OENO One
VINE AND WINE OPEN ACCESS JOURNAL
By iVES International Viticulture & Enology Society

DOI:10.20870/oeno-one.2022.56.1.4857
OPEN ACCESS

Grapevine row orientation, vintage and grape ripeness effect on anthocyanins, flavan-3-ols, flavonols and phenolic acids: I. *Vitis vinifera* L. cv. Syrah grapes

Phillip Minnaar[1], Marieta van der Rijst[2] and J.J. Hunter[3]

[1] Post-Harvest & Agro-Processing Technologies, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, 7600, South Africa
[2] Agricultural Research Council, Biometry, Private Bag X5026, Stellenbosch 7599, Stellenbosch, 7600, South Africa
[3] Plant Protection and Viticulture Division, ARC Infruitec-Nietvoorbij, Private Bag X5026, Stellenbosch, 7600, South Africa

ABSTRACT

Phenolic concentrations are affected by environmental factors and grape cultivar, as well as viticultural practices, which should be considered when a specific phenolic profile is intended. The effect of microclimate induced by row orientation on phenolic compounds of *Vitis vinifera* L. cv. Syrah/101-14 Mgt grapes was investigated. Grapes were harvested from four vintages at 22 °Brix, 24 °Brix and 26 °Brix from N-S, E-W, NE-SW and NW-SE orientated grapevine rows. Phenolics were quantified in freeze-dried grape skins using an HPLC method. A vintage effect was only evident for specific individual phenolics for the four vintages. Grapes from NE-SW rows at 22 °Brix had the highest total flavonols and anthocyanins. Catechin was highest in grapes from N-S rows at 24 °Brix, whereas total phenolic acids, caffeic acid and total flavonols were highest from NW-SE rows at 24 °Brix and 26 °Brix. Isoquercetin was highest from NE-SW rows at 22 °Brix and 26 °Brix, whereas kaempferol and quercitrin were highest from E-W rows at 24 °Brix. The majority of phenolics was highest from NW-SE rows at 24 °Brix and 26 °Brix. The results indicate that row orientation affects phenolic development in Syrah grapes, but that vintage has a limited effect on phenolics. Light induced in the fruit zone positively affected the grape phenolic development of NE-SW rows at 22 °Brix and that of NW-SE rows at 24 °Brix and 26 °Brix. However, it is unlikely that a single index can be applied to all growing conditions and grape cultivars. Rows that allow moderate radiation intensity in the canopy (i.e., NE-SW and NW-SE during the day) seem more favourable for the development of phenolics than N-S and E-W rows. The light and berry temperature conditions in the fruit zone resulting from row orientation have the potential for widening the range of Syrah wine styles. In practice, a desirable row orientation may not be applicable to all environments. Management of the fruit zone remains an option for increasing or decreasing grape light exposure, irrespective of row orientation.

KEYWORDS: Anthocyanins, Flavan-3-ols, Flavonols, Phenolic acids, Microclimate

*correspondence:
minnaarp@arc.agric.za

Associate editor:
Stefanos Koundouras

Received:
27 September 2021

Accepted:
7 March 2022

Published:
25 March 2022

INTRODUCTION

Environmental factors, such as topography, precipitation and soil type, affect the growth of grapevines and grape composition (Downey *et al*., 2004), whereas viticultural practices, such as row orientation and canopy management, primarily affect the grapevine microclimate (Hunter *et al*., 2016), which in turn has an effect on the grape chemical composition.

Grape growers attempt to minimise the heterogeneity of grape material within a single vineyard parcel/block to improve wine quality (Downey *et al*., 2004; Hunter *et al.*, 2010). The development of phenolics (secondary plant metabolites) is affected by environmental factors (Gómez-Alonso *et al.*, 2007), viticultural practices (Pérez-Magariño and González-SanJosé, 2002) and plant genetics (Czemmel *et al*., 2009).

The most common phenolic compounds present in red wine grapes are non-flavonoids (i.e., derivatives of hydroxybenzoic and hydroxycinnamic acids (Ribéreau-Gayon *et al.*, 2006)), and flavonoids (i.e., anthocyanins, flavones, flavanonols, flavan-3-ols and flavonols (Margalit, 2004; Monagas *et al.*, 2005)). Anthocyanins, flavan-3-ols and flavonols are most abundant in grape skin, whereas phenolic acids are mainly present in the grape pulp.

Phenolic concentrations increase throughout berry development (Pérez-Magariño and González-SanJosé, 2006), with differing evolution depending on phenolic group (Mateus *et al*., 2002).

The relationship between phenolic composition and grape ripeness levels has been reported by numerous authors. Phenolic evolution as a consequence of a delay in sugar accumulation during the grape development of artificially shaded Cabernet-Sauvignon has been reported by Rojas-Lara and Morrison (1989), which was likely the result of decreased photosynthetic capacity of the vine. Fournand *et al*. (2006) found that free anthocyanins change to derivatised anthocyanins during the grape ripening period, and Guidoni and Hunter (2012) reported differences between the anthocyanin profiles of grapes at different ripeness levels. Guidoni and Hunter (2012) showed that malvidin 3-O-glucoside and malvidin 3-O-(6-p-coumaroyl)-glucoside were lower when at 22 °Brix compared to 24 °Brix and 26 °Brix ripeness levels, whereas acetylated anthocyanins were higher at a lower ripeness level. The high proportion of malvidin 3-O-glucoside found at 22 °Brix appeared to occur at the expense of the acetyl derivative, which is expected to be more affected by degradation reactions in grapes. Non-acetylated and acetylated glucosides are sensitive to degradation processes induced by high temperature, whereas coumaroyl-glucoside seems to be more stable under these conditions. Treatment did not have an effect on coumaroyl-glucosides. The "insensitivity" of coumaroyl-glucoside to treatment can be ascribed to their participation in intramolecular co-pigmentation (Brouilard and Dangles, 1994).

Giacosa *et al*. (2015) found that the modification of the chemical profile of Shiraz grapes was more due to changes during grape ripening than to viticultural practices. During the over ripeness stage of grapes (> 26 °Brix), individual anthocyanins can undergo some transformation independently of light and temperature conditions.

Roubelakis-Angelakis and Kliewer (1986) suggested that temperature and light play an independent regulating role in the flavonoid biosynthetic pathway to the point of the formation of dihydroflavonols, taking into consideration that anthocyanins and flavonols share the same pathway. Hunter *et al*. (2016) and Hunter *et al*. (2021) reported that radiation profiles in the canopy of Shiraz grapevines have implications for photosynthetic rates and grape berry temperature.

Hydroxylation levels of anthocyanins can be affected by not only UV radiation, but also temperature variation (Spayd *et al*., 2002). However, Alonso *et al*. (2016) and Mori *et al*. (2005) reported that anthocyanins may not always respond to radiation. Increased temperature in the fruit zone can result in grape chemical composition variability (Romboli *et al*., 2017), since the response of anthocyanins to UV radiation depends on the interaction of grape cultivar, grape ripeness and temperature factors (Jordan, 2017).

Gallic acid and most flavan-3-ols in grape skins do not respond to UV radiation, but p-coumaric, caffeic and ferulic acid, as well as gallocatechin increase in UV blocked grape bunches (Spayd *et al*., 2002). Yu *et al*. (2016) reported that increased temperature in the fruit zone was associated with an increase in epigallocatechin and a decrease in epicatechin in Merlot and Shiraz grapes respectively. Allegro *et al*. (2019) found that increased light interception in the canopy and increased berry temperature (after leaf removal) did not affect the total flavan-3-ols in the skins of Grechetto Gentile grapes; however, high levels of epicatechin were counterbalanced by low levels of epigallocatechin.

Sternad-Lemut *et al.* (2011) showed that hydroxycinnamic acids in Pinot noir grapes respond only slightly to leaf removal (exposure to light), indicating that solar radiation has a limited role in regulating phenolic acid biosynthesis. Light exclusion (by brown paper bagging) of Cabernet-Sauvignon grape bunches have been found to affect phenolic acids less than flavonoids; however, differences in hydroxycinnamic acid contents in grape berries during ripening have been found between light-excluded grape bunches and bunches exposed to light (Sun *et al*., 2019).

Rescic *et al.* (2016) reported increased concentrations of gallic acid in Istrian Malvasia grapes harvested at 24 °Brix, with increased light in the fruit zone brought about by leaf removal/shoot thinning of grapevines planted in N-S (north-south) rows. Work by Tessarin *et al*. (2014) on grapevines planted in east-west (E-W) directions showed that caffeic acid concentrations in Uva Longanesi grapes were higher after 50 % defoliation compared to no defoliation treatment.

Martínez-Lüscher *et al.* (2014) found lower concentrations of kaempferol and quercetin in Tempranillo grapes from N-S orientated vines receiving reduced light due to artificial shading compared to low vigour vines. Friedel *et al.* (2015) reported that boxed-in Riesling grape bunches from vines planted in an E-W direction, resulted in an increase in rutin, quercitrin and isoquercetin. In a study by Downey *et al.* (2004), artificially shaded Shiraz grapes at harvest were lower in flavan-3-ol content than exposed bunches; however, the decrease in total flavan-3-ols during berry ripening was greater in exposed bunches, to the extent that the levels were virtually the same in shaded and exposed berries at harvest. Scrafidi *et al*. (2013) reported higher concentrations of total flavan-3-ols in exposed Grillo grapes at 21 °Brix and planted in N-S rows than in boxed-in grapes, whereas Cortell *et al.* (2005) found that (-)-epigallocatechin concentrations in Pinot noir grapes at harvest, planted in an E-W direction, were positively correlated to increased light in the canopy and fruit zone.

Light intensity and light quality (UV-B and blue light) have been associated with the regulation of anthocyanin biosynthesis (Jaakola, 2013). Shiraz and Pinot noir grape bunches that develop in low vigour vines, as opposed to those that develop in high vigour vines, have increased concentrations of berry skin anthocyanins (Downey *et al*., 2004; Cortell *et al*., 2007). According to Joscelyne *et al*. (2007) and Ristic *et al*. (2007), artificially shaded Syrah grapes of vines planted in an E-W direction had lower concentrations of total anthocyanins than grape bunches from low vigour vines. Feng *et al.* (2015) reported that increased light brought about by 50 % leaf removal of Pinot noir N-S planted vines, resulted in an increase in total anthocyanins, compared to no leaf removal treatment. Martínez-Lüscher *et al.* (2014) found that reduced light (artificial shading) in the canopy of Tempranillo grapevines planted in a N-S direction, resulted in an increase in acylated malvidin-glucosides in grapes at 23 °Brix. Fernandes-De Oliveira and Nieddu (2016) reported higher concentrations of delphinidin- and petunidin 3-O-(6-O-p-coumaroyl) glucosides in artificially shaded (reduced UV-radiation) Bovale Grande and Cannonau grapes from N-S orientated vines than naturally exposed grapes.

In the cited literature, flavonols, flavan-3-ols and phenolic acids generally increased in grapes of vines exposed to light due to defoliation, whereas artificial shading or high vigour vines resulted in a decrease in these phenolics. Increased light due to defoliation also resulted in an increase in total anthocyanins, but a decrease in certain monomeric anthocyanin glucosides and anthocyanin glucoside coumaroylates.

Internationally, work on the effect of grapevine row orientation under realistic field conditions has not been published, and in South Africa, only limited investigations have been undertaken on the effect of row orienation per se on phenolics. It was demonstrated that, despite total anthocyanins and phenolics in Syrah grapes from rows with different orientations being relatively similar (with highest values found for the NW-SE (northwest-southeast) rows), wooded and unwooded wines produced from E-W orientated vines contained the lowest concentrations (Hunter and Volschenk, 2017).

The aim of this study was to investigate the effect of microclimate induced by different grapevine row orientations under realistic field conditions on the concentrations of selected individual anthocyanins, flavan-3-ols, flavonols, and phenolic acids in Syrah grapes, harvested at three grape ripeness levels over four consecutive seasons.

MATERIALS AND METHODS

1. Vineyard layout and management

An experimental vineyard was established in 2003 (Figure 1). The first full harvest was carried out five years after the establishment of the vineyard. The vineyard was already in full production when this experiment was undertaken. Syrah, clone SH 9C was grafted onto 101-14 Mgt rootstock. The vineyard was established on a flat terroir with clayey loam soil on the research farm of the Agricultural Research Council (ARC) in the Breede River Valley, Robertson (33.8021° S, 19.8875° E, 187 metres above sea level), South Africa (Hunter *et al.*, 2016). Row and vine spacing were 2.7 m and 1.8 m respectively. The vines were trained to a vertical shoot positioning (VSP) trellis system. A cover crop of rye grass was sown after harvest (April) and treated with herbicide prior to bud burst (October). The grapevine canopies consisted of three to four leaf layers, side by side. The leaf layers were measured using the point quadrat method (Hunter *et al*., 2016). Grapevines were subject to supplementary irrigation every seven days.

The experimental design was a randomised complete block (Figure 1) with four different row orientations (i.e., N-S, E-W, NE-SW and NW-SE) replicated at random in each of five experimental blocks with a total surface area of 1860 m2. An experimental unit consisted of all the grapevines within a vineyard block of a specific row orientation within the experimental vineyard.

2. Temperature and radiation measurements

The methodology for the determination of ambient photosynthetic radiation profiles, berry pulp temperature profiles, seasonal canopy temperature and seasonal ambient/air temperature profiles is fully described in Hunter *et al.* (2016) and Hunter *et al.* (2021).

3. Grape samples

Sampling took place over four consecutive vintages (2008, 2009, 2010 and 2011) at three different ripeness levels; i.e., 22 °Brix, 24 °Brix and 26 °Brix. Syrah grapes in Robertson, South Africa, are normally harvested at 24 °Brix grape maturity for winemaking (Guidoni and Hunter, 2012). Part of the experimental design of this study was to include a grape ripeness level two degree below and two degrees above the harvest ripeness level, hence 22 °Brix and 26 °Brix.

FIGURE 1. Aerial view of different grapevine row orientations indicating experimental replicate blocks (1-5). 1 = East-west (low light penetration in the morning and afternoon in the canopy); 2 = North-south (high light penetration in the morning and afternoon in the canopy); 3 = Northwest-southeast (low light penetration in the afternoon and high light penetration in the morning in the canopy); 4 = Northeast-southwest (low light penetration in the morning and high light penetration in afternoon in the canopy).

Individual grape berries (100) were collected from the top, middle and bottom of the bunches before midday. Five subsamples of 100 berries each per experimental unit were selected to ensure sufficient material for further processing.

3.1 Grape berry preparation

The grape skins of each 100-berry sample were separated from the grape flesh and seeds by hand. The skins were rinsed with distilled water and blotted with laboratory absorptive paper. The skins were freeze-dried (Christ Beta 1-8 LSCbasic, Freeze-drier, 336 Osterode/Harz, Germany) and stored in airtight containers at 4 °C until required for analysis.

3.2. Extraction procedure

A method described by Castillo-Muñoz *et al*. (2007) was used for the extraction of phenolics. The freeze-dried grape skins were ground prior to extraction. Separate extractions and analyses were done for each experimental unit from the five block replicates. A volume of 30 mL methanol/formic acid/water (50:48.5:1.5) was added to 1.0 g of freeze-dried ground grape skins (n = 5). Extraction was performed in a dark room using a Variomag Poly15 Electronicrührer (Avantor, Inc. Randor, Pennsylvania, USA) stirring for 20 min at 25 °C at a rate of 450 rpm. The samples were subsequently centrifuged using a Hettich® ROTANTA 460/460R centrifuge (Merck KgaA, Darmstadt, Germany) at 1000 x g for 15 min. The supernatant was filtered through a 0.45 µm nylon membrane syringe filter [Microsep, (Pty), Ltd, Johannesburg, South Africa] prior to high performance liquid chromatographic (HPLC) analysis.

4. Reagents

All solvents and acids used were of analytical grade (Merck, Johannesburg, South Africa). De-ionised water was supplied through a Modulab® water purification system (Separations Scientific, Johannesburg, South Africa).

5. Chromatography

A reversed-phase HPLC-diode array detection (RP-HPLC-DAD) method was used to separate, identify and quantify 23 target phenolics in the freeze-dried grape skin extracts. Quantification of anthocyanins, flavonols, flavan-3-ols and phenolic acids was performed on a SpectraSYSTEM HPLC instrument (Thermo Separations Products, Inc., New Jersey, USA) equipped with an auto-sampler (injection volume 20 µL), using a published method of Waterhouse *et al.* (1999). Detection was by means of photodiode array between 190 nm and 950 nm. ChromQuest™ software was utilised for data acquisition. Calibration curves for anthocyanins, flavan-3-ols, flavonols and phenolic acids were constructed by injection of standard reference sample solutions of different concentrations of individual phenolics (Table S1).

Calibration parameters and quantification of the target phenolics were performed using ChromQuest™ software. Separation was performed at 22 °C, using a polymer reversed-phase analytical column (PLRP-S 100 Å, 5 µm, 250 x 4.6 mm) with polystyrene divinylbenzene as stationary phase (Polymer Laboratories, Massachusetts, USA). Binary gradient elution was performed at 1 mL/min flow rate. The binary mobile phases comprised water/phosphoric acid [985:15 v/v (pH 1.35) - eluent A] and water/phosphoric acid/acetonitrile [185:15:800 v/v/v (pH 1.25) - eluent B]. The gradient programme is listed in Table 1. An analysis time of 90 min was preceded by a 20-min equilibration time. Anthocyanins were detected at 520 nm, flavan-3-ols at

280 nm, phenolic acids at 316 nm and flavonols at 360 nm. The identification of phenolics was confirmed by their relative retention times based on reference standards and UV-visible absorption characteristics. All reference standards and reagents used were of HPLC grade and supplied by Merck (Pty), Ltd and Sigma-Aldrich, Johannesburg, South Africa.

6. Statistics

Analysis of variance (ANOVA) per ripeness level was performed according to the experimental design, including the factors row orientation and vintage (repeated measures), on all variables assessed, using General Linear Models Procedure of SAS software (Version 9.4; SAS Institute Inc., Cary, USA). The Shapiro and Wilk test (Shapiro and Wilk, 1965) was performed to check for deviation from normality. Fisher's least significant difference test was calculated at a 5 % level to compare means for significant effects (Ott, 1998). A probability level of 5 % was considered significant for all tests. Averages, variances and standard deviations for phenolic data were determined using XLSTAT 2016 (Version 18.06, Addinsoft, New York, USA). Principal component analysis was applied to phenolic compound data for each ripeness level, also using XLSTAT, to elucidate associations between row orientation and vintage and phenolic compounds.

RESULTS

1. Seasonal temperature trends, berry temperature, air temperature and radiation in the canopy

Seasonal temperature trends, radiation profiles, berry pulp temperature profiles and air temperature relevant to this investigation have been fully discussed in Hunter *et al.* (2016) and Hunter *et al*. (2021). Average ambient temperatures (canopy) increased from approx. mid-December (around green pea-size) and maximum temperature frequently reached more than 30 °C onwards (Hunter *et al*., 2016). Micro-temperature profiles inside the canopy were not notably different among rows, except for generally slightly lower and slightly higher morning temperatures of E-W and N-S rows respectively (Hunter *et al*., 2016).

Berry pulp temperature increased during the late ripening stage from morning to afternoon and varied by approx. 3-8 °C (Table 2) (Hunter *et al*., 2016). The largest differences among row orientations were recorded in the morning and afternoon. At midday, average berry temperatures per orientation were, however, similar.

The lowest average temperature is reported for E-W rows, being 1-1.4 °C lower than N-S, NE-SW and NW-SE rows, but still more than 2 °C higher than air temperature. In general, N-S rows resulted in grapes gaining heat in the morning, as well as the afternoon. This was also the scenario for NE-SW rows, but there was generally more heat gain during the afternoon. An E-W row mostly mitigated berry heat gain during the season. This also occurred for NW-SE rows, but with higher gains in the morning.

Depending on the month of the season, radiation outside the canopies ranged from 1400 to 1700 µmol m-2/s for all row orientations (Hunter *et al*., 2016). For all row orientations, the highest radiation inside the canopies was received during the first and second months of measurement at the beginning of the season (approx. berry set to pea berry size stage) when the canopies were not yet completely settled. Grapevines planted in E-W rows received a maximum photosynthetic active radiation (PAR) only at midday, peaking - depending on the month of the season - at ca. 45 - 1050 µmol m- 2/s inside the canopy (Hunter *et al*., 2016). During the last four months of measurement, the interior-canopy radiation reached 40 µmol m-2/s just after midday. The N-S rows received maximum PAR twice a day, peaking - depending on the month of the season - at ca. 600 µmol m- 2/s and 1050 µmol m- 2/s inside the canopy during late morning and late afternoon (highest values in the beginning of the season) (Hunter *et al*., 2016). These rows may be considered to cause a uniform light distribution in the canopy. The N-S rows maintained a higher interior canopy light interception in the morning and afternoon compared to E-W rows. During the last four months of measurement, the interior-canopy radiation consistently reached ca. 60 µmol m-2/s in the afternoon. The NE-SW rows received maximum PAR twice a day during mid-morning and afternoon (highest values in the beginning of the season), peaking - depending on the month of the season - at ca. 15 µmol m-2/s and 1050 µmol m-2/s radiation inside the canopy (Hunter *et al.*, 2016). During the last four months of

TABLE 1. Gradient programme used for the RP-HPLC-DAD separation of phenolics (flow rate 1 mL/min).

Time (min)	Eluent A composition (%)	Eluent B composition (%)
0	94.00	6.00
73	69.00	31.00
78	38.00	62.00
86	38.00	62.00
90	94.00	6.00

measurement, the interior-canopy radiation reached ca. 75 µmol m-2/s during the afternoon. Grapevines planted in a NW-SE direction also received maximum PAR twice a day during mid-morning and the afternoon (highest values at the beginning of the season), peaking - depending on the month of the season - at ca. 30 µmol m-2/s and 700 µmol m-2/s, measured inside the canopy (Hunter *et al*., 2016). During the last four months of measurement, the interior-canopy radiation reached ca. 60 µmol m-2/s during the morning.

2. Grape yield

Grape yield depends on factors such as climate, soil, grape cultivar/rootstock combination and cultivation conditions (Hunter *et al*., 2017). Light penetration within the canopy, which depends on the trellis system type, vine vigour, canopy density and management, PAR and water relations, has an effect on vine metabolism and biochemical processes and ultimately an effect on grape yield (Hunter *et al*., 2017). In this study, all the above factors were kept constant, except for PAR as it was altered by row orientation. Average grape yields for the four respective years of the experiment (2007/08–2010/11) at ripeness level 1 were 24.8, 16.4, 25.3 and 17.0 (N-S); 24.8, 13.7, 25.5 and 14.3 (E-W); 22.5, 15.3, 22.6 and 14.9 (NE-SW); and 23.7, 13.1, 26.5 and 15.0 (NW-SE) tonnes per hectare. At ripeness level 2, the yields were 23.0, 12.3, 25.3 and 16.3 (N-S); 21.9, 13.0, 23.0 and 15.1 (E-W); 21.9, 11.7 24.2 and 13.2 (NE-SW); and 21.8, 11.7, 20.8 and 14.6 (NW-SE) tonnes per hectare; whereas at ripeness level 3 the yields were 22.6, 12.2, 21.4 and 12.3 (N-S); 21.6, 10.5, 22.3 and 12.8 (E-W); 20.4, 10.1, 18.8 and 10.1 (NE-SW); and 20.9, 11.2, 21.3 and 10.8 (NW-SE) tonnes per hectare.

Average yields over the four years and three ripeness levels were 19.03, 18.23, 17.13 and 17.63 tonnes per hectare for the N-S, E-W, NE-SW and NW-SE orientations respectively. The E-W rows showed the lowest yield losses from low to high ripeness level (Hunter et al., 2017). A decreasing trend in grape yield during the last stages of ripening (> 25 °Brix) is commonly observed because of grape concentration decrease in berry mass and volume when grape ripening progresses after sugar accumulation peaks (Carlomagno *et al*., 2018). Hunter *et al.* (2017) showed that the N-S row orientation was more desirable in terms of yielding ability and stability. According to De Souza *et al.* (2019) row orientation of Syrah vines had a greater effect on grape composition than on grape yield. Considering yield differences between row orientations, Hunter and Volschenk (2017) confirmed that yield was a weak driver (if at all) of wine style/quality and that sensory profiles were rather driven by a combination of environmental (most likely mainly at microclimate level - radiation impacting on berry temperature and berry metabolism) and physiological factors; berry chemical/biochemical compounds involved in respiratory metabolism; and products of secondary metabolism that responded to radiation and temperature.

3. Analysis of variance p-values (2-factor ANOVA) for row orientation and vintage main effects and interaction to determine which effects are statistically significant

Row orientation by vintage interaction at 22 °Brix (Table 3) was not statistically significant for most compounds, except total phenolic acids, caffeic acid, ferulic acid, kaempferol, quercitrin, cyanidin 3-O-glucoside, petunidin 3-O-glucoside, petunidin 3-O-(6-O-acetyl) glucoside, delphinidin 3-O-(6-O-p-coumaroyl) glucoside and petunidin 3-O-(6-O-p-coumaroyl) glucoside. For row orientation by vintage interaction at 24 °Brix (Table 3), gallic acid, p-coumaric acid, isoquercetin, total anthocyanins, petunidin 3-O-glucoside, malvidin 3-O-glucoside, malvidin 3-O-(6-O-acetyl) glucoside and petunidin 3-O-(6-O-p-coumaroyl) glucoside were statistically significant. At 26 °Brix (Table 3), row orientation by vintage interaction was statistically significant for total flavan-3-ols, (+)-catechin, EGCG, petunidin 3-O-glucoside, peonidin 3-O-glucoside, delphinidin 3-O-glucoside, petunidin 3-O-(6-O-

TABLE 2. Average berry pulp temperature (°C) for each canopy during grape ripening of Syrah grapes planted in four different row orientations (hand-held temperature probe measurements) (values represent averages of four consecutive seasons).

Table	Average berry pulp temperature (°C)			Average berry temp per day
	Measured at 10:00 am	Measured at 13:00 pm	Measured at 16:00 pm	
N-S	29.6a	31.9a	34.6b	32.0a
E-W	27.3b	32.3a	32.4c	30.6b
NE-SW	27.6b	32.3a	35.4a	31.7a
NW-SE	29.3a	32.3a	33.3bc	31.6a
$p = 0.05$	1.2	1.0	1.4	0.7

Average ambient (air) temperature at 10:00 am, 13:00 pm and 16:00 pm = 22.8 °C, 29.5 °C and 32.3 °C respectively.

acetyl) glucoside, peonidin 3-O-(6-O-acetyl) glucoside, delphinidin 3-O-(6-O-p-coumaroyl) glucoside, petunidin 3-O-(6-O-p-coumaroyl) glucoside and malvidin 3-O-(6-O-p-coumaroyl) glucoside.

4. Phenolic compound concentration means for vintage main effects

The following results (means) are for compounds that were statistically significantly affected by the main effect only; i.e., vintage (Table 4). Certain compounds at 22 °Brix (Table 4) in 2008 and 2009 had generally lower concentrations of phenolics than in 2010 and 2011. The exception was gallic acid, for which the lowest levels were recorded in 2011 (low yield). Total flavan-3-ols and total anthocyanins were the highest in 2011 but were not different in 2010 (high yield), whereas malvidin 3-O-glucoside was the highest in 2011.

A vintage effect on phenolic compounds at 24 °Brix (Table 4) was not found, except for total flavan-3-ols in 2008 (high yield), which were lower than in 2009, 2010 and 2011. Epicatechin (-) was highest in 2010 (high yield). Statistically significant differences among vintages for the remaining compounds were not found. At 26 °Brix, only rutin was highest in 2011 (low yield) (Table 4).

5. Phenolic compound concentration means for row orientation main effects

The following results (Table 5) are for compounds statistically significantly affected by main effects only; i.e., row orientation (Table 3).

Total flavan-3-ols, (+)-catechin, gallic acid, total flavonols, and peonidin 3-O-glucoside the were lowest from the N-S rows at 22 °Brix (Table 5), but the highest concentrations of total flavonols, isoquercetin and total anthocyanins were found in grapes from NE-SW rows with peonidin 3-O-glucoside the highest in the NW-SE rows. The lowest p-coumaric acid and malvidin 3-O-(6-O-acetyl) glucoside were recorded from the NW-SE rows. Generally, the lowest phenolic concentrations were from the N-S rows. The majority of phenolics quantified were lowest in grapes form N-S rows.

Ferulic acid, EGCG, total flavonols, quercetin and kaempferol were lowest from the N-S rows at 24 °Brix (Table 5) and (+)-catechin was highest from the N-S rows. The highest levels of total phenolic acids, caffeic acid and total flavonols were found in grapes at 24 °Brix from the NW-SE rows, whereas kaempferol and quercitrin were highest from the E-W rows (Table 5). Concentration differences were evident for total phenolic acids and caffeic acid among rows with lowest levels from the NE-SW rows. Cyanidin 3-O-glucoside was lowest in grapes at 24 °Brix (Table 5) from the E-W rows.

Statistically significant differences in malvidin 3-O-(6-O-coumaroyl) glucoside were not found among any of the row orientations for grapes at 24 °Brix (Table 5). Similar to grapes at 22 °Brix, most phenolics quantified at 24 °Brix were lowest from the N-S rows.

Total phenolic acids, gallic acid, caffeic acid, total flavonols, kaempferol, quercitrin and malvidin 3-O-(6-O-acetyl) glucoside were highest from the NW-SE rows at 26 °Brix (Table 5). Statistically significant differences in caffeic acid and kaempferol among all row orientations were evident. Caffeic- and p-coumaric acid were lowest from the NE-SW rows, whereas ferulic acid was highest in grapes from E-W rows. Ferulic acid was not significantly different among the remaining rows.

Isoquercetin and quercetin were both highest from the NE-SW rows at 26 °Brix (Table 5). Malvidin 3-O-glucoside was highest from the N-S rows with no statistically significant differences among the E-W, NE-SW and NW-SE rows, whereas malvidin 3-O-(6-O-acetyl) glucoside was highest from the NW-SE rows. No statistical significant differences among the E-W, N-S and NE-SW rows for malvidin 3-O-(6-O-acetyl) glucoside were found. The majority of phenolics of this study quantified in grapes at 26 °Brix were highest from the NW-SE rows.

Generally, caffeic acid was lowest from the NE-SW rows at 24 °Brix and 26 °Brix, whereas total flavonols and kaempferol lowest from the N-S rows at 22 °Brix and 24 °Brix and 24 °Brix and 26 °Brix (Table 5). Highest total phenolics, caffeic acid and total flavonols were found in grapes from the NW-SE rows at 24 °Brix and 26 °Brix and isoquercetin was highest from the NE-SW rows at both 22 °Brix and 26 °Brix. NW-SE (low light exposure in the morning) was found to lead to increased concentrations of most phenolics measured at 24 °Brix and 26 °Brix ripeness.

6. Principal component analysis

The PCA biplot (Figure 2) of the first two principal components (PC1 and PC2) illustrates the association of phenolic compounds at 22 °Brix in 2008, 2009, 2010 and 2011 with the N-S, E-W, NE-SW and NW-SE rows, explaining 59.42 % of the variation in the data. The major source of variation is row orientation with PC1 separating the NE-SW row from the E-W, N-S and NW-SE rows, while PC2 separates the NW-SE row from the E-W and N-S rows. Variables with the highest squared cosine values (data not shown) on PC1 are (+)-catechin, caffeic acid, ferulic acid, total flavonols, isoquercetin, quercetin, kaempferol, total anthocyanins, petunidin 3-O-glucoside, malvidin 3-O-glucoside, delphinidin 3-O-(6-O-p-coumaroyl) glucoside and petunidin 3-O-(6-O-p-coumaroyl) glucoside. These variables were positively associated with the NE-SW rows.

Flavan-3-ols, epigallocatechin 3-O-gallate, total phenolic acids, p-coumaric acid, peonidin 3-O-(6-O-acetyl) glucoside and malvidin 3-O-(6-O-acetyl) glucoside had the highest squared cosine values on PC2. The NW-SE rows were positively associated with flavan-3-ols, but negatively associated with total phenolic acids.

Principal component 3 explains only an additional 13.85 % of variation (data not shown) and does not improve interpretability; it was therefore not included in the biplot. Only quercitrin, (-)-epicatechin and peonidin

TABLE 3. Analysis of variance p-values (2-factor ANOVA) for row orientation and vintage main effects and interaction for Syrah grapes at 22 °Brix, 24 °Brix and 26 °Brix to determine which effects are statistically significant.

Phenolic compounds	22 °Brix			24 °Brix			26 °Brix		
	Main effects		Interaction	Main effects		Interaction	Main effects		Interaction
	Row orientation	Vintage	Row orientation x vintage	Row orientation	Vintage	Row orientation x vintage	Row orientation	Vintage	Row orientation x vintage
Total flavan-3-ols	< 0.0001	0.0009	0.4099	0.0005	0.0143	0.5144	< 0.0001	0.2066	0.0284
(+)-Catechin	0.0015	0.2171	0.8429	< 0.0001	0.1517	0.9102	< 0.0001	0.0006	0.0137
(-)-Epicatechin	0.0037	0.0501	0.3766	0.1870	< 0.0001	0.1582	0.3470	0.2872	0.6771
EGCG[1]	< 0.0001	0.0027	0.1352	< 0.0001	0.4110	0.5803	0.0008	0.9208	0.0469
Total phenolic acids	< 0.0001	0.0064	0.0015	< 0.0001	0.7496	0.8200	< 0.0001	0.0636	0.4158
Gallic acid	0.0025	0.0039	0.4090	< 0.0001	0.0055	0.0078	< 0.0001	0.3873	0.1293
Caffeic acid	< 0.0001	< 0.0001	0.0005	< 0.0001	0.9951	0.9502	< 0.0001	0.0838	0.4907
p Coumaric acid	< 0.0001	0.5169	0.1445	< 0.0001	0.5285	0.0526	< .00001	0.0158	0.4288
Ferulic acid	< 0.0001	0.5144	0.0042	< 0.0001	0.5399	0.5249	0.0007	0.4721	0.8558
Rutin	0.3820	0.0341	0.3493	0.0228	0.0468	0.5053	0.0180	0.0116	0.3489
Total flavonols	< 0.0001	0.8483	0.0656	< 0.0001	0.2568	0.1704	< 0.0001	0.1640	0.4008
Isoquercetin	< 0.0001	0.4161	0.1723	< 0.0001	0.0140	0.0023	< 0.0001	0.0091	0.4195
Quercetin	< 0.0001	0.0605	0.2818	< 0.0001	0.2752	0.4879	0.0079	0.0503	0.0737
Kaempferol	< 0.0001	0.0895	0.0393	< 0.0001	0.1663	0.5133	< 0.0001	0.6634	0.4476
Quercitrin	< 0.0001	0.7451	0.0152	< 0.0001	0.3409	0.1366	< 0.0001	0.5543	0.4541
Total anthocyanins	0.0012	0.0010	0.6308	0.0023	0.0002	0.0374	0.9236	0.0006	0.1170
CyGluc[2]	< 0.0001	0.6716	< 0.0001	0.0020	0.0530	0.2107	0.0085	0.4473	0.4327
PetGluc[2]	< 0.0001	< 0.0001	0.0021	0.0003	0.0001	0.0002	< 0.0001	0.6105	< 0.0001
PeoGluc[2]	0.0017	0.0822	0.6622	0.2300	0.1910	0.5479	0.0070	0.0007	0.0351
MalGluc[2]	0.0051	< 0.0001	0.6973	0.0232	< 0.0001	0.0238	< 0.0001	0.0038	0.8793
DelGluc[2]	0.6010	0.0781	0.5539	0.1015	< 0.0001	0.1731	0.0111	0.0001	0.0304
PetGlucAc[3]	0.0005	0.2785	< 0.0001	0.2054	0.0423	0.9217	< 0.0001	0.2598	< 0.0001
PeoGlucAc[3]	0.0487	0.3406	0.5767	0.2725	< 0.0001	0.5022	0.0005	0.0167	< 0.0001
MalGlucAc[3]	0.0241	0.0887	0.2734	0.0249	0.0002	0.0175	0.0005	0.5906	0.8032
DelGlucCoum[4]	0.0017	0.0050	0.0216	0.0672	0.0333	0.9756	< 0.0001	0.0114	< 0.0001
PetGlucCoum[4]	< 0.0001	0.6020	< 0.0001	< 0.0001	0.0004	< 0.0001	< 0.00001	0.0042	< 0.0001
MalGlucCoum[4]	0.0623	0.2131	0.4000	0.0280	0.0828	0.2584	0.0008	< 0.0001	0.0216

[1]Epigallocatechin 3-O-gallate; [2]Cyanidin-, petunidin-, peonidin-, malvidin- and delphinidin 3-O-glucoside; [3]Petunidin-, peonidin- and malvidin 3-O-(6-O-acetyl) glucoside; [4]Delphinidin-, petunidin- and malvidin 3-O-(6-O-p-coumaroyl) glucoside.

TABLE 4. Phenolic compound concentration (mg/L) means for vintage (2008, 2009, 2010 and 2011) main effects only at 22 °Brix, 24 °Brix and 26 °Brix.

	Vintage	2008	2009	2010	2011	*p*-value
	*Ambient min/max Temp	15.4-29.5 °C	15.0-29.7 °C	15.5-30.5 °C	16.7-31.0 °C	
			22 °Brix			
Phenolics	Total flavan-3-ols	**11.820 ± 1.29c	12.137 ± 1.38bc	12.403 ± 1.34ab	12.713 ± 1.04a	0.0009
	(-)-Epicatechin	4.958 ± 0.18b	5.022 ± 0.33ab	5.202 ± 0.39a	5.199 ± 0.39a	0.0501
	EGCG[1]	1.596 ± 0.91b	1.666 ± 1.16b	1.890 ± 0.95a	1.941 ± 0.83a	0.0027
	Gallic acid	1.510 ± 0.29a	1.680 ± 0.39a	1.515 ± 0.27a	1.313 ± 0.35b	0.0039
	Rutin	0.652 ± 0.12a	0.585 ± 0.15ab	0.514 ± 0.18b	0.657 ± 0.13a	0.0341
	Total anthocyanins	372.115 ± 59.87c	375.960 ± 60.70bc	411.303 ± 57.41ab	439.961 ± 69.47a	0.0010
	Malvidin[2]	80.369 ± 18.65c	87.711 ± 26.64bc	95.139 ± 16.02b	120.428 ± 25.48a	< 0.0001
			24 °Brix			
Phenolics	Total flavan-3-ols	**12.387 ± 0.66b	12.811 ± 0.77a	13.157 ± 0.65a	12.897 ± 0.58a	0.0143
	(-)-Epicatechin	5.005 ± 0.29b	5.072 ± 0.29b	5.670 ± 0.39a	5.119 ± 0.39b	< 0.0001
	Rutin	0.685 ± 0.07ab	0.669 ± 0.09ab	0.723 ± 0.15a	0.635 ± 0.07b	0.0468
	DelGluc[3]	1.765 ± 0.22c	2.156 ± 0.38ab	1.971 ± 0.31bc	2.351 ± 0.29a	< 0.0001
	PetGlucAc[4]	2.884 ± 0.43b	3.185 ± 0.37ab	3.316 ± 0.67a	3.368 ± 0.34a	0.0423
	PeoGlucAc[4]	10.201 ± 1.42b	12.493 ± 1.85a	11.270 ± 1.55b	13.179 ± 1.56a	< 0.0001
	DelGlucCoum[5]	6.841 ± 1.38b	7.333 ± 1.25ab	8.014 ± 1.36a	7.685 ± 1.45ab	0.0333
			26 °Brix			
Phenolics	*p* Coumaric acid	**24.071 ± 3.96ab	24.059 ± 3.89ab	25.346 ± 4.92a	23.588 ± 3.75b	0.0158
	Rutin	0.589 ± 0.06b	0.594 ± 0.06b	0.613 ± 0.07b	0.666 ± 0.05a	0.0116
	Isoquercetin	2.406 ± 1.01bc	2.713 ± 1.01a	2.622 ± 1.01ab	2.198 ± 0.78c	0.0091
	Quercetin	7.291 ± 1.19a	6.642 ± 1.29ab	6.440 ± 1.12b	6.362 ± 1.16b	0.0503
	Total anthocyanins	334.007 ± 34.54c	350.910 ± 39.76bc	360.768 ± 30.63ab	380.778 ± 40.48a	0.0006
	Malvidin[2]	73.751 ± 17.24c	86.864 ± 19.58ab	82.221 ± 15.83bc	92.965 ± 18.46a	0.0038

*Average ambient temperatures for January, February and March (harvest time). **Means given for compounds with significant vintage main effects as indicated in Table 3. Different letters in the same row indicate significant differences in the content of the measured variables among the different row orientations according to Fischer's least significant difference test ($p = 0.05$). [1]Epigallocatechin 3-O-gallate; [2]Malvidin 3-O-glucoside; [3]Delphinidin 3-O-glucoside; [4]Petunidin- and Peonidin 3-O-(6-O-acetyl) glucoside; [5]Delphinidin 3-O-(6-O-p-coumaroyl) glucoside.

TABLE 5. Phenolic compound concentration (mg/L) means for row orientation main effects only at 22 °Brix, 24 °Brix and 26 °Brix.

Table 5 (part 1/3)

Phenolics	Row orientation				*p*-value
	E-W[1]	NE-SW[1]	N-S[1]	NW-SE[1]	
	22 °Brix				
Total flavan-3-ols	*11.401 ± 0.84b	13.199 ± 0.52a	11.008 ± 0.79c	13.404 ± 0.72a	< 0.0001
(+)-Catechin	5.386 ± 0.45a	5.588 ± 0.35a	5.100 ± 0.50b	5.529 ± 0.48a	0.0015
(-)-Epicatechin	5.230 ± 0.40a	4.986 ± 0.35b	4.931 ± 0.14b	5.214 ± 0.32a	0.0037
EGCG[2]	0.784 ± 0.34b	2.625 ± 0.35a	0.976 ± 0.35b	2.660 ± 0.37a	< 0.0001
Gallic acid	1.504 ± 0.24a	1.669 ± 0.36a	1.173 ± 0.35b	1.621 ± 0.23a	0.0025
p Coumaric acid	31.723 ± 4.53a	25.671 ± 3.62b	29.117 ± 5.47ab	16.031 ± 2.11c	< 0.0001
Total flavonols	16.056 ± 1.44b	24.302 ± 2.10a	13.032 ± 1.38c	15.534 ± 1.28b	< 0.0001
Isoquercetin	1.711 ± 0.29c	3.095 ± 0.47a	1.569 ± 0.25c	2.693 ± 0.40b	< 0.0001
Quercetin	5.041 ± 0.79a	4.065 ± 1.95b	5.419 ± 0.90a	4.600 ± 0.64ab	< 0.0001
Total anthocyanins	407.968 ± 67.24b	452.845 ± 70.78a	387.571 ± 46.59bc	352.615 ± 41.01c	0.0012
PeoGluc[3]	14.802 ± 2.68ab	14.159 ± 2.07b	11.221 ± 2.18c	16.286 ± 4.64a	0.0017
MalGluc[3]	101.381 ± 29.37ab	113.582 ± 28.18a	86.223 ± 19.71bc	84.368 ± 19.97c	0.0051
PeoGlucAc[4]	11.203 ± 2.46a	10.909 ± 2.07a	10.429 ± 1.52ab	9.242 ± 1.47b	0.0487
MalGlucAc[4]	57.721 ± 11.69a	60.936 ± 16.43a	56.496 ± 13.93a	43.638 ± 12.06b	0.0240

Table 5 (part 2/3)

Phenolics	Row orientation				*p*-value
	E-W[1]	NE-SW[1]	N-S[1]	NW-SE[1]	
	24 °Brix				
Total flavan-3-ols	*12.475 ± 0.57b	13.209 ± 0.74a	12.477 ± 0.61b	13.102 ± 0.59a	0.0005
(+)-Catechin	5.121 ± 0.26c	5.452 ± 0.33b	5.774 ± 0.49a	5.214 ± 0.28c	< 0.0001
EGCG[2]	1.993 ± 0.42b	2.663 ± 0.32a	1.460 ± 0.16c	2.772 ± 0.33a	< 0.0001
Total phenolic acids	85.961 ± 6.62b	57.244 ± 3.23d	63.865 ± 5.10c	117.728 ± 6.84a	< 0.0001
Caffeic acid	48.391 ± 6.25b	15.036 ± 1.20d	24.648 ± 3.24c	83.658 ± 6.31a	< 0.0001
p Coumaric acid	28.301 ± 3.51b	34.413 ± 3.03a	34.723 ± 4.89a	25.084 ± 1.82c	< 0.0001
Ferulic acid	6.996 ± 1.12a	5.541 ± 1.21b	2.905 ± 0.75c	6.915 ± 0.91a	< .00001
Total flavonols	16.711 ± 2.06b	16.440 ± 1.33b	13.836 ± 1.94c	34.406 ± 2.72a	< .00001
Rutin	0.643 ± 0.10b	0.632 ± 0.11b	0.713 ± 0.09a	0.715 ± 0.09a	0.0228
Quercetin	5.921 ± 1.48a	6.012 ± 1.01a	5.358 ± 1.09b	5.976 ± 1.95a	< 0.0001
Kaempferol	1.569 ± 0.21a	1.304 ± 0.07b	0.726 ± 0.28c	1.317 ± 0.074b	< 0.0001
Quercitrin	7.226 ± 1.02a	6.531 ± 0.92b	6.286 ± 0.99b	4.572 ± 1.56c	< 0.0001
CyGluc[3]	1.964 ± 0.26c	2.288 ± 0.48b	2.654 ± 0.57a	2.406 ± 0.54ab	0.0020
MalGlucCoum[5]	15.276 ± 2.71bc	14.547 ± 2.33c	16.403 ± 2.90ab	16.871 ± 2.69a	0.0280

Table 5 (part 3/3)

Phenolics	Row orientation				*p*-value
	E-W[1]	NE-SW[1]	N-S[1]	NW-SE[1]	
	26 °Brix				
Total phenolic acids	*62.689 ± 4.05b	45.687 ± 5.90c	65.727 ± 4.22b	83.101 ± 5.83a	< .0001
Gallic acid	1.728 ± 0.24b	1.656 ± 0.32b	1.630 ± 0.24b	2.207 ± 0.34a	< .0001
Caffeic acid	29.942 ± 2.22c	18.491 ± 4.04d	31.803 ± 3.48b	48.986 ± 5.09a	< .0001
p Coumaric acid	27.207 ± 2.620a	18.567 ± 2.80b	25.866 ± 1.58a	26.007 ± 1.51a	< .0001
Ferulic acid	7.217 ± 1.01a	6.333 ± 1.02b	5.832 ± 0.57bc	5.266 ± 0.53c	0.0007
Total flavonols	16.921 ± 0.95c	19.041 ± 1.06b	16.530 ± 1.62c	27.448 ± 2.72a	< .0001
Rutin	0.593 ± 0.06b	0.637 ± 0.06a	0.594 ± 0.07b	0.633 ± 0.07a	0.0180
Isoquercetin	1.629 ± 0.26c	3.561 ± 0.57a	1.623 ± 0.26c	2.962 ± 0.56b	< .0001
Quercetin	6.102 ± 0.85b	7.330 ± 0.74a	6.658 ± 1.67b	6.558 ± 1.18b	0.0079
Kaempferol	1.682 ± 0.17b	1.403 ± 0.164c	1.219 ± 0.06d	1.993 ± 0.23a	< .0001
Quercitrin	7.510 ± 0.72b	6.745 ± 0.91c	7.029 ± 1.15bc	15.933 ± 1.60a	< .0001
CyGluc[3]	2.000 ± 2.00b	2.209 ± 2.20ab	2.324 ± 2.32a	2.426 ± 2.42a	0.0085
MalGluc[3]	78.425 ± 13.25b	74.599 ± 13.98b	103.821 ± 18.84a	78.957 ± 13.32b	< .0001
MalGlucAc[4]	65.654 ± 15.01b	60.996 ± 8.42b	63.239 ± 14.31b	86.945 ± 12.41a	0.0005

*Means given for compounds with significant row orientation main effects as indicated in Table 3. Different letters in the same row indicate significant differences in the content of the measured variables among the different row orientations according to Fischer's least significant difference test (p = 0.05). [1]East-West; [1]Northeast-Southwest; [1]North-South; [1]Northwest-Southeast. [2]Epigallocatechin 3-O-gallate; [3]Peonidin-, malvidin and cyanidin 3-O-glucoside; [4]Peonidin- and malvidin 3-O-(6-O-acetyl) glucoside; [5]Malvidin 3-O-(6-O-p-coumaroyl) glucoside.

3-O-glucoside had the highest squared cosine value on PC3. Close groupings of the same rows from different vintages may be an indication that the largest variation in phenolic content are between rows, while variation between vintages are less pronounced.

At 24 °Brix, the PCA biplot (Figure 3) of the first two principal components (PC and PC2) illustrates the association of phenolic compounds in 2008, 2009, 2010 and 2011 with the N-S, E-W, NE-SW and NW-SE rows, explaining 57.61 % of the variation in the data. In general, the different vintages and rows separated and grouped reasonably well.

The main source of variation is row orientation with PC1 separating NW-SE from E-W, NE-SW and N-S rows, while PC2 separates individual vintages within rows. Variables with the highest squared cosine values (≥ 0.5) on PC1 were EGCG, phenolic acids, caffeic acid, p-coumaric acid, ferulic acid, total favonols, quercetin, quecitrin and petunidin 3-O-glucoside. Phenolic acids and flavonols were mainly associated with the NW-SE rows.

Catechin (+), total anthocyanins, malvidin 3-O-glucoside, delphinidin 3-O-glucoside, peonidin 3-O-(6-O-acetyl) glucoside and malvidin 3-O-(6-O-acetyl) glucoside had the highest squared cosine values on PC2. Gallic acid, rutin and cyanidin 3-O-glucoside had the highest squared cosine values on PC3, but it explained only an additional 15.17 % of variation in the biplot. It does not improve interpretability and was therefore not included in the biplot.

At 26 °Brix, the PCA biplot (Figure 4) of the first two principal components (PC1 and PC2) illustrates the association of phenolic compounds in 2008, 2009, 2010 and 2011 with the N-S, E-W, NE-SW and NW-SE rows, explaining 54.75 % of the variation in the data. The different vintages and rows showed excellent separation and grouping. PC1 mainly separates NW-SE from the other rows, while PC2 separates NE-SW from N-S and E-W. The phenolic compounds with the highest squared cosine values (≥ 0.5) on PC1 were total flavan-3-ols, (+)-catechin, total phenolic acids, gallic acid, caffeic acid, ferulic acid, total flavonols, kaempferol, quercitrin and malvidin 3-O-(6-O-acetyl) glucoside. NW-SE rows were mainly associated with flavonols and phenolic acids. Epigallocatechin 3-O-gallate, p-coumaric acid, isoquercetin, quercetin and petunidin 3-O-(6-O-acetyl) glucoside have the highest squared cosine values on PC2 (data not shown).

Most anthocyanins were associated with E-W rows and flavan-3-ols with NE-SW. Peonidin 3-O-glucoside, malvidin 3-O-glucoside and malvidin 3-O-(6-O-coumaryol) glucoside have the highest squared cosine values on PC3, but they

contributed only an additional 13.76 % to the variability in the PCA biplot; PC3 does not improve interpretability and was therefore not included in the biplot.

DISCUSSION

The results show that row orientation and grape ripeness effects were detectable for individual phenolic compounds and phenolic classes. Generally, vintage effect was not evident among the phenolics quantified, except for gallic acid and malvidin 3-O-glucosides in 2011 (22 °Brix), epicatechin and total flavan-3-ols in 2008 and 2010 (24 °Brix) respectively, and rutin in 2011 (26 °Brix). Hunter *et al.* (2016) reported that climatic conditions among vintages were similar, reaching comparable maturity indices in all four seasons; therefore, the vintage factor was not taken into account and the grapes per ripeness level were considered as replications.

Ambient (air) temperature of the experimental vineyard during grape development never exceeded 35 °C (Hunter *et al.*, 2016). Ambient temperature between 30 °C and 35 °C is the critical temperature range for flavonoid and non-flavonoid biosynthesis (Tarara *et al.*, 2008; Bonada *et al.*, 2015). Temperatures above 35 °C can be detrimental to, for example, anthocyanin and flavonol accumulation, since they share the same flavonoid biosynthetic pathway (Bonada *et al.*, 2015).

Considering the relatively uniform vegetative characteristics of the canopy architecture (Figure 1), canopy interior temperature primarily reflected ambient (air) temperature at meso-level (Hunter *et al.*, 2017) and therefore temperature profiles inside the canopy did not show marked differences among the different rows. Canopy temperature per se consequently played a minor role in the final berry temperature of VSP-trellised vines of the different rows. Despite this, berry temperature and light penetration were slightly affected by the canopy orientation. Berry temperature of grapes from the E-W and NE-SW rows were slightly lower than grapes from the N-S and NW-SE rows in mid-morning (Table 2). However, at around midday, significant differences in temperature among the rows were not found. In late afternoon, grapes from the NE-SW rows had the highest temperature. This may be a consequence of the high levels

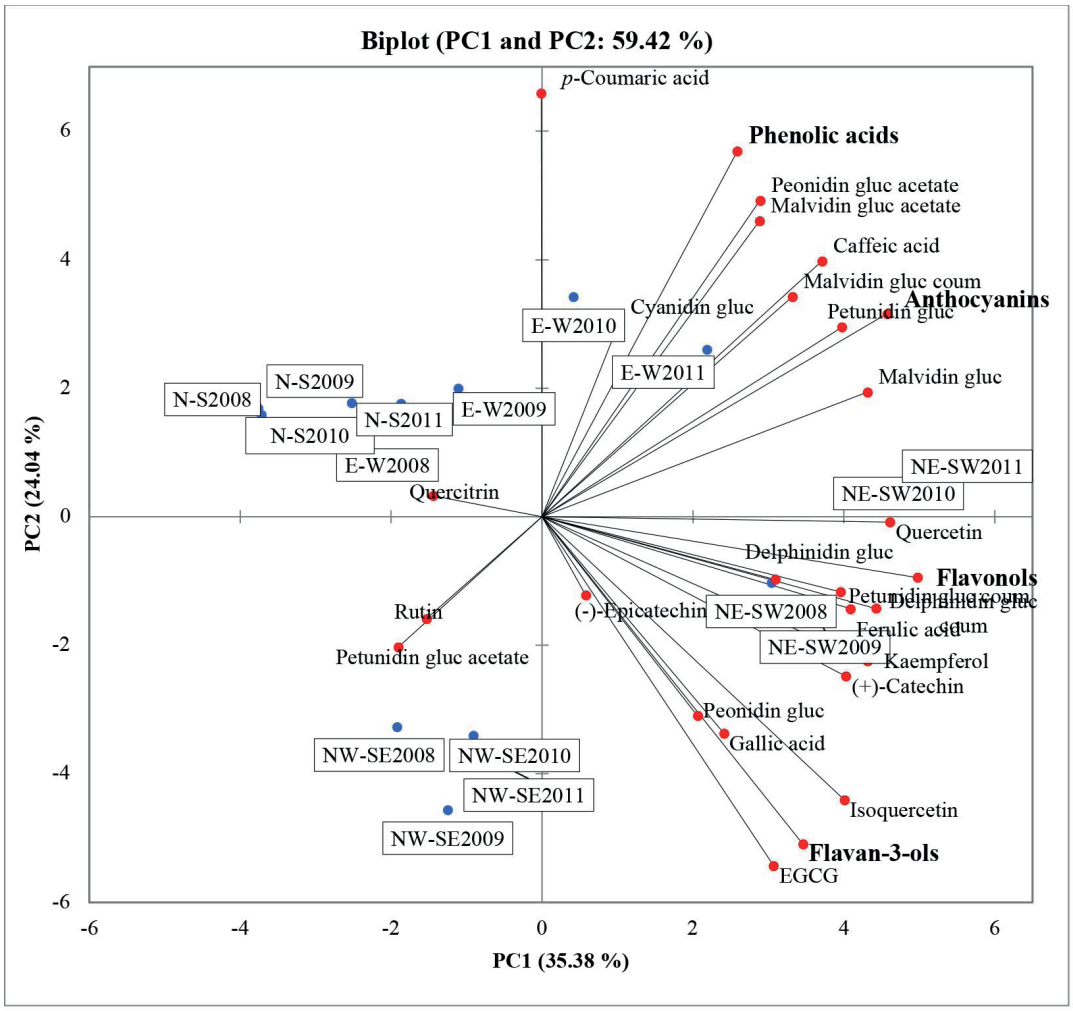

FIGURE 2. Principal component analysis bi-plot illustrating the association of 23 individual phenolic compounds measured in grapes harvested in 2008, 2009, 2010 and 2011 at 22 °Brix from N-S, E-W, NE-SW and NW-SE rows. Gluc = glucoside; Coum = coumaroyl; EGCG = Epigallocatechin 3-O-gallate.

measured for total flavonols, isoquercetin and total malvidin 3-O-glucosides at 22 °Brix and isoquercetin and quercetin measured at 26 °Brix. The respective berry temperatures of the N-S and E-W rows in the late afternoon were significantly different, but those of the N-S and NW-SE rows were not different. Nevertheless, on average over the four consecutive seasons, only grapes from the E-W rows were significantly lower in temperature (Table 2).

Beggs and Wellman (1994) reported that increased light exposure of grape berries, and not temperature, enhances anthocyanin biosynthesis. Downey *et al*. (2006) reported that increased grape bunch temperature can result in a decrease in total anthocyanin content, and Cagnasso *et al*. (2011) reported that cooler climates seem to favour anthocyanin accumulation in Carema grapes. Anthocyanins can increase at higher grape ripeness levels as reported by Guidoni and Hunter (2012), who showed that increased anthocyanin levels in Shiraz grapes is related to cooler temperatures and moderate light penetration under the control of photoreceptors during the final stages of ripening, which may prolong anthocyanin synthesis. Despite this, these flavonoids can also undergo polymerisation during the last ripening phase before harvest, leading to a decrease in anthocyanin levels. Therefore, separating the effects of temperature and light on grape composition is difficult because many of the biochemical pathways are light and temperature (solar radiation) sensitive (Spayd *et al*., 2002).

Anthocyanins reached their highest levels at 22 °Brix from the N-S rows, whereas flavonols and flavan-3-ols seemed to be the highest at 24 °Brix from the same row orientation (Minnaar, 2018). Anthocyanin concentrations tended to decrease as grape ripeness levels increased. Hunter *et al.* (2021) reported that anthocyanins in Shiraz grapes from N-S rows decreased as the grapes ripend. Phenolic acids were not substantially affected by grape ripeness levels but were highest at 24 °Brix from N-S rows.

For E-W rows, flavan-3-ols and phenolic acids reached their highest levels at 22 °Brix, after which a decrease occurred as the grapes ripened further (Minnaar, 2018). Jordão *et al.* (2001) reported flavan-3-ols of Castelão Francês

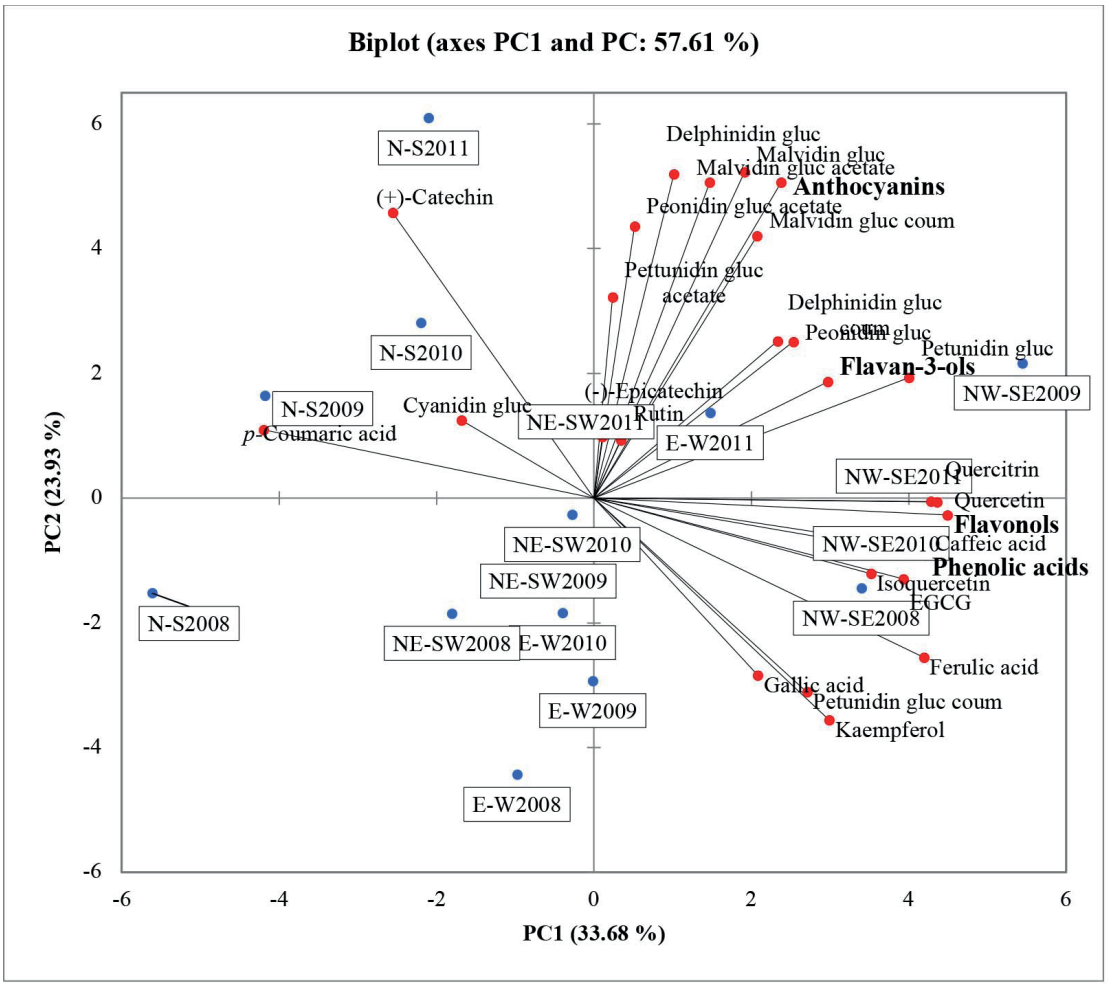

FIGURE 3. Principal component analysis bi-plot illustrating the association of 23 phenolic compounds measured in grapes harvested in 2008, 2009, 2010 and 2011 at 24 °Brix from N-S, E-W, NE-SW and NW-SE row orientations. Gluc = glucoside; Coum = coumaroyl; EGCG = Epigallocatechin-3-O-gallate.

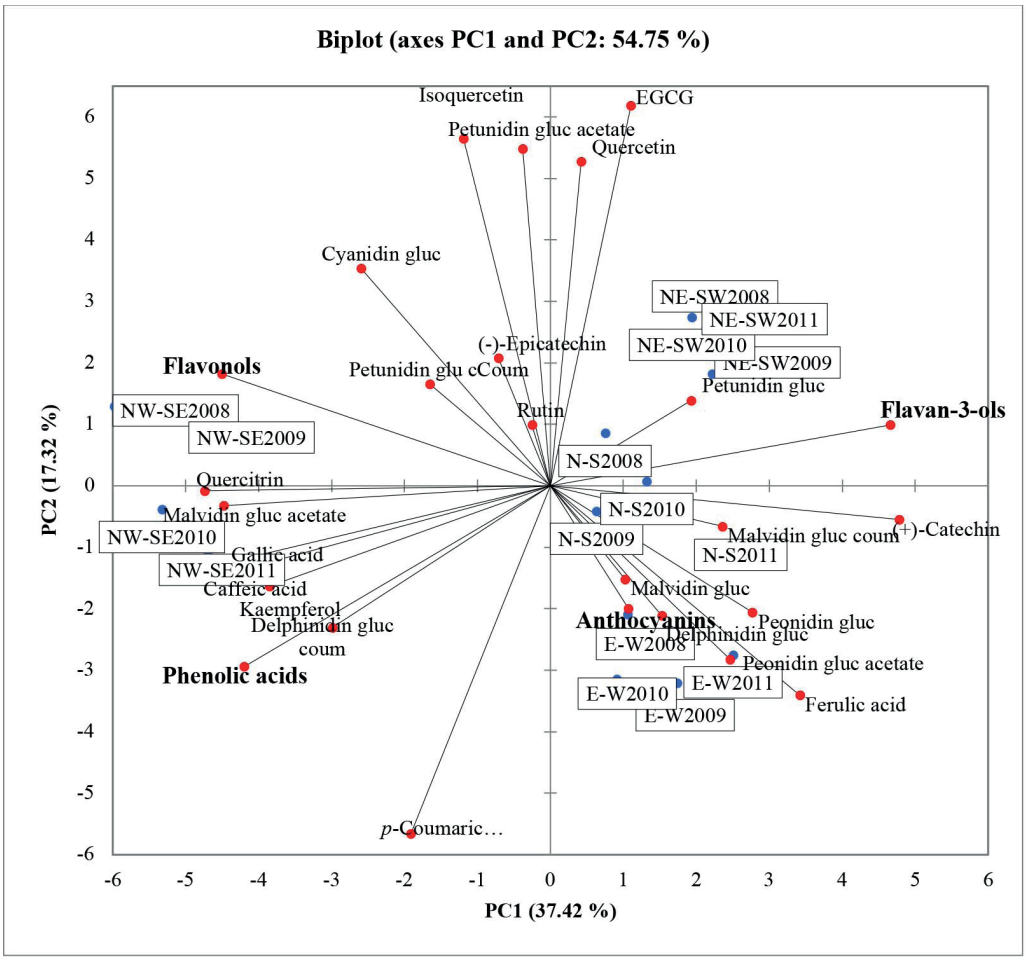

FIGURE 4. Principal component analysis bi-plot illustrating the association of 23 phenolic compounds measured in grapes harvested in 2008, 2009, 2010 and 2011 at 26 °Brix from the N-S, E-W, NE-SW and NW-SE rows. Gluc = glucoside; Coum = coumaroyl; EGCG = Epigallocatechin-3-O-gallate.

and Touriga Francesa grapes to decrease during the grape maturation process. Flavonols, on the other hand, appeared to be highest at a ripeness level of 24 °Brix. This is similar for grapes from N-S rows of this study. Anthocyanins tended to already be highest at 22 °Brix, after which a decrease occurred as the grapes ripened. This is similar to the findings for the grapes from N-S rows. The grapes from both the N-S and E-W rows tended to reach their highest levels of anthocyanins and flavonol at 22 °Brix and 26 °Brix respectively.

The NE-SW rows brought about only a slight variation in flavan-3-ol levels from 22 °Brix to 26 °Brix ripeness level. Flavonols and phenolic acids ostensibly reached their highest levels at 22 °Brix, after which a decrease occurred as the grapes ripened (Minnaar, 2018). Anthocyanins seemed to reach their highest levels at 22 °Brix, as was the case for the grapes from N-S and E-W rows.

Northwest-southeast rows were the cause of flavan-3-ols already reaching their highest levels at 22 °Brix, after which a decrease occurred as the grapes ripened. Flavonols, phenolic acids and anthocyanins tended to be highest at 24 °Brix. Grapes from NE-SW rows seemed to reach their highest anthocyanin, phenolic acid and flavonol levels before those from NW-SE rows, but they tended to reach their highest flavan-3-ol levels before those from the NE-SW rows (Minnaar, 2018). Certain treatments (rows) had no effect on phenolics (no main effects, p-values not significant) which means that row direction prevented sufficient UV-B radiation from entering the canopy, thereby delaying accumulation, but did not completely inhibit synthesis.

Monomeric anthocyanins not affected by treatment (not significant main effects) maybe a result of the shift in anthocyanin composition during grape maturation due to the hydroxylation of the B-ring (Ryan and Revilla, 2003); this is an indication of post maturity and the process in which cells irreversibly stop dividing and enter a state of permanent growth arrest without undergoing cell death, also known as senescence. In contrast to findings by Guidoni and Hunter (2012), Ryan and Revilla (2003), and Pérez-Magarino and González-SanJosé (2006) reported that maximum accumulation of anthocyanins occurred when grape sugar levels reached between 20 and 25 °Brix in Tempranillo

and Cabernet-Sauvignon grapes planted in an E-W direction. This is in agreement with the results of this study, in which maximum anthocyanin accumulation already occurred at 22 °Brix, but in grapes from NE-SW rows. Furthermore, De la Hera Orts *et al.* (2005) reported that maximum anthocyanin levels were reached at between 20 and 24 °Brix in Monastrell grapes, but Fourmand *et al.* (2006) found that anthocyanins remained unchanged from 20 °Brix to 26 °Brix.

Chorti *et al.* (2010) reported increased anthocyanins in artificially (partially) shaded Nebbiolo grapes at harvest and planted in N-S rows (northern-hemisphere), compared to grape bunches without shading. Partially shaded grapes from N-S rows with limited light penetration in the canopy can be considered to be similar to the NW-SW rows of this study in terms of light penetration (low light penetration in the afternoon). Contrary to Chorti *et al.* (2010), Bubola *et al*. (2017) and Feng *et al.* (2017) have reported increased total anthocyanins in Teran grapes at 23 °Brix and Pinot noir grapes at 22 °Brix from N-S-orientated vines. Vines underwent 50 % defoliation thereby increasing the light penetration and temperature in the fruit zone, compared to no leaf removal.

Ultraviolet-B radiation can upregulate chalcone synthase; i.e., flavonoid biosynthesis and phenylalanine ammonia-lyase in the phenylpropanoid genes pathway (Jansen *et al*., 1998). Flavonols can therefore increase when grapes are exposed to increased light penetration in the canopy (Alonso *et al*., 2016; Pastore *et al*., 2017a) and expression of gene encoding flavonol synthase in grape skins (Pastore *et al.*, 2013). Flavonols are sensitive to changes in environmental conditions and can respond as a protectant against UV-containing light. Temperatures above 35 °C can, however, have a detrimental effect on flavonol biosynthesis (Pastore *et al.*, 2017b). In contrast to the above, flavonol levels in this study were lowest in grapes from N-S rows (high light penetration all day) at 22 °Brix, 24 °Brix and 26 °Brix; this is similar to work by Cohen *et al.* (2012), who found lower concentrations of flavonols in Merlot grapes at 22 °Brix of vines planted in N-S rows subjected to heating in the fruit zone (via heat blowers), compared to grapes at ambient temperature. Increased temperature in the fruit zone may have inhibit flavonol biosynthesis.

In this study, flavonols that decreased during grape ripening (Table 5) may be attributed to oxidation through coupled reactions and may act as co-pigments with anthocyanins in co-pigmentation processes (Bimpilas *et al*., 2015). The increase in flavonols (i.e., grapes from NW-SE rows at 24 °Brix and 26 °Brix) could be due to the high light penetration in the canopy in the morning, resulting in increased grape bunch temperature (Table 2), which stimulates flavonol biosynthesis; some UV radiation is required to stimulate gene transcription to produce flavonol synthase (Holton and Cornish, 1995). Flavonol synthase catalysis the conversion of dihydrokaemferol to kaempferol and dihydroquercetin to quercetin, among other flavonols. Friedel *et al*. (2015) have also reported that flavonols increased in Riesling grape bunches at harvest with increased light penetration in the canopy (E-W direction) due to leaf removal in the fruit zone. It seems that flavonol biosynthesis favours increased light penetration in the fruit zone in the morning; i.e., in NW-SE rows.

In the present study, the decrease in total phenolic acids in grapes from the E-W, NE-SW and NW-SE rows as grapes ripened (24 °Brix to 26 °Brix) may be a result of down-regulated flavonoid biosynthesis due to limited light penetration in these canopies. Grapes from N-S rows showed a slight increase in phenolic acids with ripeness. Additionally, the differences in grape phenolic acid levels among rows may be a consequence of these reactive compounds changing to galloylated derivatives when exposed to increased PAR, thereby decreasing free acids (Jordão *et al*., 2001). Trihydroxybenzoic acids in grapes do not usually respond to UV radiation; hydroxycinnamic acids, however, respond to variation in light penetration in the canopy (Spayd *et al*., 2002), which could have contributed to the observed differences in their concentrations in grapes depending on the row orientation in this study (Table 5). Rescic *et al*. (2016) reported that total phenolic acids were higher in Uva Longanesi and Istrian Malvasia grapes from E-W and N-S rows respectively at 22 °Brix after 50 % defoliation, compared to vines with no defoliation treatment. In this study, the phenolic acid levels were highest in grapes from NW-SE rows at 24 °Brix and 26 °Brix.

Flavan-3-ol levels in grapes from the NE-SW and NW-SE rows at both 22 °Brix and 24 °Brix were higher than those in grapes from to N-S and E-W rows. Similar results have been reported by Romboli *et al.* (2017), who found increased flavan-3-ol levels in low vigour Sangiovese grape planted in NE-SW rows at harvest, compared to high vigour vines. In the present study, total flavan-3-ols generally increased until grape ripeness reached 24 °Brix, after which it was not affected by the treatment as grapes ripened to 26 °Brix.

In a study by Downey *et al*. (2004), flavan-3-ol levels in artificially shaded Shiraz grapes during berry ripening were found to be lower than in exposed bunches; however, the decrease in total flavan-3-ols during berry ripening was greater in exposed bunches, to the extent that the levels were virtually the same in shaded and exposed berries at harvest. Pastore *et al.* (2017a) reported flavan-3-ols to have minimal response to environmental factors; however, PAR rather than UV radiation had a positive effect on flavan-3-ol synthesis. In addition, Scrafidi *et al.* (2013) reported higher concentrations of total flavan-3-ols in exposed Grillo grapes at 21 °Brix and planted to N-S rows than in boxed-in grapes. In contrast to work by Pastore *et al*. (2017a) and Scrafidi *et al*. (2013), the results of this study show that grapes from N-S rows (high light penetration all day) had the lowest levels of total flavan-3-ols. However, in agreement with the flavan-3-ol levels found in this study, Allegro *et al.* (2019) reported that increased light penetration in the canopy/fruit zone of Grechetto Gentile grapes did not increase the accumulation of flavan-3-ols. Higher levels of total flavan-3-ols, phenolic acids and flavonols are to be expected

in N-S rows (high light penetration in the canopy), compared to E-W, NE-SW and NW-SE rows; however, this was not the case in this study. The low levels of total flavan-3-ols and total flavonols at 22 °Brix and total flavonols at 24 °Brix may be due to over-exposure of grape bunches to light (i.e., high initial PAR at 22 to 24 °Brix grape ripeness), which could have inhibited some metabolic processes (Downey *et al.*, 2006).

The variation in phenolic concentrations reported in the cited literature was likely caused by defoliation and artificial shading, resulting in grape bunches being over- or under-exposed to light. Artificial shading of grape bunches (light manipulation/boxed-in) caused a decrease in certain phenolic compounds and over-exposure/artificial heating an increase. The results reported by international authors were therefore primarily due to the manipulation of the canopy and fruit zone in addition to "natural" row orientation creating a microclimate. The cited results may, however, be linked to the results and deductions of this study regarding the effect of different row orientations on the light penetration in the fruit zone. Hunter *et al*. (2016) have reported that the microclimate profiles (light) of canopies are affected by changes in row orientation.

This is the first investigation in South Africa to demonstrate the effect of grapevine row orientation on individual phenolic compounds in Syrah grapes, as well as the combined effect of vintage and grape ripeness level, although vintage did not play a major role. The results showed that phenolic compound biosynthesis involves the interaction of light and grape ripeness (and temperature). The effect of different light regimes brought about by the different row orientations and grape ripeness on phenolic compound development was shown.

Under realistic field conditions (i.e., different row orientations), planting grapevines in N-S and NE-SW rows can result in higher temperatures in the fruit zone (Hunter *et al*., 2016) than planting in E-W and NW-SE rows. An increase in temperature in the fruit zone resulting from grapevine row direction has been found to cause a decrease in certain phenolics, which is probably due to a combination of both degradation and biosynthesis inhibition (Mori *et al.*, 2005; Tarara *et al.*, 2008; Feng *et al*., 2017). In this study, NE-SW and NW-SE row orientation generally had a positive effect on phenolic development, which may be linked to shorter periods of light penetration/exposure in the fruit zone; i.e., low light exposure in the morning for NE-SW and low light exposure in the afternoon for NW-SE rows. However, the effect of light on the biosynthesis of phenolics can vary depending on the grape cultivar (Rustioni *et al*., 2011; Bonada and Sadras, 2014). Ambient temperatures above 35 °C may inhibit phenolic biosynthesis even under different light regimes. Nevertheless, light intensity differences in the fruit zone are an important factor for phenolic compound development/accumulation, irrespective of row orientation per se (Koyama *et al*., 2012; Zoratti *et al.*, 2014). Accumulation is also dependent on a combination of moderate exposure of grape bunches to light and moderate temperature in the canopy.

Within the context of global warming, many grape growers are facing premature véraison due to early-season temperature increases, which could lead to sunburn of grape-berry skin tissue, as well as the inhibition of the biosynthesis of, for example, anthocyanins and flavan-3-ols (Hunter *et al*., 2010). Achieving phenolic maturity when grape sugar content and acidity are at optimum levels can sometimes be challenging in warm, semi-arid wine-growing regions, such as in Robertson in South Africa. As a result, growers have to either wait for phenolic maturity, at the cost of producing wines with high alcohol content and sometimes too low acidity, or harvest at desired sugar-acid levels and risk producing wines with poor colour and unripe tannins.

The desirable conditions for grapevines growing in a warm semi-arid environment are moderate to low light penetration of the canopy. However, the orientation of vineyard rows has proven to be one of the viticultural practices enabling successful canopy microclimate manipulation, leading to grape phenolic improvement. Since in practice, it may not be possible to achieve suitable grapevine row orientation in all environments, the management of the fruit zone by means of pruning, shoot positioning, removal of infertile shoots and leaf removal remains an option for increasing/decreasing grape exposure to light, irrespective of row orientation.

CONCLUSION

The results indicate that row orientation and ripeness level can be applied to control phenolic development. The effect of vintage, however, cannot be controlled. In the southern hemisphere, light conditions induced in the grape bunch zone primarily in the NE-SW (at 22 °Brix) and NW-SE (at 24 °Brix and 26 °Brix) rows positively affected most phenolics. Grapes from these row orientations could result in Syrah wines with an improved phenolic profile and have the potential to extend the range of Syrah wine styles. However, it is unlikely that a single factor can be applied to all growing conditions and grape cultivars. It is advised that viticulture-related factors, such as fertility, berry set, berry size and berry yield, be taken into account before making a decision on a specific row orientation. It is important to consider the characteristics of specific growing regions when determining the effect of row orientation on grape phenolic content. In the light of this, the results of this study can be applied to grapevines planted in specific row orientations in clayey loam soil and trained to a VSP trellis system. Commercial vineyard parcels/blocks, different terroirs and the hombre influencia can also have an effect on grape phenolic profiles. Therefore, further research is needed to understand the associations that exist among vine phenology, microclimate/light penetration and grape phenolic content.

ACKNOWLEDGEMENTS

The authors would like to thank the Agricultural Research Council and Winetech for providing funding and infrastructure. This research forms part of a PhD thesis obtained by P Minnaar at Stellenbosch University, South Africa.

REFERENCES

Allegro, G., Pastore, C., Valentini, G., & Filippetti, I. (2019). Effects of sunlight exposure on flavonol content and wine sensory of the white wine grape Grechetto Gentile. *American Journal of Enology and Viticulture*, 70, 277-285. https://doi.org/10.5344/ajev.2019.17108

Alonso, R., Berli, F.J., Picolli, P., & Bottini, R. (2016). Ultraviolet-B radiation, water deficit and abscisic acid: a review of independent and interactive effects on grapevines. *Theoretical and Experimental Plant Physiology*, 28, 11-12. https://doi.org/10.1007/s40626-016-0053-y

Beggs, C.J., & Wellman, E. (1994). Photocontrol of flavonoid biosynthesis. In: Photomorphogenisis in plants. 2nd ed. Kendrick, R.E., Kronenberg, G.H.M. Eds: Kluwer Academic Publishers, Dordrecht, The Nederlands, pp.733-751. https://doi.org/10.1007/978-94-011-1884-2_26

Bimpilas, A., Tsimogiannis, D., Balta-Brouma, K., Lymperopoulou, T., & Oreopoulou, V. (2015). Evolution of phenolic compounds and metal content of wine during alcoholic fermentation and storage. *Food Chemistry*, 178, 164-171. https://doi.org/10.1016/j.foodchem.2015.01.090

Bonada, M., & Sadras, V.O. (2014). Review: critical appraisal of methods to investigate the effect of temperature on grapevine berry composition. *Australian Journal of Grape and Wine Research*, 21, 1-17. https://doi.org/10.1111/ajgw.12102

Bonada, M., Jeffery, D.W., Petrie, P.R., Moran, M.A., & Sadras, V.O. (2015). Impact of elevated temperature and water deficit on the chemical and sensory profiles of Barossa Shiraz grapes and wines. *Australian Journal of Grape and Wine Research*, 21, 240-253. https://doi.org/10.1111/ajgw.12142

Bubola, M., Sivilotti, P., Janjanin, D., & Poni, S. (2017). Early leaf removal has a larger effect than cluster thinning on grape phenolic composition in cv. Teran. *American Journal of Enology and Viticulture*, 68, 234-242. https://doi.org/10.5344/ajev.2016.16071

Brouilard, R., & Dangles, O. (1994). Anthocyanin molecular interactions, the first step in the formation of new pigments during wine aging. *Food Chemistry*, 51, 365-372. https://doi.org/10.1016/0308-8146(94)90187-2

Cagnasso, E., Torchio, F., Gerbi, V., Rio Segade, S., Giacosa, S., & Rolle, L. (2011). Evolution of the phenolic content and extractability indices during ripening of Nebbiolo grape from Piedmont growing areas over six consecutive years. *South African Journal of Enology and Viticulture*, 32, 229-241. https://doi.org/10.1016/j.foodchem.2014.10.155

Carlomagno, A., Novello, V., Ferrandino, A., Genre, A., Lovisolo, C., & Hunter, J. J. (2018). Pre-harvest berry shrinkage in cv. "Shiraz" (Vitis vinifera L.): Understanding sap flow by means of tracing. *Scientia Horticulturae*, 233, 394-406. https://doi.org/10.1016/j.scienta.2018.02.014

Castillo-Muñoz, N., Gómez-Alonso, S., Garcia-Romero, E., & Hermosin-Gutierrez, I. (2007). Flavonol profiles of Vitis vinifera red grapes and their single-cultivar wines. *Journal of Agricultural and Food Chemistry*, 55, 992-1002. https://doi.org/10.1021/jf062800k

Chorti, E., Guidoni, S., Ferrandino, A., & Novello, V. (2010). Effect of different cluster sunlight-exposure levels on ripening and anthocyanin accumulation in Nebbiolo grapes. *American Journal of Enology and Viticulture*, 61, 23-30. ISSN: 0002-9254

Cohen, S.D., Tarara, J.M., & Kennedy, J.A. (2012). Diurnal temperature range compression hastens berry development and modifies flavonoid partitioning in grapes. *American Journal of Enology and Viticulture*, 63, 112-120. https://doi.org/10.5344/ajev.2011.11015

Cortell, J.M., Halbleib, M., Gallagher, A.V., Righetti, T.L., & Kennedy, J.A. (2005). Influence of vine vigour on grape (Vitis vinifera L. cv. Pinot noir) and wine proanthocyanidins. *Journal of Agricultural and Food Chemistry*, 53, 5798-5808. https://doi.org/10.1021/jf0504770

Cortell, J.M., Halbleib, M., Gallagher, A.V., Righetti, T.L., & Kennedy, J.A. (2007). Influence of vine vigour on grape (Vitis vinifera L. cv. Pinot Noir) anthocyanins. 1. Anthocyanin concentration and composition in fruit. *Journal of Agricultural and Food Chemistry*, 55, 6575-6584. https://doi.org/10.1021/jf070195v

Czemmel, S., Stracke, R., Weisshaar, B., Cordon, N., Harris, N.N., Walker, A.R., & Bogs, J. (2009). The grapevine R2R3-MYB transcription factor VvMYBF1 regulates flavonol synthesis in developing grape. *Plant Physiology*, 151, 1513-1530. https://doi.org/10.1104/pp.109.142059

De la Hera Orts, M.L., Martinez-Cutillas, A., López-Roca, J.M., Pérez-Prieto, L.J., & Gómez-Plaza, E. (2005). Effect of deficit irrigation on anthocyanin content of Monastrell grapes and wines. *Journal International des Sciences de la Vigne et du vin*, 39, 47-55. https://doi.org/10.20870/oeno-one.2005.39.2.899

De Souza, C.R., Da Mota, R.V., Carvalho-Silva, C.P., Raimundo, R.H.P., De Paula-Fernandes, F., & Peregrino, I. (2019). Row orientation effects grapevine performance during winter growing seasons. *Revista Ceres*, 66, 184-190. https://doi.org/10.1590/0034-737X201966030004

Downey, M.O., Harvey, J.S., & Robinson, S.P. (2004). The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. *Australian Journal of Grape and Wine Research*, 10, 55-73. https://doi.org/10.1111/j.1755-0238.2004.tb00008.x

Downey, M.O., Dokoozlian, N.K., & Krstic, M.P. (2006). Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review on recent research. *American Journal of Enology and Viticulture*, 57, 257-268.

Feng, H., Yaun, F., Skinkis, P.A., & Qian, M.C. (2015). Influence of cluster-zone leaf removal on Pinot noir grape chemical and volatile composition. *Food Chemistry*, 173, 414-423. https://doi.org/10.1016/j.foodchem.2014.09.149

Feng, H., Skinkis, P., & Qian, M.C. (2017). Pinot noir wine volatile and anthocyanin composition under different levels of vine fruit zone leaf removal. *Food Chemistry*, 214, 736-744. https://doi.org/10.1016/j.foodchem.2016.07.110

Fernandes-De Oliveira, A., & Nieddu, G. (2016). Accumulation and partitioning of anthocyanins in two red grape cultivars under natural and reduced UV solar radiation. *Australian Journal of Grape and Wine Research*, 22, 96-104. https://doi.org/10.1111/ajgw.12174

Fournand, D., Vicens, A., Sidhoum, L., Souquet, J.M., Moutounet, M., & Cheynier, V. (2006). Accumulation and extractability of grape skin tannins at different advanced physiological stages. *Journal of Agricultural and Food Chemistry*, 54, 7331-7338. https://doi.org/10.1021/jf061467h

Friedel, M., Stoll, M., Patz, C.D., Will, F., & Dietrich, H. (2015). Impact of light exposure on fruit composition of white 'Riesling' grape berries (*Vitis vinifera* L.). Vitis, 54, 107-116. ISSN 2367-4156

Giacosa, S., Marengo, F., Guidoni, S., Rolle, L., & Hunter, J. (2015). Anthocyanin yield and skin softening during maceration as affected by vineyard row orientation and grape ripeness of *Vitis vinifera* L. cv. Shiraz. Food Chemistry, 174, 8-15. https://doi.org/10.1016/j.foodchem.2014.10.155

Gómez-Alonso, S., García-Romero, E., & Hermosín-Gutiérrez, I. (2007). HPLC analysis of diverse grape and wine phenolics using direct injection and multi-detection by DAD and fluorescence. *Journal of Food Composition and Analysis*, 20, 618-626. https://doi.org/10.1016/j.jfca.2007.03.002

Guidoni, S., & Hunter, J.J. (2012). Anthocyanin profile in berry skins and fermenting must/wine as affected by grape ripeness level of Vitis vinifera cv. Shiraz/R99. *European Food Research and Technology*, 235, 397-408. https://doi.org/10.1007/s00217-012-1744-5

Holton, T.A., & Cornish, E.C. (1995). Genetics and biochemistry of anthocyanin biosynthesis. *Plant Cell*. 7, 1071-1083. https://doi.org/10.1105/tpc.7.7.1071

Hunter, J.J., Archer, E., & Volschenk, C.G. (2010). Vineyard management for environment valorisation. Proc. VIIIth Int. Terroir Zoning Congress, 14-18 June, Soave, Italy. pp. 7-3-7-15. Corpus ID: 110648410

Hunter, J.J., & Volschenk, C.G. (2017). Chemical composition and sensory properties of non-wooded and wooded Shiraz (*Vitis vinifera* L.) wine as affected by vineyard row orientation and grape ripeness level. *Journal of the Science of Food and Agriculture*, 98, 2689-2704. https://doi.org/10.1002/jsfa.8763

Hunter, J.J., Volschenk, C.G., & Zorer, R. (2016). Vineyard row orientation of Vitis vinifera L. cv. Shiraz/101-14 Mgt: Climatic profiles and vine physiology. *Agricultural and Forest Meteorology*, 228, 104-119. https://doi.org/10.1016/j.agrformet.2016.06.013

Hunter, J.J., Volschenk, C.G., & Booyse, M. (2017). Vineyard row orientation and grape ripeness level effects on vegetative and reproductive growth characteristics of *Vitis vinifera* L. cv. Shiraz/101-14 Mgt. European Journal of Agronomy, 84, 47-57. https://doi.org/10.1016/j.eja.2016.12.004

Hunter, J.J., Volschenk, C.G., Mania, E., Vincente-Castro, A., Booyse, M., Guidoni, S., Pisciotta, A., Di Lorenzo, R., & Zorer, R. (2021). Grapevine row orientation mediated temporal and cumulative microclimate effects on grape berry temperature and composition. *Agricultural and Forest Meteorology*, 310, 108660. https://doi.org/10.1016/j.agrformet.2021.108660

Jaakola, L. (2013). New insights into the regulation of anthocyanin biosynthesis in fruits. *Trends in Plant Science*, 18, 477-83. https://doi.org/10.1016/j.tplants.2013.06.003

Jansen, M.A.K., Gaba, V., & Greenberg, B.M. (1998). Higher plants and UV-B radiation: balancing damage, repair and acclimation. Trends in Plant Science, 3, 131-135. https://doi.org/10.1016/S1360-1385(98)01215-1

Jordan, B.R. (2017). Effects of ultraviolet B on *Vitis vinifera*-how important is UV-B for grape biochemical composition? In: UV-B Radiation and Plant Life: Molecular Biology to Enology, ed. By Jordan B.R. CAB International, Wallingford, pp. 144-160. https://doi.org/10.1079/9781780648590.0144

Jordão, A.M., Ricardo da Silva, J.M., & Laureano, O. (2001). Evolution of Catechins and Oligomeric Procyanidins during Grape Maturation of Castelão Francês and Touriga Francesa. *American Journal of Enology and Viticulture*, 52, 230-234. ISSN:002-9254

Joscelyne, V.L., Downey, M.O., Mazza, M., & Bastian, S.E.P. (2007). Partial shading of Cabernet-Sauvignon and Shiraz vines altered wine colour and mouth-feel attributes but increased exposure had little impact. *Journal of Agricultural and Food Chemistry*, 55, 10888-10896. https://doi.org/10.1021/jf0721621

Koyama, K., Ikeda, H., Poudel, P.R., & Goto-Yamamoto, N.G. (2012). Light quality affects flavonoid biosynthesis in young berries of Cabernet-Sauvignon grapes. *Phytochemistry*, 78, 54-64. https://doi.org/10.1016/j.phytochem.2012.02.026

Margalit, Y. (2004). *Concepts in Wine Chemistry*. San Francisco: The Wine Appreciation Guild.

Martínez-Lüscher, J., Torres, N., Hilbert, G., Richard, T., Sánchez-Díaz, M., Delrot, S., Aguirreolea, J., Pascal, I., & Gomès, E. (2014). Ultraviolet-B radiation modifies the quantitative and qualitative profile of flavonoids and amino acids in grape berries. *Phytochemistry*, 102, 106-114. https://doi.org/10.1016/j.phytochem.2014.03.014

Mateus, N., Machado, J.M., & de Freitas, V. (2002). Development changes of anthocyanins in Vitis vinifera grapes grown in the Douro Valley and concentration in respective wines. *Journal of the Science of Food and Agriculture*, 82, 1689-1695. https://doi.org/10.1002/jsfa.1237

Minnaar, P.P. (2018). "Microclimate and Grape Ripeness Effects on the Phenolic Composition of Grapes and Wine (*Vitis vinifera* L. cv. Syrah/101-14 Mgt)". PhD Thesis, Stellenbosch University, Stellenbosch. http://hdl.handle.net/10019.1/104947

Monagas, M., Bartaolomé, B. & Gómez-Cordovés, C. (2005). Updated knowledge about the presence of phenolic compounds in wine. *Critical Reviews in Food Science and Nutrition*, 45, 85-118. https://doi.org/10.1080/10408690490911710

Mori, K., Sugaya, S., & Gemma, H. (2005). Decreased anthocyanin biosynthesis in grape berries grown under elevated night temperature conditions. *Scientia Horticulturae*, 105, 319-330. https://doi.org/10.1016/j.scienta.2005.01.032

Ott, R.L. (1998). An Introduction to Statistical methods and data analysis. Duxbury Press: Belmont, California.

Pastore, C., Zenoni, S., Fasoli, M., Pezzotti, G.B., & Filippetti, I. (2013). Selective defoliation effects plant growth, fruit transcriptional ripening program and flavonoid metabolism in grapevine. *BMC Plant Biology*, 13, 30. https://doi.org/10.1186/1471-2229-13-30

Pastore, C., Allegro, G., Valentini, G., Muzzi, E., & Filippetti, I. (2017a). Anthocyanin and flavonol composition response to veraison leaf removal on Cabernet-Sauvignon, Nero d'Avola, Rabosa Piave and Sangiovese *Vitis vinifera* L. cultivars. Scientia. Horticulturae. 218, 147-155. https://doi.org/10.1016/j.scienta.2017.01.048

Pastore, C., Dal Santo, S., Zenoni, S., Movahed, N., Allegro, G., Filippetti, I., & Tornielli, G.B. (2017b). Whole plant temperature manipulation affects flavonoid metabolism and the transcriptome of grapevine berries. *Frontiers in Plant Science*, 8, 929. https://doi.org/10.3389/fpls.2017.00929

Pérez-Magariño, S., & González-SanJosé, M.L. (2002). Physicochemical parameters justifying the vintage qualification in wines from Spanish Protected Designation of Origin. *European Food Research and Technology*, 214, 444-448. https://doi.org/10.1007/s00217-001-0468-8

Pérez-Magariño, S., & González-SanJosé, M.L. (2006). Polyphenols and colour variability of red wines made from grapes harvested at different ripeness grades. *Food Chemistry*, 96, 197-208. https://doi.org/10.1016/j.foodchem.2005.02.021

Rescic, J., Mikulic-Petkovsek, M., & Rusjan, D. (2016). The impact of canopy managements on grape and wine composition of cv.

'Istrian Malvasia' (*Vitis vinifera* L.). *Journal of the Science of Food and Agriculture*, 96, 2595-2623. https://doi.org/10.1002/jsfa.7778

Ribéreau-Gayon, P., Glories, Y., Maujean, A., & Du Bourrdieu, D. (2006). Handbook of Enology. (2nd ed.). The chemistry of wine stabilisation and treatment. New York: John Wiley & Sons. https://doi.org/10.1002/0470010398

Ristic, R., Downey, M.O., Iland, P.G., Bindon, K.A., Francis, I.L., Herderich, M., & Robinson, S.P. (2007). Exclusion of sunlight from Shiraz grapes alters wine colour, tannin and sensory properties. *Australian Journal of Grape and Wine Research*, 13, 53-65. https://doi.org/10.1111/j.1755-0238.2007.tb00235.x

Rojas-Lara, B.A., & Morrison, J.C. (1989). Differential effects of shading fruit or foliage on the development and composition of grape berries. *Vitis*, 29, 199-208. https://doi.org/10.5073/vitis.1989.28.199-208

Romboli, Y., Di Gennaro, S.F., Mangani, S., Buscioni, G., Matese, A., Genesio, L., & Vincenzini, M. (2017). Vine vigour modulates bunch microclimate and affects the composition of grape and wine flavonoids: an unmanned aerial vehicle approach in a Sangiovese vineyard in Tuscany. *Australian Journal of Grape and Wine Research*, 23, 368-377. https://doi.org/10.1111/ajgw.12293

Roubelakis-Angelakis, K.A., & Kliewer, W.M. (1986). Effects of exogenous factors on phenylalanine ammonia-lyase activity and accumulation of anthocyanins and total phenols in grape berries. *American Journal of Enology and Viticulture*, 37, 275-280. Corpus ID:85904533

Rustioni, L., Rossoni, M., Calatroni, M., & Failla, O. (2011). Influence of bunch exposure on anthocyanins extractability from grape skins (*Vitis vinifera* L.). *Vitis*, 50, 137-143. https://doi.org/10.5073/vitis.2011.50.137-143

Ryan, I.M., & Revilla, E. (2003). Anthocyanin composition of Cabernet-Sauvignon and Tempranillo grapes at different stages of ripening. *Journal of Agricultural and Food Chemistry*, 51, 3372-3378. http://doi.org/10.1021/jf020849u

Scrafidi, P., Pisciotta, A., Patti, D., Tamborra, P., Di Lorenzo, R., & Barbagallo, M.G. (2013). Effect of artificial shading on the tannin accumulation and aromatic composition of the Grillo cultivar (*Vitis vinifera* L.). *BMC Plant Biology*, 13, 175-197. https://doi.org/10.1186/1471-2229-13-175

Shapiro, S.S., & Wilk, M.B. (1965). An analysis of Variance Test for Normality (complete samples). *Biometrika*, 52, 591-611. https://doi.org/10.1093/biomet/52.3-4.591

Spayd, S.E., Tarara, J.M., Mee, D.L., & Ferguson, J.C. (2002). Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *American Journal of Enology and Viticulture*, 53, 171-182. ISSN: 002-9254

Sternad-Lemut, M., Trost, K., Sivilotti, P., & Vrhovsek, U. (2011). Pinot Noir grape colour related phenolics as affected by leaf removal treatments in the Vipava Valley. *Journal of Food Composition and Analysis*, 24, 777-784. https://doi.org/10.1016/j.jfca.2011.03.003

Sun, R-Z, Cheng, G., Li, Q., Zhu, Y-R., Zhang, X., Wang, Y., He, Y-N., Li, S-Y., He, L., Chen, W., Pan, Q-H., Duan, C-Q., & Wang, J. (2019). Comparative physiological, metabolomic, and transcriptomic analyses reveal developmental stage-dependent effects of cluster bagging on phenolic metabolism in Cabernet-Sauvignon grape berries. *BMC Plant Biology*, 19, 583. https://doi.org/10.1186/s12870-019-2186-z

Tarara, J.M., Lee, J., Spayd, S.E., & Seagal, C.F. (2008). Berry temperature and solar radiation alter acylation, proportion and concentration of anthocyanin in Merlot grapes. *American Journal of Enology and Viticulture*, 59, 235-247. Corpus ID: 87523932

Tessarin, P., Boliani, A.C., Botelho, R.V., Rusin, C., Versari, A., Parpinello, G.P., & Rombolà, A.D. (2014). Effects of late defoliation on chemical and sensory characteristics of cv. Uva Longanesi wines. *Journal of Soil Science and Plant Nutrition*, 14, 1021-1038. https://doi.org/10.4067/S0718-95162014005000079

Waterhouse, A.L., Price, S.F., & McCord, J.D. (1999). Reversed-phase high-performance liquid chromatography methods for analysis of wine polyphenols. *Methods in Enzymology*, 299, 113-122. https://doi.org/10.1016/S0076-6879(99)99014-6

Yu, R., Cook, M.G., Yacco, R.S., Watrelot, A.A. , Gambetta, G., Kennedy, J.A., & Kurtural, S.K. (2016). Effect of leave removal and applied water on flavonoid accumulation in grape vine (*Vitis vinifera* L. cv. Merlot) berry in hot climate. *Journal of Agricultural and Food Chemistry*, 64, 3118-8127. https://doi.org/10.1021/acs.jafc.6b03748

Zoratti, L., Karppinen, K., Luengo-Escobar, A., Häggman, H., & Jaakola, L. (2014). Light-controlled flavonoid biosynthesis in fruits. *Frontiers in Plant Science*, 5, 534. https://doi.org/10.3389/fpls.2014.00534.