# RIESLING AROMA COMPOSITION IN LIGHT OF CHANGING GLOBAL TEMPERATURES – DELVING INTO THE EFFECTS OF WARMER NIGHTS ON THE VOLATILE PROFILE OF RIESLING GRAPES

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## Abstract:

**Context and purpose of the study:** Climate is a key parameter when the modulation of berry and subsequent wine composition is considered. Recent decades have already seen an increase in global surface temperatures, with a more pronounced effect on night temperatures. In Australia, very warm monthly minimum temperatures (two standard deviations higher than the historical average) increased from a 2% to 11% frequency of occurrence, and very cool monthly night temperatures have declined by about a third (Barlow and Daly, 2017). Night time temperatures are known to influence transcriptomic responses in ripening grapes (Rienth et al., 2014), however, the effect on grape chemical composition, in particular on the aroma compounds, remains to be elucidated. Aroma compounds such as the terpenes and norisoprenoids are key to the quality of white wine varieties such as Riesling. Understanding both the synthesis and loss of these desirable compounds due to the effects of warmer night temperatures, is critical to understanding the need for implementation of suitable mitigation strategies to help cope with the effects of warming projected in the future.

**Materials and Methods:** Four sites in the Canberra wine region (Australian Capital Territory and New South Wales, Australia) were chosen based on climatic data and, historic cool night index. As such, sites were catalogued as having either warmer, cooler or intermediate temperature nights. Temperature, humidity and light sensors were installed from the véraison stage to monitor meso- and microclimatic parameters throughout the ripening period. Berries were collected every two weeks from véraison until commercial harvest for chemical analysis. Midday stem water potential was also measured at sampling to assess water stress levels. Chemical analyses included total soluble sugars, titratable acidity, pH, yeast assimilable nitrogen, carotenoids, and free and bound volatile compounds.

**Results**: Higher temperature summations significantly depressed the synthesis of important aroma compounds such as norisoprenoids and terpenes, with carotenoid concentrations also being significantly decreased. Conversely, the concentration of aldehydes such as *E*-2-octenal and *E*-2-nonanal were positively correlated with higher temperature summation throughout the overall ripening season. Night temperature appeared to have a more pronounced effect, particularly on the synthesis of terpenes, during the later stages of berry development, as previously observed by Rienth et al. (2014). At harvest, warmer night temperatures resulted in lower concentrations of terpenes (e.g. linalool and  $\alpha$ -terpineol) and the C6 alcohols (e.g. 1-hexanol) whilst a direct correlation to heat summation was less significant.

Keywords: Riesling, climate, night temperature, chemical composition, volatiles, carotenoids

#### 1. Introduction

During the ripening season an intrinsic program, partially triggered by hormone signals, regulate vine physiology together with a circadian rhythm. This circadian rhythm is regulated by environmental factors such as light and temperature, and mandates the timing of several physiological and developmental occurrences during the course of a day (Carbonell-Bejerano et al., 2014; McClung, 2001).

Up to 1843 genes, including several responsible for the synthesis of aroma compounds, have been shown to be specifically night-modulated, (Rienth et al., 2014). Nocturnal temperature influences parameters that are key to wine quality such as pH, acidity, colour and aromas. When compared to cool nights, higher night temperatures have been shown to increase pH, reduce acid levels and depress anthocyanin synthesis leading to a decrease in wine colour (Gaiotti et al., 2018; Kliewer, 1973; Sweetman et al., 2014). While cooler night temperatures have been observed to improve wine sensory characteristics, in particular aroma (Tonietto et al., 2014), higher night temperatures have been associated with lower aroma potential (Jackson & Lombard, 1993) and with the repression of key enzymes related to the synthesis of aroma precursors (Rienth et al., 2014), however specific evidence is lacking.

Since 1950, Australian climate has warmed by 0.4-0.7 °C, with nocturnal temperatures increasing at a higher rate than diurnal temperatures (CSIRO & The Bureau of Meteorology, 2016; Jarvis, et al., 2019). This trend only seems set to continue. More recently, the 2019 summer broke all previous temperature with mean average minimum temperatures 1.67 °C higher than the previous record, set during the 2018 summer (+1.09 °C, Bureau of Metereology, 2019). The impact of higher night temperatures is restricted to anecdotal claims and limited generalised evidence in grapevines. However it is recognised that specific aroma compounds are key to the overall quality white wine varieties such as Riesling. Therefore, understanding both the synthesis and loss of these desirable compounds due to the effects of warmer night temperatures, is critical to understanding the need for implementation of suitable mitigation strategies to help ameliorate the effects of warming projected in the future.

## 2. Materials and Methods

Field trials were conducted over the 2017-2018 season in four commercial Riesling vineyards (S1-S4) in the Canberra wine region (Australian Capital Territory and New South Wales, Australia; (34°59'32.7S, 149°03'30.7E; 34°59'30.7S, 149°02'12.1E; 35°13'51.1S, 149°11'39.8E and 35°12'39.0S, 149°23'33.1E).

Climatic monitoring was conducted at the meso- and microclimatic level from véraison onwards. Canopy temperature and relative humidity were recorded every 10 minutes using a dual-channel logger (Tinytag TGP-4500, Gemini Dataloggers). Photosynthetically active radiation (PAR) was measured hourly using a sun quantum sensor (Apogee, Campbell Scientific Australia Pty Ltd). Bunch temperature was monitored every 10 minutes using thermocouples positioned in the middle of the bunch. Thermocouples and quantum sensor signals were scanned and recorded by two data acquisition systems (AM-25T and CR-1000, Campbell Scientific Australia Pty Ltd). Midday stem water potential was measured at sampling to assess water stress levels.

Starting at véraison, berries (n=150) were collected every two weeks in triplicate from each vineyard for chemical analysis. One hundred berries were reserved for aroma analysis and frozen immediately using liquid nitrogen and stored at -80 °C until analysis. Total soluble sugars (TSS), pH, titratable acidity, yeast assimilable nitrogen (YAN) and berry weight were measured using the remaining 50 fresh berries. Frozen berry samples for aroma analysis were prepared according to (Matarese et al., (2013). 2 g of frozen berry powder were placed in a 10 mL SPME vial containing 2 mL of phosphate-citrate buffer (pH 5), 40  $\mu$ L of ascorbic acid (50 g/L) and 1g of NaCl. A 20  $\mu$ L aliquot of internal standards in methanol (2-octanol, 5.08 mg/L; d<sub>3</sub>-linalool, 1.89 mg/L; d<sub>13</sub>-1-hexanol, 5.00 mg/L; d<sub>5</sub>-ethyl trans-cinnamate, 6.03 mg/L and d<sub>12</sub>-hexanal, 50.1 mg/L) was added. Vials were immediately capped, vortexed, and analysed by headspace solid-phase microextraction (HS-SPME) with a PDMS-CAR-DVB fibre. Chromatographic conditions were as outlined by Šuklje et al. (2016). Carotenoids were quantified in frozen berry powder using the method described by Wehrens et al. (2013). Samples were analysed using a Waters Acquity UPLC system (Milford, MA, USA) equipped with an Acquity PDA e $\lambda$  detector (Waters as previously described (Young et al., 2016).

Basic chemical data were processed using Microsoft Excel and are presented as mean values with standard deviation. One-way and two-way analysis of variance (ANOVA) with subsequent pairwise multiple comparison, using site and sampling date as explanatory variables, Pearson correlation analysis ( $p \le 0.05$ ) and principal components analysis (PCA) were performed using Matlab version 9.5.0.944444 (The Mathworks, Natick, MA, U.S.A). Data were mean-centred and normalised (1/standard deviation) prior to Pearson correlation and principal component analysis.

#### 3. Results and discussion

Heat summation (base 10) was calculated for each site from January 15<sup>th</sup> (véraison) until harvest. By harvest, S1-S4 had accumulated 639, 675, 592 and 549 °C days respectively. Located at the highest

altitude (800 m.a.s.l.), S4 was the coolest site, and accordingly, harvest occurred later than at all other sites (March 29<sup>th</sup>, 24 days after S1, Table 1). The Cool night index (Cl30, average of minimum temperatures during the 30 days preceding harvest) in addition to a modified version, Cl15 (15 days preceding harvest) were calculated for each site. Cl30 and Cl15 for all sites are listed in Table 1. All Cl30 were lower than 12 °C, and were therefore classified as having very cool nights (Tonietto & Carbonneau, 2004).

Grape compositional analyses revealed clear differences in free volatile and carotenoid final concentration according to site. A 2-way ANOVA revealed that site was a significant source of variation (p < 0.05) for all 32 quantified free volatile compounds and eight carotenoids (except zeaxanthin). A significant timing of sampling effect was also observed for all compounds with the exception of geraniol whilst the site × sampling date interaction showed that effects remained significant for all compounds except pheophytin, hexyl acetate, *E*-2-nonenal, *Z*-2-hexenol and berry weight.

The two first dimensions of the PCA (Fig. 1) accounted for 64% of the total variation of the data. A clear separation could be observed along PC1 by a distinct, consistent progression of samples in a chronological date order. The differences along this first dimension were primarily driven by an increase in TSS and pH, and to a lesser extent, by increases in the concentration of C6 compounds and aldehydes (E-2-nonenal, E-2-hexenal, nonanal, hexanal, 1-heptanal, 1-hexanol and E-2-hexen-1-ol) as well as linalool, as the berries ripened. Conversely, 1,4-cineole, Z-3-hexen-1-ol and most carotenoids in particular chlorophyll B are associated with earlier fruit sampling dates, indicating that these compounds decreased with ripening as observed by a previous study (Kalua & Boss, 2010). PC2 shows a separation of samples collected from different sites. The main differences can be observed in the lower side of the loadings plot. The location of the S4 samples appears to be predominantly due to higher concentrations of the terpenes terpinolene,  $\alpha$ -terpinene and  $\alpha$ -terpineol. The position of all S1-S3 samples appears to mostly a result of lower amounts of the same compounds. As noted previously by Marais et al. (1999), the lower heat summation and cool night index of S4 would have helped to preserve and produce more terpenes than in the other sites. Interestingly, the positioning of S1-S3 along PC2 seems to be related more closely with their cool night index rather than heat summation.Pearson correlation analysis was used to test the correlation between climatic variables and berry chemical composition. Heat summations between véraison and harvest were significantly correlated with the content of 1,8-cineole, terpinene-4-ol,  $\alpha$ -ionone, E-nonenal, E-2-octenal, 1-octanol and benzyl alcohol and negatively correlated with zeaxanthin and 1,4-cineole, violaxanthin, zeaxanthin, and chlorophyll and b. Nineteen out of the 32 aroma compounds evaluated were significantly negatively correlated with night temperature. Among all chemical classes, only benzenoids were not correlated to night temperature, these however were affected by maximum temperature, increasing when maximum temperatures were highest. In general correlations were stronger when only the last 15 days before harvest were considered. The effect of these different parameters also varied according to berry ripening state.

# 4. Conclusions

Four sites located at different altitudes in the Canberra wine region were selected and their micro- and mesoclimate monitored during ripening period. Temperature during the ripening season was shown to have a significant influence on berry composition both through heat summation and night temperature. Higher night temperatures contributed to a lower synthesis and higher degradation of important aroma compounds such as terpenes and norisoprenoids in Riesling berries. This study is the first to investigate the effect of night temperature on aroma synthesis and degradation over the whole ripening period

#### 5. Acknowledgements

This study was financially supported by the Faculty of Science of Charles Sturt University. Joanna would also like to acknowledge the continuing support of all grape growers and wineries involved in this study.

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**Figure 1.** (A) PCA scores plots showing the distribution of all samples according to site and sampling date. Different symbols denote sampling date: (o) 1/2/2018, ( $\Box$ ) 20/2/2018, (x) 1/3/2018, (+) 13/3/2018 and (\*) denote commercial harvest. (B) Loadings of variables.

Site	CI15	CI30	Harvest
S1	11.7	11.7	05/03/2018
S2	11.1	11.1	18/03/2018
<b>S</b> 3	10.8	11.9	13/03/2018
S4	9.1	9.9	29/03/2018