis pathways to better understand the impacts of UV attenuation. α-Humulene is formed via the humulyl carbocation pathway, 1-epi-cubenol and cubenol are formed via the nerolidol diphosphate pathway and γ-eudesmol is derived from (*E*, *E*)-germacradienyl cation. The rest of the sesquiterpenes are all biosynthesised through the germacrene D pathway [2,25]. Minimal influences of UV attenuation on the concentrations of sesquiterpenes were observed in both vintages, except for α-muurolene and γ-cadinene, which account for over 80% of total sesquiterpene contents (Tables S2 and S3). Out of five compounds detected only in vintage 2017, δ-cadinene, zonarene, ω-cadinene and cubenol were only found in UV-attenuated treatments. Increased α-muurolene was observed in the UV attenuation treatment in vintage 2016, but not in vintage 2017 (Figure 3C). In contrast, a clear reduction of γ-cadinene was observed in UV-attenuated treatments. Importantly, UV attenuation from intermediate ripeness to harvest tended to significantly increase the concentration of sesquiterpenes at harvest (Tables S2 and S3). This phenomenon was very clear in the case of α-muurolene and γ-cadinene, where UV-attenuated grape from intermediate ripeness to harvest had the highest concentration of α-muurolene and γ-cadinene in both vintages (Figure 4).

Sesquiterpenes in grapes were present in free forms rather than glycosidic bound forms [2,25], thus the changes in the concentration of sesquiterpenes were likely due to the influences of UV on their biosynthesis. Previous research studies reported that the concentration of some sesquiterpenes in grape flower such as β-farnesene and α-panasinsene could be dramatically reduced by UV-B exclusion, while other sesquiterpenes such as farnesol could be increased by UV-B exclusion, and others, such as α-caryophyllene, β-caryophyllene and nerolidol, remain unchanged [40]. A study on peach also found elevated sesquiterpene, namely α-farnesene, after 48 h of UV-B radiation due to the upregulation of *PpTPS2* from a TPS-b terpene synthase subfamily [22]. Despite this, the same study also reported a reduced concentration of valencene in peach after UV-B radiation, while the concentration of α-cedrene decreased after 6 h of UV-B exposure and increased afterward. These examples in the literature suggest that the impacts of UV radiation on individual sesquiterpenes may vary from each other depending on the extent of UV exposure, and the sesquiterpenes related to plant defence are likely to be upregulated by UV-B [15,22,40].

Regarding the impacts of UV attenuation film's application stage, our previous research studies and others have demonstrated that the concentration of most sesquiterpenes decreased from fruit-set to veraison and gradually increased at the late stage of ripening, and many sesquiterpenes only appeared at the end of the grape ripening period [25–27,38,41]. Sesquiterpenes are biosynthesised through distinct biological pathways, and the responses of sesquiterpenes to UV attenuation at different phenological stages could vary among different biosynthesis pathways [2]. In the current study, no clear changes were observed in sesquiterpenes derived from the humulyl carbocation pathway, the nerolidol diphosphate pathway and the (*E*, *E*)-germacradienyl cation pathway, whereas the two sesquiterpenes (α-muurolene and γ-cadinene) derived from the germacrene D pathway had the most obvious changes. Our previous study observed the highest concentration of α-muurolene before veraison with a decreasing trend afterward until harvest, whereas γ-cadinene could only be detected at harvest [26,38]. The current study observed higher α-muurolene and γ-cadinene in the UV attenuation treatment from intermediate ripeness to harvest, which was consistent with our previous study where grape bunch light exclusion from intermediate ripeness to harvest resulted in a higher γ-cadinene concentration at harvest than light exclusion at other phenological stages [42]. This suggested that UV shading at this period may upregulate the biosynthesis of α-muurolene and γ-cadinene or prevent

them from being converted into other metabolites. Further molecular study is required to confirm the expression of relevant terpene synthase genes.

4. Conclusions

This research investigated the impacts of UV attenuation at different phenological stages on the concentration of free terpenes in Shiraz grapes at harvest. Results showed a small reduction in total monoterpenes and increases in selected norisoprenoids and sesquiterpenes in UV-attenuated treatments. The application stage of UV shading also have an impact on the final concentration of terpenes in grapes at harvest, where UV attenuation after veraison tended to modify the concentration of γ-terpinene, cymene, linalool, geranylacetone and β-damascenone. These impacts could be due to the UV modulation of relevant terpene synthase and/or through the impacts on relevant β-D-glucosyltransferases responsible for the conversion between free and bound monoterpenes and norisoprenoids. Two representative sesquiterpenes from the germacrene D pathway, α-muurolene and γ-cadinene, were likely to be upregulated by UV attenuation from intermediate ripeness to harvest. This may be due to UV regulation of their biosynthesis or conversion into other metabolites. Future research should look into the impacts of UV radiation on the expression of relevant terpene synthase to further confirm the findings of the current study.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/9/1431/s1, Table S1: Weather conditions during vintages 2016 and 2017 (obtained from the website of the Australian Government Bureau of Meteorology using the Ararat Prison Observation Station data), Table S2: Free terpene concentrations in Shiraz grape at harvest under different UV attenuation treatments in vintage 2016, Table S3: Free terpene concentrations in Shiraz grape at harvest under different UV attenuation treatments in vintage 2017.

Author Contributions: Conceptualization, K.H. and P.Z.; Data curation, W.M., J.L. (Jiaqiang Luo) and J.L. (Junda Liu); Formal analysis, W.M., J.L. (Jiaqiang Luo), J.L. (Junda Liu) and P.Z.; Investigation, J.L. (Jiaqiang Luo) and P.Z.; Methodology, J.L. (Jiaqiang Luo), J.L. (Junda Liu), K.H. and P.Z.; Project administration, P.Z.; Supervision, K.H. and P.Z.; Writing—original draft, W.M.; Writing—review and editing, W.M., K.H., and P.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This project was funded by the School of Agriculture and Food, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, and Veski through its Veski Sustainable Agricultural Fellow program awarded to P.Z.

Acknowledgments: The authors gratefully thank Mount Langi Ghiran for donating grape samples to support this study and Damien Sheehan, Ben Haines and Jess Robertson for their expert technical support.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Gershenzon, J.; Dudareva, N. The function of terpene natural products in the natural world. *Nat. Chem. Biol.* **2007**, *3*, 408. [CrossRef] [PubMed]
2. Li, Z.; Howell, K.; Fang, Z.; Zhang, P. Sesquiterpenes in grapes and wines: Occurrence, biosynthesis, functionality, and influence of winemaking processes. *Compr. Rev. Food Sci. Food Saf.* **2020**, *19*, 247–281. [CrossRef]
3. Black, C.A.; Parker, M.; Siebert, T.E.; Capone, D.L.; Francis, I.L. Terpenoids and their role in wine flavour: Recent advances. *Aust. J. Grape Wine Res.* **2015**, *21*, 582–600. [CrossRef]
4. Vilanova, M.; Sieiro, C. Determination of free and bound terpene compounds in albariño wine. *J. Food Compos. Anal.* **2006**, *19*, 694–697. [CrossRef]
5. Yu, F.; Utsumi, R. Diversity, regulation, and genetic manipulation of plant mono- and sesquiterpenoid biosynthesis. *Cell. Mol. Life Sci.* **2009**, *66*, 3043–3052. [CrossRef] [PubMed]
6. Jackson, R.S. 6-chemical constituents of grapes and wine. In *Wine Science*, 3rd ed.; Jackson, R.S., Ed.; Academic Press: San Diego, CA, USA, 2008; pp. 27–331.
7. Guimarães, A.C.; Meireles, L.M.; Lemos, M.F.; Guimarães, M.C.C.; Endringer, D.C.; Fronza, M.; Scherer, R. Antibacterial activity of terpenes and terpenoids present in essential oils. *Molecules (Basel Switz.)* **2019**, *24*, 2471. [CrossRef]

8. Dahham, S.S.; Tabana, Y.M.; Iqbal, M.A.; Ahamed, M.B.; Ezzat, M.O.; Majid, A.S.; Majid, A.M. The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of aquilaria crassna. *Molecules (Basel Switz.)* **2015**, *20*, 11808–11829. [CrossRef]
9. González-Burgos, E.; Gómez-Serranillos, M.P. Terpene compounds in nature: A review of their potential antioxidant activity. *Curr. Med. Chem.* **2012**, *19*, 5319–5341. [CrossRef]
10. Kalua, C.M.; Boss, P.K. Comparison of major volatile compounds from riesling and cabernet sauvignon grapes (*Vitis vinifera* L.) from fruitset to harvest. *Aust. J. Grape Wine Res.* **2010**, *16*, 337–348. [CrossRef]
11. Coelho, E.; Rocha, S.M.; Delgadillo, I.; Coimbra, M.A. Headspace-spme applied to varietal volatile components evolution during *Vitis vinifera* L. Cv. 'Baga' ripening. *Anal. Chim. Acta* **2006**, *563*, 204–214. [CrossRef]
12. Zhang, P.; Howell, K.; Krstic, M.; Herderich, M.; Barlow, E.W.R.; Fuentes, S. Environmental factors and seasonality affect the concentration of rotundone in *Vitis vinifera* L. Cv. Shiraz wine. *PLoS ONE* **2015**, *10*, e0133137. [CrossRef] [PubMed]
13. Coelho, E.; Rocha, S.M.; Barros, A.S.; Delgadillo, I.; Coimbra, M.A. Screening of variety- and pre-fermentation-related volatile compounds during ripening of white grapes to define their evolution profile. *Anal. Chim. Acta* **2007**, *597*, 257–264. [CrossRef] [PubMed]
14. De Oliveira, A.F.; Nieddu, G. Vine growth and physiological performance of two red grape cultivars under natural and reduced uv solar radiation. *Aust. J. Grape Wine Res.* **2016**, *22*, 105–114. [CrossRef]
15. Gil, M.; Pontin, M.; Berli, F.; Bottini, R.; Piccoli, P. Metabolism of terpenes in the response of grape (*Vitis vinifera* L.) leaf tissues to uv-b radiation. *Phytochemistry* **2012**, *77*, 89–98. [CrossRef]
16. Song, J.; Smart, R.; Wang, H.; Dambergs, B.; Sparrow, A.; Qian, M.C. Effect of grape bunch sunlight exposure and uv radiation on phenolics and volatile composition of *Vitis vinifera* L. Cv. Pinot noir wine. *Food Chem.* **2015**, *173*, 424–431. [CrossRef]
17. Gregan, S.; Wargent, J.; Liu, L.; Shinkle, J.; Hofmann, R.; Winefield, C.; Trought, M.; Jordan, B. Effects of solar ultraviolet radiation and canopy manipulation on the biochemical composition of sauvignon blanc grapes. *Aust. J. Grape Wine Res.* **2012**, *18*, 227–238. [CrossRef]
18. Šuklje, K.; Antalick, G.; Coetzee, Z.; Schmidtke, L.M.; Baša Česnik, H.; Brandt, J.; du Toit, W.J.; Lisjak, K.; Deloire, A. Effect of leaf removal and ultraviolet radiation on the composition and sensory perception of *Vitis vinifera* L. Cv. Sauvignon blanc wine. *Aust. J. Grape Wine Res.* **2014**, *20*, 223–233. [CrossRef]
19. Gil, M.; Bottini, R.; Berli, F.; Pontin, M.; Silva, M.F.; Piccoli, P. Volatile organic compounds characterized from grapevine (*Vitis vinifera* L. Cv. Malbec) berries increase at pre-harvest and in response to uv-b radiation. *Phytochemistry* **2013**, *96*, 148–157. [CrossRef]
20. Sasaki, K.; Takase, H.; Matsuyama, S.; Kobayashi, H.; Matsuo, H.; Ikoma, G.; Takata, R. Effect of light exposure on linalool biosynthesis and accumulation in grape berries. *Biosci. Biotechno. Biochem.* **2016**, *80*, 2376–2382. [CrossRef]
21. Jug, T.; Rusjan, D. Advantages and disadvantages of uv-b radiations on grapevine (*vitis* sp.). *Emir. J. Food Agric.* **2012**, *24*, 576. [CrossRef]
22. Liu, H.; Cao, X.; Liu, X.; Xin, R.; Wang, J.; Gao, J.; Wu, B.; Gao, L.; Xu, C.; Zhang, B. Uv-b irradiation differentially regulates terpene synthases and terpene content of peach. *Plant Cell Environ.* **2017**, *40*, 2261–2275. [CrossRef]
23. Coombe, B.G. Growth stages of the grapevine: Adoption of a system for identifying grapevine growth stages. *Aust. J. Grape Wine Res.* **1995**, *1*, 104–110. [CrossRef]
24. Iland, P.; Bruer, N.; Edwards, G.; Weeks, S.; Wilkes, E. *Chemical Analysis of Grapes and Wine: Techniques and Concepts*; Patrick Iland Wine Promotions Pty Ltd: Adelaide, Australia, 2004.
25. Zhang, P.; Fuentes, S.; Siebert, T.; Krstic, M.; Herderich, M.; Barlow, E.W.R.; Howell, K. Terpene evolution during the development of *Vitis vinifera* L. Cv. Shiraz grapes. *Food Chem.* **2016**, *204*, 463–474. [CrossRef]
26. Luo, J.; Brotchie, J.; Pang, M.; Marriott, P.J.; Howell, K.; Zhang, P. Dataset of concentrations of free terpenes at different phenological stages in *Vitis vinifera* L. Shiraz, cabernet sauvignon, riesling, chardonnay and pinot gris. *Data Brief* **2019**, *27*, 104595. [CrossRef] [PubMed]
27. Luo, J.; Brotchie, J.; Pang, M.; Marriott, P.J.; Howell, K.; Zhang, P. Free terpene evolution during the berry maturation of five *Vitis vinifera* L. Cultivars. *Food Chem.* **2019**, *299*, 125101. [CrossRef] [PubMed]
28. Zhang, H.; Fan, P.; Liu, C.; Wu, B.; Li, S.; Liang, Z. Sunlight exclusion from muscat grape alters volatile profiles during berry development. *Food Chem.* **2014**, *164*, 242–250. [CrossRef] [PubMed]

29. Zhang, E.; Chai, F.; Zhang, H.; Li, S.; Liang, Z.; Fan, P. Effects of sunlight exclusion on the profiles of monoterpene biosynthesis and accumulation in grape exocarp and mesocarp. *Food Chem.* **2017**, *237*, 379–389. [CrossRef]
30. Berli, F.; Fanzone, M.; Piccoli, P.; Bottini, R. Solar uv-b and aba are involved in phenol metabolism of *Vitis vinifera* L. Increasing biosynthesis of berry skin polyphenols. *J. Agric. Food Chem.* **2011**, *59*, 4874–4884. [CrossRef]
31. Lücker, J.; Bowen, P.; Bohlmann, J. Vitis vinifera terpenoid cyclases: Functional identification of two sesquiterpene synthase cdnas encoding (+)-valencene synthase and (−)-germacrene d synthase and expression of mono- and sesquiterpene synthases in grapevine flowers and berries. *Phytochemistry* **2004**, *65*, 2649–2659. [CrossRef]
32. Bönisch, F.; Frotscher, J.; Stanitzek, S.; Rühl, E.; Bitz, O.; Schwab, W.; Wüst, M. Enantioselectivities of uridine diphosphate-glucose:Monoterpenol glucosyltransferases from grapevine *vitis vinifera* l.). In *Importance of Chirality to Flavor Compounds*; American Chemical Society: Washington, DC, USA, 2015; Volume 1212, pp. 77–83.
33. Liu, D.; Gao, Y.; Li, X.X.; Li, Z.; Pan, Q.H. Attenuated uv radiation alters volatile profile in cabernet sauvignon grapes under field conditions. *Molecules* **2015**, *20*, 16946–16969. [CrossRef]
34. Lafontaine, M.; Schultz, H.R.; Lopes, C.; Bálo, B.; Varadi, G. Leaf and fruit responses of riesling grapevines to uv-radiation in the field. In Proceedings of the Seventh International Symposium on Grapevine Physiology and Biotechnology, Davis, CA, USA, 21–25 June 2004.
35. Bureau, S.M.; Baumes, R.L.; Razungles, A.J. Effects of vine or bunch shading on the glycosylated flavor precursors in grapes of *Vitis vinifera* L. Cv. Syrah. *J. Agric. Food Chem.* **2000**, *48*, 1290–1297. [CrossRef] [PubMed]
36. Kwasniewski, M.T.; Vanden Heuvel, J.E.; Pan, B.S.; Sacks, G.L. Timing of cluster light environment manipulation during grape development affects C13 norisoprenoid and carotenoid concentrations in riesling. *J. Agric. Food Chem.* **2010**, *58*, 6841–6849. [CrossRef] [PubMed]
37. Mathieu, S.; Terrier, N.; Procureur, J.; Bigey, F.; Günata, Z. A carotenoid cleavage dioxygenase from *Vitis vinifera* L.: Functional characterization and expression during grape berry development in relation to C13-norisoprenoid accumulation. *J. Exp. Bot.* **2005**, *56*, 2721–2731. [CrossRef]
38. Zhang, P.; Fuentes, S.; Siebert, T.; Krstic, M.; Herderich, M.; Barlow, E.W.R.; Howell, K. Comparison data of common and abundant terpenes at different grape development stages in shiraz wine grapes. *Data Brief* **2016**, *8*, 1127–1136. [CrossRef]
39. Bönisch, F.; Frotscher, J.; Stanitzek, S.; Rühl, E.; Wüst, M.; Bitz, O.; Schwab, W. Activity-based profiling of a physiologic aglycone library reveals sugar acceptor promiscuity of family 1 udp-glucosyltransferases from grape. *Plant Physiol.* **2014**, *166*, 23–39. [CrossRef]
40. Gil, M.; Bottini, R.; Pontin, M.; Berli, F.J.; Salomon, M.V.; Piccoli, P. Solar uv-b radiation modifies the proportion of volatile organic compounds in flowers of field-grown grapevine (*Vitis vinifera* L.) cv. Malbec. *Plant Growth Regul.* **2014**, *74*, 193–197. [CrossRef]
41. May, B.; Lange, B.M.; Wust, M. Biosynthesis of sesquiterpenes in grape berry exocarp of *Vitis vinifera* L.: Evidence for a transport of farnesyl diphosphate precursors from plastids to the cytosol. *Phytochemistry* **2013**, *95*, 135–144. [CrossRef]
42. Zhang, P. The Influence of Climate Factors and Plant Physiological Responses on the Accumulation of Rotundone in Vitis Vinifera cv. Shiraz Grapevines. Ph.D. Thesis, The University of Melbourne, Melbourne, Victoria, Australia, 2015.