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SHORT COMMUNICATION

Vine spacing of *Vitis vinifera* cv. Shiraz/101-14 Mgt. III. Grape and wine quality

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ABSTRACT

Vine spacing effects of Shiraz/101-14 Mgt on a relatively high potential soil in the Breede River Valley, Robertson, South Africa, on grape composition and wine quality, were investigated. Canopies were VSP trellised and orientated approximately NNE-SSW. Spacing between rows was fixed at 2.2 m. In-row vine spacing changed from 0.3 – 4.5 m with increments of 30 cm (from 15151 – 1010 vines/ha). Grape composition and wine quality were monitored over six seasons and two grape ripeness levels. Decreasing trends of soluble solids (°B) and pH and increasing titratable acidity from narrow to wide spacing were observed. Sugar accumulation of wider spacings seemed delayed. Berry skin total anthocyanin index and phenol content showed virtually no change. Malvidin mono-glucoside and its p-coumaroyl and acetyl derivatives were present in the highest concentrations, followed by peonidin-, petunidin-, delphinidin- and cyanidin mono-glucosides and derivatives, each vine spacing treatment displaying a unique grape anthocyanin profile. The narrower spacing of vines seemed to favour skin anthocyanin accumulation compared to wider spacing. Decreasing trends in wine total anthocyanin intensity, anthocyanin density, and phenols were observed from narrow to wide spacing; all had higher levels in wines from riper grapes. Individual wine anthocyanin concentrations also showed qualitative profile changes. The first regression join points for all anthocyanins occurred around 1.8 – 2.4 m vine spacing; those for malvidin and derivatives consistently appeared at 1.8 m spacing. Although volatile compounds only seemed to respond to ripeness level and not to vine spacing, wine sensory quality generally decreased with wider vine spacing at both grape ripeness levels. Given the changes in biotic and abiotic growth conditions with wider vine spacing, the results likely displayed adaptation to the combined impact of increasing light exposure, berry temperature, physiological functioning, vegetative and reproductive growth ratios, and plant stress. The study showed the prominent role of plant spacing in sustainable grape growing and wine outcomes and thus the importance of judicious decision-making during the establishment and management of vines under any growth conditions and in any terroir.

KEYWORDS: Vine spacing, plant density, grape composition, wine composition/quality, anthocyanins, sensory quality, sustainability

INTRODUCTION

The spacing of vineyard rows and vines is an integral, major impacting factor in the quest for optimal soil surface utilisation, water and nutrient availability, radiation energy interception, vine physiological functioning, growth:yield balances, and overall sustainability of grapevine growing. Together with vine training, trellising, row orientation, pruning and canopy management (Smart *et al*., 1990; Hunter *et al*., 2020), the spacing of grapevines directly affects the dimensions and functioning of the root system, vegetative growth and accommodation, canopy space and functioning, yields, grape composition, and wine quality (Archer and Strauss, 1991; Reynolds *et al*., 1995; Hunter, 1998a; Hunter, 1998b). Plant spacing is, thus, a vital link in the valorisation of the environment in which the grapevine is grown and in reaching the full yield and quality potential of a scion-rootstock combination (Hunter *et al*., 2010).

Bioletti and Winkler (1934) stated that close planting under favourable growth conditions leads to shoot crowding that interferes with the setting, growth, colouring and ripening of the fruit, whereas it increases the difficulty of pest and disease control and escalates production costs. Although water availability is always a limiting factor, other, mostly soil-related, restricting conditions usually require greater plant densities to fully utilise land surface (Richards, 1983). In a study on Concord grapevines, Cawthon (2002) demonstrated interactions between vine spacing and rooting depth, recommending shorter distances between vines in shallow soils and wider distances in deep soils, the latter conditions leading to higher yields and pruning weights. Differences in quality parameters were not pronounced in deeper soils, but the widest spacing led to delayed ripening in shallow soil. In previous studies on Pinot noir, in which both row and vine spacing were changed under dryland and supplementary irrigated conditions, plant spacing affected the exposure of the soil and canopy right through the season (Archer and Strauss, 1991; Hunter, 1998a; Hunter, 1998b). The root system responded to spacing differences by changing its density, while canopy microclimate and photosynthetic activity, water potential, yields, grape composition, and wine quality were also governed. The leaf area per fresh mass ratio of widely spaced vines was much lower than the generally accepted norm of 10 – 12 cm^2 per gram of fruit (Hunter and Visser, 1990; and references therein), pointing to over-cropping. These trends were also found by Hunter and Volschenk (2024a) and Hunter and Volschenk (2024b) in a field experiment on Shiraz under relatively high soil potential conditions in which only the vine spacing was changed. Grape musts of widely spaced vines had less soluble solids and titratable acidity, whereas the pH increased progressively from widely to closely spaced vines, with optimum skin colour obtained for medium-spaced vines (Archer and Strauss, 1991; Hunter, 1998b). Sensorially, wines from closer-spaced vines scored distinctly higher. It was clear that efficient accommodation of aboveground growth is of primary importance to sustain production, grape and wine quality, and vine longevity.

Extensive previous research showed the effects that a change in canopy microclimate and ripeness level may have on berry physical characteristics and the composition matrix of sugars, organic acids, pH, terpenoids, and flesh, skin and seed phenolics, all of which may alter the occurrence of quality-determining compounds in wine (Smart *et al.*, 1990; Hunter *et al.*, 1991; Coombe, 1992; Allen and Lacey, 1993; Marais *et al.*, 1999; Spayd *et al.*, 2002; Hunter *et al.*, 2004a; Hunter *et al.*, 2004b; Pérez-Magariño and Gonzales-San José, 2004; Fournand *et al.*, 2006; Pereira *et al.*, 2006; Tarara *et al.*, 2008; Chorti *et al.*, 2010; Barbagallo *et al.*, 2011; Guidoni and Hunter, 2012; Hunter *et al.*, 2014a; Hunter *et al.*, 2014b; Giacosa *et al.*, 2015; Asproudi *et al.*, 2016; Hunter and Volschenk, 2017; Sun *et al.*, 2017; Carlomagno *et al.*, 2018; Asproudi *et al.*, 2020; Yan *et al.*, 2020; Hunter *et al.*, 2021; Matsuda *et al.*, 2021). As reviewed by Lovisolo *et al*. (2010), many physiological strategies of grapevines to endure abiotic stress, particularly to tolerate dehydration, may also alter primary and secondary compound accumulation in berries. This is often stimulated by hydraulic (xylem-related) and chemical (hormone-related, e.g., cytokinin, gibberellic acid, abscisic acid) signalling, followed by physiological regulation by the grapevine to maintain homeostasis. Intense heat is ensued by the transmittance of heat stress signals using transporters (such as aquaporins, calcium anions, etc.), initiating transcription networks to establish stress tolerance via heat shock proteins and reactive oxygen species scavenging enzymes (Venios *et al*., 2020). High seasonal temperatures and evaporative demand and heat waves may lead to higher berry sugar:organic acid ratios, accompanied by thermal degradation of colour and aroma compounds (Hunter and Volschenk, 2017; Hunter *et al*., 2021).

The importance of judicious growth management and the role that it has in radiation interception, growth balances, metabolism, water use efficiency, yield, grape composition, and the steering of wine quality, have been demonstrated thoroughly over the years (Smart *et al*. 1990; Hunter *et al*., 1994; Hunter *et al.,* 1995; Marais *et al*. 1999; Hunter, 2000; Hunter *et al*., 2016; Hunter and Volschenk, 2017; Hunter *et al.*, 2017; Hunter *et al.,* 2020). Proper canopy size (re physical dimensions/sufficiency), composition (re leaf age/potential output), porosity (re microclimate/photosynthetic efficiency), uniformity (re utilization of space/maximal productive output), and protection (re judicious light exposure of both leaves and grapes/grape health/grape quality) are essential for sustainable, product-focused grape and wine production (Smart *et al.*, 1990; Poni *et al*., 1994; Hunter, 2000; Hunter *et al*., 2004a; Hunter *et al*., 2004b; Hunter *et al*., 2016; Hunter *et al*., 2021). All these factors are directly or indirectly affected by the spacing of grapevines.

Previous research on vine spacing mostly focused on changing both row and vine spacing under various soil conditions. The most comprehensive work in this regard was done under medium-potential, shallow soil conditions in a temperate, Mediterranean climate where young vines were studied under dryland conditions and mature vines under supplementary irrigation (Archer and Strauss, 1991; Hunter,

1998b). Hence, in this study and in companion papers (Hunter and Volschenk, 2024a; Hunter and Volschenk, 2024b), the global uncertainty on the spacing of vines under fertile soil conditions with unrestricted depth was addressed. The target was to explore compensatory vine reactions to varying spacing and to provide sufficient information that would allow extrapolation to miscellaneous growth conditions to aid sustainable grape and wine production. In the companion papers, physiological and vegetative and reproductive growth responses were detailed. In this systematic study, the effects of a wide range of vine spacings on grape composition and wine quality/style were determined.

MATERIALS AND METHODS

1. Experiment layout and practices

Vitis vinifera cv. Shiraz (clone SH 9C)/101-14 Mgt was planted during the spring of 2008 with an approximate NNE-SSW (30 °) row orientation at the Robertson experiment farm of ARC Infruitec-Nietvoorbij, located in the Breede River Valley, South Africa (GPS coordinates 33°49'29.87"S; 19°52'50.67"E). Vines were spaced from 0.3 – 4.5 m with incremental increases of 30 cm from narrowest to widest, representing 15 treatments (15,000 – 1000 vines/ha). Row spacing was fixed at 2.2 m. Vines were double-cordon-trained on a VSP trellis with four sets of movable foliage wires. Irrigation was applied according to crop factors. Two-bud spurs with 15 cm inter-spur spacing were pruned since the winter of 2012. Vines were irrigated and fertilised similarly. Each treatment was replicated four times with two buffer rows per treatment, on a total surface of approximately 2 ha. Further details of the experiment layout are given in Hunter and Volschenk (2024a) and Hunter and Volschenk (2024b).

2. Berry composition

2.1 Basic grape must analyses

Grapes were harvested at two grape ripeness level stages (approximately 24 °B and 26 °B, respectively) per treatment replicate. The soluble solid content [by refractometry, °Brix (°B)], titratable acidity (TA) (as g/L tartaric acid equivalents using titration), and pH of the supernatant of settled, strained grape must of each treatment replicate were determined immediately after crushing.

2.2 Berry skin anthocyanin extraction

All the bunches from five shoots that were sampled to determine vegetative and reproductive characteristics were removed, de-berried, and properly mixed. Thirty berries per replicate were used for determination of skin total anthocyanin index by spectrophotometric measurement (Guidoni and Hunter, 2012; Giacosa *et al*., 2015; based on Di Stefano and Cravero, 1991) as well as individual skin anthocyanins [by High Performance Liquid Chromatography (HPLC), see below] according to Guidoni and Hunter (2012) and Giacosa *et al*. (2015). Berry skins were carefully removed from pulps with a scalpel, blotted dry with a paper towel, and immediately immersed in 120 mL of an extracting solution containing 12 % ethanol, 5 g/L D-tartaric acid, and 2 g/L sodium metabisulfite, with pH adjusted to 3.2 with addition of 1N NaOH. Samples were then frozen at – 20 °C. To continue the analyses, frozen samples were left overnight at 4 °C to thaw and then incubated at 30 °C for 72 hours for extraction to take place. At the end of the extraction period, samples were homogenised using a macerator (Ultra-Turrax T25 – IKA, Labortechnik, Staufen, Germany) for 60 s at 8000 rpm and extracting solution added to 150 mL. Skin residues were removed by centrifugation at 12,000 rpm for 10 min.

2.3 Skin anthocyanin spectrophotometry

For spectrophotometric analyses, absorbance was read with an Agilent Technologies Cary 60 UV-VIS Spectrophotometer using a fibre optics system with a 10 mm path length dip-probe. The total skin anthocyanin index and total phenol content of supernatants were determined as follows:

Total anthocyanin index =

Total phenol content = $A_{540} \times 16.17 \times d \times \left(\frac{v}{w}\right)$

where A_{540} = absorban $A_{280} \times d$ nm, A_{280} = absorbance at 280 nm, 16.17 = extinction coefficient of malvidin-3-O-glucoside, d = dilution factor (50), v = volume of extract (150), and w = weight of 30 fresh berries.

2.4 Skin anthocyanin HPLC analyses

For individual anthocyanin determination by HPLC, a 2 mL sample of the centrifuged skin extract was further purified by pushing samples through a Chromabond® octadecyl-modified silica C_{18} cartridge (Macherey-Nagel, Düren, Germany). Phenolic compounds were eluted from the cartridge with 5 mL methanol, the solution evaporated to dryness, re-dissolved in 1 mL of methanol:water:formic acid 50:40:10 (v/v), and membrane filtered at 0.22 µm. Samples were stored at – 20 °C before analyses. Anthocyanin profiles were determined using a Hewlett Packard series 1100 HPLC, equipped with a series 1200 automatic degasser, quaternary pump, autosampler, UV detector, and LiChroCart 250-4 Purospher RP-18 column (Merck, Darmstadt, Germany), measuring 25 × 0.4 cm with 5 µm particle size. Formic acid:water (10:90, v/v) was used as solvent A and formic acid:methanol:water (10:50:40, v/v/v) as solvent B. Anthocyanin compounds were identified by comparing retention times of peaks with literature (Di Stefano and Cravero, 1991; Guidoni and Hunter, 2012). Mono-glucoside anthocyanins (malvidin-3-glucoside; delphinidin-3-glucoside; cyanidin-3-glucoside; peonidin-3-glucoside; petunidin-3-glucoside) and their acetyl (malvidin acetyl-glucoside; delphinidin acetyl-glucoside; cyanidin acetyl-glucoside; peonidin acetyl-glucoside; petunidin acetyl-glucoside) and p-coumaroyl (malvidin p-coumaroyl-glucoside; delphinidin p-coumaroyl-glucoside; cyanidin p-coumaroyl-glucoside; peonidin p-coumaroyl-glucoside; petunidin p-coumaroyl-glucoside) derivatives were quantified at 520 nm using malvidin 3-O-glucoside chloride (Extrasynthèse, Genay, France) as external standard. Results were expressed in mg/L.

3. Winemaking and analyses

Approximately 40 kg of grapes per replicate were harvested at each of the two ripeness levels mentioned above. Grapes were cooled overnight to the same temperature (20 °C) before processing. Batches of grapes were individually de-stemmed and crushed using a custom-made de-stemmer/crusher suitable for small batches. Wines were made according to a standard winemaking procedure prescribed by the Experimental Cellar. Juice volume was measured and 50 mg/L SO_2 was added (determination of SO_2 based on the Ripper method). After one hour of skin contact, the pomace was inoculated with 30 g/hL re-hydrated pure yeast (VIN 13). As a nutrient source for yeasts, di-ammonium phosphate was added at 50 g/hL. Fermentation was conducted in 60 L food-grade plastic containers with lids, in a temperature-controlled room at 24 °C. The pomace cap was punched down three times per day throughout the fermentation period. Fermentation on skins averaged four days, after which the pomace was pressed once with a custom-built balloon press at 2 bar. Pressed and free-run parts of wine were mixed and allowed to further ferment at 25 °C until dry (less than 5 g/L residual sugar). Wines were racked into 20 L stainless steel canisters and cold-stabilised at 0 °C for approximately 21 days. No malolactic fermentation was performed. The SO_2 levels were adjusted before bottling. Wines were stored in 750 mL bottles under screw caps. All winemaking procedures were strictly controlled and repeatedly followed for the duration of the experiment.

3.1 Basic wine analyses

Bottled wines were analysed for ethanol (picnometric), extract (picnometric), residual sugar (Fehling), pH, titratable acidity (auto-titration), volatile acidity (distillation) and free and total SO_2 (Ripper–hand titration) by a commercial laboratory using standard chemistry methods.

3.2 Wine anthocyanin Spectrophotometric and HPLC analyses

Wines were analysed spectrophotometrically using an Agilent Technologies Cary 60 UV-VIS Spectrophotometer (manufactured in Malaysia) for total anthocyanin (A_{520}) and phenolic (A_{280}) contents after proper dilution (5× with deionised water), as described above for grape skin anthocyanins. For individual anthocyanin determination by HPLC, a 2 mL wine sample was purified using a Chromabond® octadecyl-modified silica C_{18} cartridge (Macherey-Nagel, Düren, Germany). Phenolic compounds were eluted from the cartridge with methanol, the solution evaporated to dryness, re-dissolved in 1 mL of methanol:water:formic acid 50:40:10 (v/v), and membrane filtered (0.22 µm). The HPLC wine analysis was then performed as described above for skin anthocyanins.

3.3 Wine volatile compound extraction

Experimental wine (5 mL) was transferred into a sealable sample tube, followed by 100 µL of 175 ppm 4-methyl-2-pentanol as internal standard and 1.5 mL diethyl ether as extraction solvent. The sample was vortexed and then sonicated for 30 mins, and liquid–liquid extraction was performed, followed by centrifugation at 3000 rpm for 1 min. The upper diethyl ether layer containing the volatiles was transferred into a 2 mL vial with sodium sulphate to remove excess H_2O from the extract. It was then transferred into a vial with a glass insert and capped. An aliquot of 1 µl was injected in splitless mode into a Gas Chromatograph-Mass Spectrometer (GC-MS).

3.4 Wine volatile compound GC–MS detection

Wine volatiles (higher alcohols, esters, volatile acids, and C-6 compounds) were analysed with a GC (6890N, Agilent technologies network) coupled to an Agilent technologies inert XL EI/CI Mass Selective Detector (MSD) (5975B, Agilent Technologies Inc., Palo Alto, CA). The GC-MS system was coupled to a CTC Analytics PAL autosampler. A polar ZB-Wax (60 m, 0.25 mm ID, 0.50 µm film thickness) capillary column (Phenomenex, Torrance, CA) was used. Helium was used as carrier gas at a flow rate of 1 mL/min. The injector temperature was maintained at 240 °C. An aliquot of 1 µl of sample was injected in splitless mode. The oven temperature was programmed as follows: 40 °C for 5 min. and raised to 200 °C at a rate of 5 °C/min. for 0 min. and finally raised to 250 °C at a rate of 25 °C/min. for 6 min. The MSD was operated in full scan mode and source and quad temperatures were maintained at 230 °C and 150 °C, respectively, while the transfer line temperature was maintained at 250 °C. The Mass Spectrometer was operated under electron impact mode at an ionisation energy of 70 eV, scanning from 30 to 650 m/z. Results were expressed in mg/L.

4. Wine sensory evaluation

The expert tasting panel consisted of 10 trained and experienced persons from ARC Infruitec-Nietvoorbij Institute. They regularly taste wines made in an experimental environment. Tasters were trained on an ongoing basis during the weeks before the tasting of the wines from each season, as follows: to recognise descriptors used during tastings (using prepared mixtures of different aromas); to understand the relation of descriptors to the evaluation method; to synchronise them in the use of the evaluation method and intensity line-scale; and to familiarise them with nuances generally associated with young experimental wines. Additionally, and together with an explanation of the project treatments, tasting methodology, descriptor terminology and recognition by the tasters were refreshed before each tasting, using unmarked wines from the wine batches to be tasted.

Wine batches were tasted approximately four months after bottling. Wines of all replicates were tasted in batches of 30. Treatments and replicates were completely randomised within a ripeness level. Tasters were presented with numbered tasting sheets in a random order without any identification that could be traced to wine origin/treatment; tastings were therefore blind. A non-structured 100 mm line-scale method was used to indicate the level of intensity of a specific pre-selected sensory attribute/descriptor (Jackson, 2002) and was marked by the taster according to perception intensity.

Sensory descriptors were repeated from year to year and comprised the following: colour intensity; overall aroma

intensity; fruity aroma intensity; vegetative (fresh, cooked, dry) intensity; spicy aroma intensity; pepper aroma intensity; jammy aroma intensity; acidity intensity; tannin (astringency) intensity; alcohol intensity; body (mouthfeel); finish (persistence); overall quality. Scores of tasters were averaged per descriptor and wine replicate and used as a quantitative measurement of sensory perception. To avoid sensory fatigue, tasting batches were limited to approximately 30 wines per tasting panel per day. The temperature-controlled tasting room comprised a set-up of separate cubicles, each taster being allocated an individual private cubicle. The tasting room interior, tasting set-up and procedure, wine-tasting glasses and wine volumes were standardised from year to year. Wines were served individually and sequentially to tasters. They were not given any time limit for tasting.

5. Statistical design and methods

The experimental design was a randomised block design with 15 spacing treatments and four block replications. Sampling mostly comprised two grape ripeness levels. Destructive sampling was done on different vines. Data were subjected to ANOVA using the General Linear Models Procedure (PROC GLM) of SAS software (Version 9.4; SAS Institute Inc, Cary, NC) to determine polynomial trends. Data were then either subjected to the regression procedure (PROC REG) to fit linear and quadratic regressions or to a non-linear regression procedure (PROC NLIN). Join point regression was fitted to the data to describe the changes in trends over vine spacing. Join point regression, also known as change point regression, broken stick, or segmented regression, assumes that data can be divided into subsets, each with their own unique linear trend (Hudson, 1966). PROC NLIN was performed to fit more than one line to the data to determine the "join point/s". Outliers were determined using the Shapiro–Wilk test on standardised residuals from the model to verify normality (Shapiro and Wilk, 1965). Outliers were replaced by predicted values of the model.

RESULTS AND DISCUSSION

1. Grape must composition

The average grape must composition from narrow to wide spacing at the two harvest dates over the last five years followed trends of decreasing °B and pH, and slightly increasing TA, resulting in °B:TA also decreasing (Figure 1). Similar results were found by Archer and Strauss (1991), Hunter (1998b), Cawthon (2002) and Murisier and Zufferey (2006). The °B:TA ratio was previously found to correlate with secondary compound and sensory quality profiles and proved to be a very important and useful practical indicator of wine quality and style categories (Hunter *et al*., 2004a; Nadal and Hunter, 2007; Hunter *et al*., 2014a). Closely spaced vines ended up being slightly under-cropped and widely spaced vines over-cropped (Hunter and Volschenk, 2024b), the latter confirmed by lower/delayed berry sugar accumulation, despite the generally found sugar-concentrating effect of a more open canopy. Although differences were small, trends of TA and pH differed in slope. Delayed/inhibited sugar accumulation with wide vine spacing is in line with trends found for the physiological parameters water potential, photosynthesis and water use efficiency that indicated that wider spaced vines experienced more stress, most likely caused by a combination of under-supply of water, photo-inhibition, excessive crop load, insufficient leaf area and premature leaf ageing, all leading to sub-optimal photosynthetic sucrose output and imbalanced carbon distribution (Hunter and Volschenk, 2024a; Hunter and Volschenk, 2024b). Overcropping was clear from the available leaf area/fresh grape mass that decreased from generally acceptable values of approx. 13 – 15 cm^2/g for narrow-spaced vines to less than two-thirds of that for wider-spaced vines (> 2.7 m) (Hunter and Volschenk, 2024b). Such source:sink modifications may activate leaf senescence and if nutrient demand exceeds supply, nutrients may be withdrawn from older leaves and the root system (Hunter, 2000; Rankenberg *et al*., 2021). This may affect plasticity and accelerate leaf ageing, especially if the root system is lacking sufficient fine roots and thus activity to absorb water and nutrients, as in this case where wider spaced vines had fewer fine roots than narrower spaced vines (Hunter and Volschenk, 2024a). Such a scenario would have a negative effect on stress tolerance and resilience. This points to the necessity of creating a well-balanced canopy and root system, each comprising a composition of young and older tissues to reduce the repercussions of abiotic stresses under field conditions and avoid a scenario where physiological integrity, growth activity, yield, grape ripening, and reserve accumulation are compromised. Tissue-age-related synchronised physiological mechanisms and the regulation and distribution/recycling of inorganic and organic elements are essential to maintaining whole plant homeostasis (Hunter, 2000). This also emphasises the importance of judicious manipulation at the practical level to accommodate the morphological and physiological needs of the plant in growth, yield, and quality as well as the necessity for disease and virus control, to optimise and maintain grapevine activity in any scenario.

Although a poorer canopy light microclimate would normally also have a reducing effect on berry sugar accumulation (Smart *et al*., 1985), this was probably counteracted by the better leaf area/grape mass and the denser root system of narrow-spaced vines (Hunter and Volschenk, 2024a; Hunter and Volschenk, 2024b). The above nonetheless indicates differential irrigation/fertilisation/soil type needs per spacing group, with widely spaced vines requiring higher supply (either using irrigation and fertilisation or via better edaphic conditions regarding nutrient yielding and water holding capacity) to optimise physiological and growth performance that would equal that of narrower spaced vines under the conditions of this experiment. This emphasises the important dictating effect that soil conditions have on plant spacing. Changes in total canopy size, leaf size and age composition, and canopy porosity at different vertical and horizontal levels would certainly affect the canopy-environment exchange (wind flow and turbulence, light penetration and reflection, humidity, and temperature; Hunter *et al*., 2016; Hunter *et al*., 2021), with implications for microclimate, fertility, yield,

and grape ripening rate and composition. As demonstrated in this study, the canopy body evolved from narrow to wide plant spacing, gradually changing from a more solid to a more porous/sparse condition, resulting in multiple adaptive responses (Hunter and Volschenk, 2024a; Hunter and Volschenk, 2024b).

Organic acids are synthesised mainly during the pre-véraison period (Ruffner, 1982a; Hunter and Ruffner, 2001). In practice, and especially under warm climatic conditions favouring malate respiration, it would therefore be of the utmost importance to create and maintain efficient microclimatic and sufficient water status conditions in the grapevine canopy during the pre-véraison period that would favour photosynthetic activity/high sugar production and sucrose translocation to berries to guarantee high organic acid, particularly tartaric acid, formation (Hunter and Ruffner, 2001). Grapes would then enter the ripening period at higher organic acid levels, providing a buffer for adverse conditions during this period, favouring a lower berry pH at ripeness, and limiting deleterious effects on grape quality. A higher tartaric acid:malic acid ratio and lower must pH are normally found under better canopy microclimate conditions (Smart *et al*., 1990 ; Hunter *et al*., 1991). Considering the higher radiation in canopies of wider-spaced vines (Hunter and Volschenk, 2024a), a shift in organic acid balance may have led to a lower-than-expected pH in the grape must of these vines, despite the only slight increase in titratable acidity. Furthermore, malic acid normally decreases, whereas tartaric acid is more or less stable, also under high ambient temperatures, from berry softening onwards (Ruffner, 1982a; Ruffner, 1982b; Coombe, 1992; Hunter and Ruffner, 2001). A reduction in tartaric acid is mainly ascribed to dilution (Ruffner *et al*., 1983), while some free acid is converted to salt forms, mainly due to the influx of potassium during the ripening phase (Iland, 1987a; Iland, 1987b; Gutiérrez-Granda and Morrison, 1992; Hunter and Ruffner, 2001; Hunter *et al*., 2014b). Accumulation of potassium is normally also associated with poor canopy microclimate conditions (Smart *et al*., 1985; Smart *et al*., 1990), but a phloem turgor maintenance-related accumulation and translocation are guaranteed when water or other physiological stress conditions that restrict optimal photosynthetic activity (and thus sucrose availability), prevail (Hunter and Ruffner, 2001). In the case of malic acid, suggested additional reducing mechanisms include a decrease in malate formation by inhibition of glycolytic carbon flow (which would result in an accumulation of imported sugar); this seemed not to be the case, or the initiation process was delayed for wider spaced treatments. Rather, re-metabolisation of stored malic acid (Steffan and Rapp, 1979; Possner and Kliewer, 1985; Gutiérrez-Granda and Morrison, 1992) and increased catabolic enzyme activity (Possner *et al*., 1983; Ruffner *et al.*, 1983) could be more plausible mechanisms to satisfy continuing demand for respiratory substrates under conditions of the wider spaced vines with more open canopies and higher radiation (Hunter

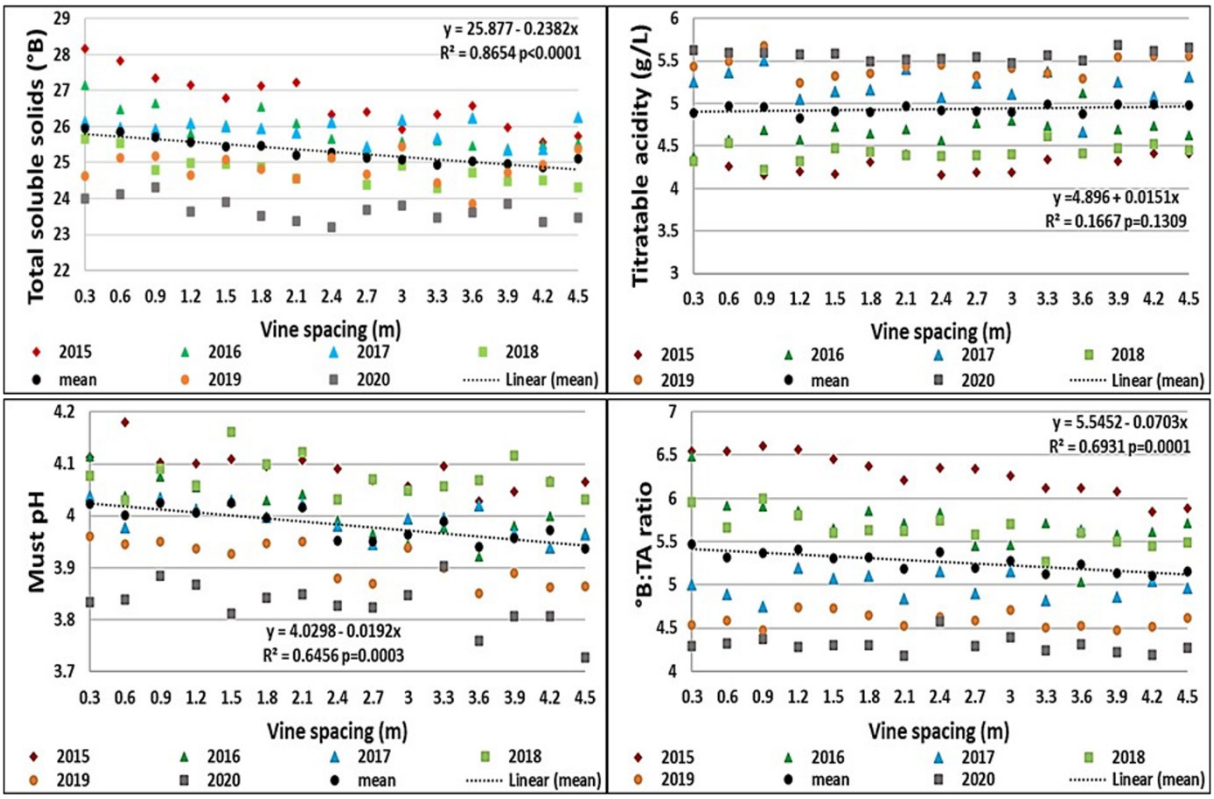


FIGURE 1. Grape must composition (means of two ripeness levels) (Soluble solids - °Brix: Top left; Titratable acidity: Top right; pH: Bottom left; °B:TA ratio: Bottom right) of Shiraz/101-14 Mgt, Breede River Valley, Robertson, South Africa (2015–2020 seasons).

and Volschenk, 2024a). Grapevines may clearly compensate at various levels for the consequential effects of plant spacing.

Despite increased compensatory photosynthetic activity that essentially might have occurred and the accompanied export of photo-assimilates because of an increase in sink:source relationship per shoot (Johnson *et al*., 1982; Hunter and Visser, 1988; Candolfi-Vasconcelos and Koblet, 1990; Hunter *et al*., 1995; Koblet *et al*., 1996; Hunter, 2000), the very low leaf area/grape mass of wider spaced vines was insufficient for maintaining carbohydrate reserve accumulation and uninterrupted quantitative sucrose flow to berries to prevent a decrease in soluble solid accumulation. It is likely that the bunches on the shoots of these vines were the preferred allocations for photo-assimilates, but a further contribution to the build-up of soluble solids in the berries by the importing of storage carbohydrates from the rest of the vine structure (spurs, cordon, trunk, roots) cannot be excluded. This was previously found to occur in experiments involving leaf darkening and excessive defoliation (Quinlan and Weaver 1970; Hunter and Visser, 1988; Candolfi-Vasconcelos and Koblet, 1990). Concomitantly, given the ostensible stressful physiological status that prevailed for widely spaced vines, it is likely that carbohydrate reserve accumulation in storage compartments may have been neglected, with a slowly deteriorating effect which, in time, would shorten the productive lifespan and sustainability of these vines, despite this not being apparent in root starch contents at the stage of measurement (Hunter and Volschenk, 2024a).

2. Grape skin composition

Notwithstanding the decrease in °B of the grape must from narrow to wide spacing (Figure 1), the grape skin total anthocyanin index and total phenol content showed virtually no change (except for the decreasing trend for the former at the higher ripeness level) with plant spacing or grape ripeness level (Figure 2). The almost invariable trend with an increase in vine spacing may be related to the more or less stable TA and decrease in pH, indicating a delay in ripening with wider spacing (Figure 1). The effect of ripeness level on total anthocyanin and phenolic contents confirms previous findings with different irrigation treatments and vineyard row orientations, showing peak concentrations already at an early grape ripening stage (Hunter *et al*., 2014a; Hunter *et al*., 2021). At the physiological level, an increase in anthocyanin concentration might have been expected with wider spacing, considering the lower pH and higher canopy light exposure than narrow spacings. However, research showed a negative relationship between anthocyanins and high temperature (and over-exposure) (Spayd *et al*., 2002; Downey *et al*., 2004; Mori *et al*., 2007; Pisciotta *et al*., 2013; Gouot *et al*., 2019), even though not all berries would be affected to the same extent (Hunter *et al*., 2021). The results also again point to overcropping, as confirmed by the leaf area/grape mass that decreased with wider spacing of vines (Hunter and Volschenk, 2024b). Contrary to this, the higher secondary leaf area/grape mass, root density, and ratio of fine:thick roots and deeper root penetration of narrower spaced vines most likely favoured

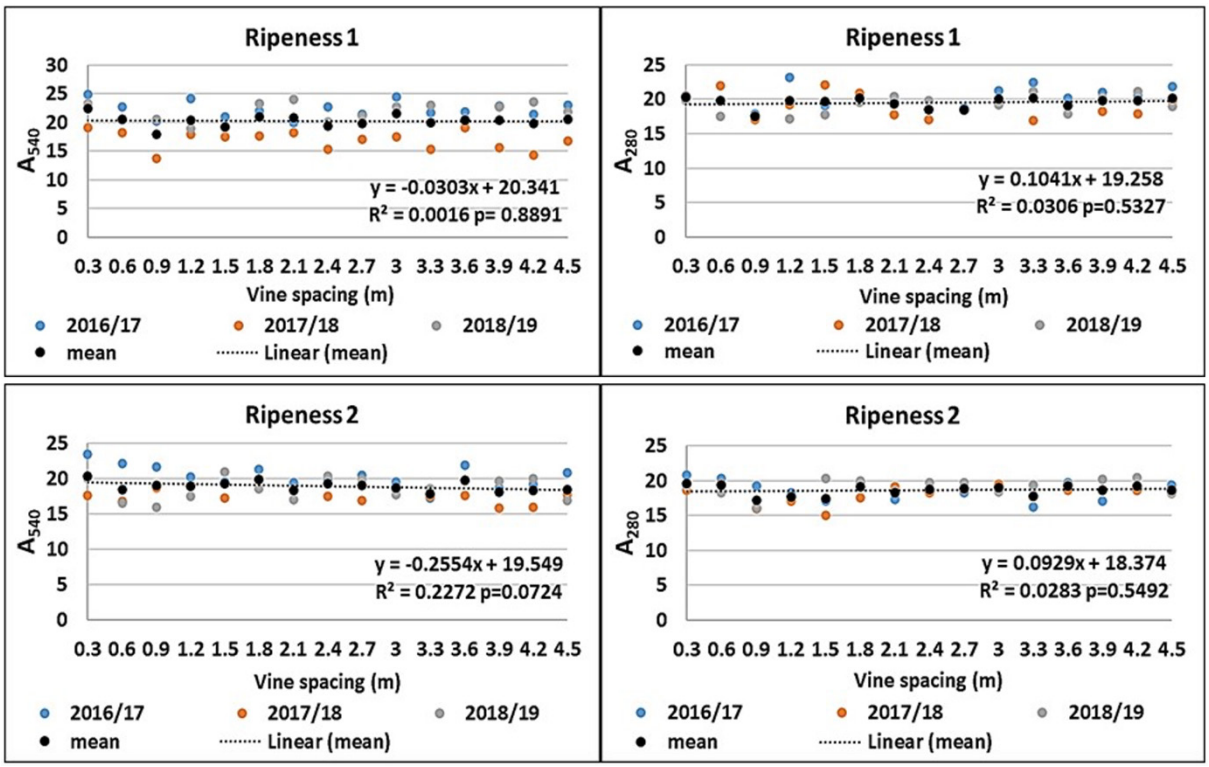


FIGURE 2. Grape total anthocyanin index and total phenol content at two grape ripeness levels (Top – grape ripeness level 1; Bottom – grape ripeness level 2) of NNE-SSW oriented Shiraz/101-14 Mgt, Breede River Valley, Robertson, South Africa (2017–2019 seasons).

ripening of the grapes of these vines (Hunter, 2000; Hunter and Volschenk, 2024a). The hormone abscisic acid showed a positive relationship with grape soluble solid and skin anthocyanin concentrations and increased in the xylem sap with high-density planting (Hunter, 1998b). Similarly, cytokinin formation is increased by a root system with higher fine root composition, thus regulating shoot and fruit development (Davies *et al*., 1986; Field *et al*., 2009). In previous research, an increase in grape soluble solids and skin colour as well as wine quality was found with denser planting/higher population per surface area (Archer and Strauss, 1991; Hunter, 1998b). Recent transcriptomic studies on the effects of vine root restriction showed phenolics, stilbenes, flavonols, anthocyanins and proanthocyanidins being induced to accumulate, implicating the role of functional genes in this response and stimulations involving multiple environmental stress factors, such as water deficit, high temperature, phytohormones and sunlight exposure in the eventual outcomes at whole plant and berry composition levels (Leng *et al*., 2020).

Although an increase in grape colour and phenolics could have been expected under the better canopy microclimate conditions of wider-spaced vines (Hunter and Volschenk, 2024a), this may be further evidence of the grapes being deleteriously affected by stressful conditions experienced by these vines. It also confirms the complexity of the physiological homeostasis being maintained in the grapevine under field conditions. Even if it can be accepted that the lower stem water potential, non-responsive/compensating photosynthetic activity, and lower source:sink ratio played a role in vine performance and thus grape composition (Hunter and Volschenk, 2024a; Hunter and Volschenk, 2024b), the increase in canopy exposure would have impacted on both physical and chemical composition of the grapes. Despite this, berry mass and volume per se appeared largely unaffected

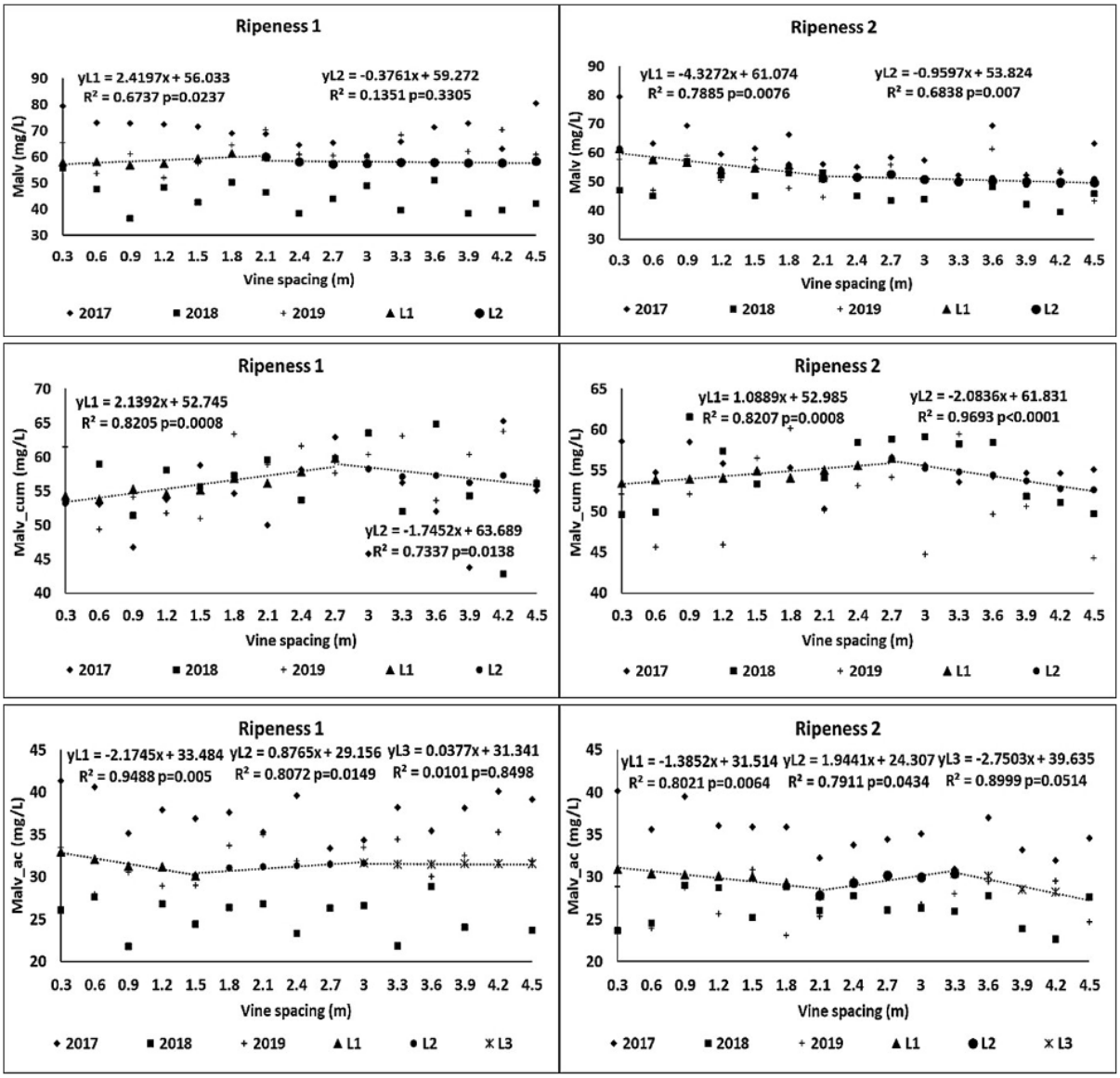


FIGURE 3. Polynomial linear trends of grape skin individual anthocyanin concentrations at two grape ripeness levels (Left – grape ripeness level 1; Right – grape ripeness level 2) of NNE-SSW oriented Shiraz/101-14 Mgt, Breede River Valley, Robertson, South Africa (2017–2019 seasons).

by vine spacing (Hunter and Volschenk, 2024b) and likely had no direct role in the eventual berry condition and composition at ripeness. After an initial increase, almost all bunch parameters showed an apparent flattening of the curve with wider spacing (Hunter and Volschenk, 2024b). Rachis mass and volume decreased less than that of berry mass and volume from the first to the second harvest stage, changing the ratio of rachis mass:berry mass in favour of the former as ripening proceeded, in agreement with earlier findings (Hunter *et al*., 2014b). Along with an increase in ripeness level, the decrease in berry volume with further ripening would have increased the skin:pulp ratio, all favouring the transfer of skin components into the wine (Nadal and Hunter, 2007; Guidoni and Hunter, 2012; Hunter *et al*., 2014a; Melo *et al*., 2015).

Escobar-Bravo *et al*. (2017) found that phenolic catabolism may be stimulated under a combination of UV-B radiation and increased temperature. Berry temperature ranges during pre- and post-véraison in the afternoon (Hunter and Volschenk, 2024a) were well within those that may alter gene ontology (Lecourieux *et al*., 2019), whereas air temperatures were conducive to the suppression of anthocyanin accumulation (Hunter and Bonnardot, 2011; Matsuda *et al*., 2021). Occasional heat waves may even be more deleterious to grape composition in a grapevine canopy where low source: sink relationships prevail and physiological homeostasis is disturbed (Hunter, 2000). Heat stress may push plants beyond their intrinsic thermotolerance boundaries, particularly in combination with drought, rendering natural response mechanisms incapacitated or totally ineffective to guard against stress injury (Bokszczanin, 2013). This is relevant within a climate change scenario and particularly critical for terroirs that are already pushing extreme climatic boundaries for grape growing.

Individual grape skin anthocyanin differences between plant spacing treatments are shown in Figure 3. As found previously (Guidoni and Hunter, 2012; Giacosa *et al*., 2015; Hunter *et al*., 2021), malvidin mono-glucoside and its p-coumaroyl and acetyl derivatives were present in the highest concentrations. They were followed by peonidin-, petunidin-, delphinidin- and cyanidin mono-glucosides and derivatives (see Supplementary Material for all these component profiles). Irrespective of ripeness level, each vine spacing displayed a unique anthocyanin profile. The overall canopy exposure and physiological conditions of narrower-spaced vines were more favourable to skin anthocyanin accumulation. Most of the anthocyanins and respective derivatives showed first regression join points around 1.8/2.1 m vine spacing, where after concentrations mostly remained relatively stable or decreased. The trends agree with subterranean and aboveground vegetative and reproductive parameters and growth relationships found by Hunter and Volschenk (2024a) and Hunter and Volschenk (2024b).

3. Wine composition

Chemical wine composition of the different vine spacing treatments showed no differences between treatments at any ripeness level (see Supplementary Material for wine chemical composition). However, alcohol, extract, residual sugar, and pH generally increased from lower to higher ripeness levels, whereas TA remained more or less the same. A trend for higher extract seemed to occur for narrower spacings at the second ripeness level.

In contrast to the relatively stable grape total anthocyanin index and total phenol content across the different plant spacing treatments (Figure 2), a decreasing trend in wine total anthocyanin intensity, total anthocyanin density and total phenols occurred with wider spacing (Table 1, Figure 4). These parameters increased in wine made from the grapes at higher ripeness levels. Along with a quantitative change, the anthocyanin qualitative profiles may also vary, i.e., ratios of 3-glucoside, 3-acetyl-glucoside, and 3-p-coumaroyl-glucoside derivatives may change with bunch exposure, berry temperature and grape ripeness level, affecting development, conversion, and degradation dynamics as well as extractability from the skins of berries during maceration (Guidoni and Hunter, 2012; Hunter *et al*., 2021). This is confirmed by the individual wine anthocyanin concentrations, displaying malvidin and derivatives (Figure 5) (see Supplementary Material for profiles of peonidin-, petunidin-, delphinidin- and cyanidin and their derivatives). Anthocyanins are known to be unstable and to undergo enzymatic and chemical reactions during the winemaking process, from which new compounds may form, for example, anthocyanin polymers, tannin oligomers and proanthocyanidin polymers, which may change wine sensory properties and thus wine quality (Cheynier *et al*., 2006). Further cleavages and polymerisations within such a rich pool of mono- and polymers in a wine medium may yet again lead to other derivatives. The structural diversity and complexity of wine are therefore continuously changing, also affecting the stability of co-pigmentation and structural changes of anthocyanidins/anthocyanins, by which different shades of red colour and sensory attributes are manifested (Paissoni *et al*., 2018). However, the contribution of the various individual anthocyanins and derivatives to the sensory palate is far from clear, salivary proteins apparently having a pivotal interactive role in influencing perception that would nonetheless seem not to go beyond astringency and bitterness, but the sensation of which that would inevitably be enriched by the abundant presence of other wine chemical components and interactions.

The first regression join points for all anthocyanins occurred around 1.8 – 2.4 m vine spacing, but join points for the anthocyanins that were present in the highest concentrations (malvidin and derivatives) consistently appeared at 1.8 m spacing. Given the changes in biotic as well as abiotic conditions, as the vines were spaced wider and wider, the grape and wine phenolic contents most likely displayed the combined impact of increasing light exposure, berry temperature, and plant stress (Bokszczanin, 2013; Escobar-Bravo *et al*., 2017; Hunter *et al*., 2021; Hunter and Volschenk, 2024a).

TABLE 1. Wine composition at two grape ripeness levels of NNE–SSW oriented Shiraz/101-14 Mgt, planted to different vine spacings, Breede River Valley, Robertson, South Africa (2015–2019 seasons).

	Wine total anthocyanins & phenolics																	
Vine	Anthocyanins (A_{520})						Anthocyanins (A_{420})						Phenolics (A_{280})					
Spacing (m)	2015	2016	2017	2018	2019	Ave	2015	2016	2017	2018	2019	Ave	2015	2016	2017	2018	2019	Ave
	Ripe 1	Ripe 1	Ripe 1	Ripe 1	Ripe 1	Ripe 1	Ripe 1	Ripe 1	Ripe 1	Ripe 1	Ripe 1	Ripe 1	Ripe 1	Ripe 1	Ripe 1	Ripe 1	Ripe 1	Ripe 1
	Ripe 2	Ripe 2	Ripe 2	Ripe 2	Ripe 2	Ripe 2	Ripe 2	Ripe 2	Ripe 2	Ripe 2	Ripe 2	Ripe 2	Ripe 2	Ripe 2	Ripe 2	Ripe 2	Ripe 2	Ripe 2
0.3	0.72	0.56	0.66	0.38	0.62	0.59	0.60	0.43	0.54	0.34	0.54	0.49	10.99	10.16	7.99	8.59	10.53	9.65
	0.84	0.70	0.74	0.48	0.57	0.66	0.78	0.55	0.63	0.42	0.51	0.58	12.84	10.66	10.89	8.76	13.62	11.35
0.6	0.71	0.48	0.48	0.36	0.59	0.53	0.58	0.35	0.44	0.32	0.53	0.45	10.40	9.06	7.66	7.78	10.59	9.10
	0.72	0.50	0.70	0.46	0.58	0.59	0.62	0.40	0.61	0.39	0.52	0.51	12.16	9.52	10.74	8.66	13.48	10.78
0.9	0.58	0.40	0.49	0.32	0.61	0.48	0.48	0.30	0.45	0.28	0.56	0.41	10.34	8.57	7.55	7.69	10.62	8.96
	0.80	0.50	0.66	0.37	0.54	0.57	0.68	0.40	0.59	0.33	0.50	0.50	12.10	10.40	10.79	8.56	13.79	10.99
1.2	0.63	0.38	0.48	0.34	0.56	0.48	0.52	0.29	0.44	0.31	0.50	0.41	9.67	8.71	7.53	7.56	9.98	8.69
	0.69	0.45	0.71	0.43	0.59	0.58	0.60	0.37	0.63	0.38	0.51	0.50	11.08	9.07	11.17	8.62	13.78	10.83
1.5	0.58	0.38	0.51	0.33	0.59	0.48	0.49	0.29	0.45	0.30	0.51	0.41	9.31	8.68	7.59	7.95	10.44	8.79
	0.63	0.49	0.66	0.39	0.53	0.54	0.55	0.37	0.58	0.34	0.47	0.46	10.58	9.27	11.35	8.27	13.25	10.54
1.8	0.58	0.46	0.58	0.24	0.65	0.50	0.49	0.34	0.51	0.23	0.56	0.43	9.33	9.16	8.29	6.93	10.55	8.76
	0.75	0.54	0.65	0.48	0.58	0.61	0.65	0.43	0.56	0.42	0.53	0.52	11.69	9.69	10.75	9.30	13.52	11.08
2.1	0.64	0.37	0.53	0.27	0.59	0.49	0.54	0.29	0.47	0.26	0.53	0.42	9.52	8.60	7.66	7.29	10.61	8.81
	0.66	0.50	0.69	0.32	0.62	0.56	0.58	0.41	0.61	0.40	0.55	0.51	10.91	10.21	11.68	9.15	13.32	11.06
2.4	0.56	0.38	0.51	0.32	0.55	0.46	0.46	0.29	0.45	0.28	0.47	0.39	9.12	8.82	7.68	7.26	9.57	8.49
	0.63	0.46	0.67	0.40	0.62	0.56	0.52	0.37	0.58	0.34	0.55	0.47	10.12	9.29	10.92	7.96	12.40	10.14
2.7	0.52	0.36	0.49	0.26	0.56	0.44	0.43	0.26	0.43	0.23	0.49	0.37	8.42	7.72	6.97	6.77	10.49	8.07
	0.73	0.43	0.59	0.32	0.59	0.53	0.61	0.34	0.54	0.28	0.51	0.46	10.41	8.77	10.45	8.28	13.12	10.21
3.0	0.52	0.37	0.54	0.27	0.62	0.46	0.42	0.28	0.45	0.24	0.55	0.38	7.99	8.56	7.71	7.31	9.85	8.23
	0.65	0.48	0.70	0.39	0.66	0.58	0.54	0.36	0.60	0.33	0.59	0.48	10.42	9.05	11.01	7.85	12.91	10.25
3.3	0.54	0.34	0.44	0.29	0.56	0.44	0.44	0.26	0.36	0.25	0.49	0.36	8.47	7.78	6.71	6.99	10.05	7.77
	0.63	0.44	0.60	0.31	0.60	0.53	0.54	0.36	0.54	0.32	0.53	0.46	9.64	9.13	10.35	8.04	12.76	9.98
3.6	0.58	0.42	0.52	0.29	0.62	0.49	0.47	0.31	0.46	0.25	0.53	0.41	8.57	8.30	7.77	7.18	10.38	8.44
	0.66	0.48	0.67	0.41	0.60	0.56	0.56	0.41	0.65	0.37	0.52	0.50	10.72	9.48	11.03	8.31	13.06	10.20
3.9	0.52	0.31	0.40	0.24	0.57	0.41	0.44	0.25	0.36	0.22	0.49	0.35	8.99	7.79	6.79	6.91	9.90	8.08
	0.59	0.37	0.65	0.32	0.56	0.49	0.51	0.30	0.56	0.29	0.51	0.43	9.67	8.37	10.11	7.80	12.75	9.46
4.2	0.45	0.35	0.47	0.21	0.50	0.40	0.38	0.25	0.46	0.19	0.44	0.35	8.33	7.93	7.73	6.24	9.49	7.95
	0.64	0.39	0.55	0.32	0.66	0.50	0.54	0.31	0.49	0.30	0.60	0.45	10.32	9.10	9.52	8.31	13.16	9.92
4.5	0.50	0.34	0.50	0.20	0.58	0.41	0.42	0.24	0.47	0.19	0.53	0.36	8.52	7.36	7.52	6.38	10.25	8.01
	0.64	0.47	0.69	0.29	0.65	0.54	0.54	0.36	0.60	0.27	0.59	0.47	10.22	8.49	10.83	8.19	13.15	9.85
LSD (p = 0.05)																		
Ripe 1	0.086	0.095	0.173	0.096	0.166	0.078	0.066	0.070	0.124	0.070	0.120	0.06	1.287	1.281	1.216	0.814	1.920	0.732
Ripe 2	0.141	0.096	0.151	0.135	0.110	0.068	0.114	0.074	0.121	0.105	0.089	0.05	1.255	1.047	1.156	1.146	1.698	0.697

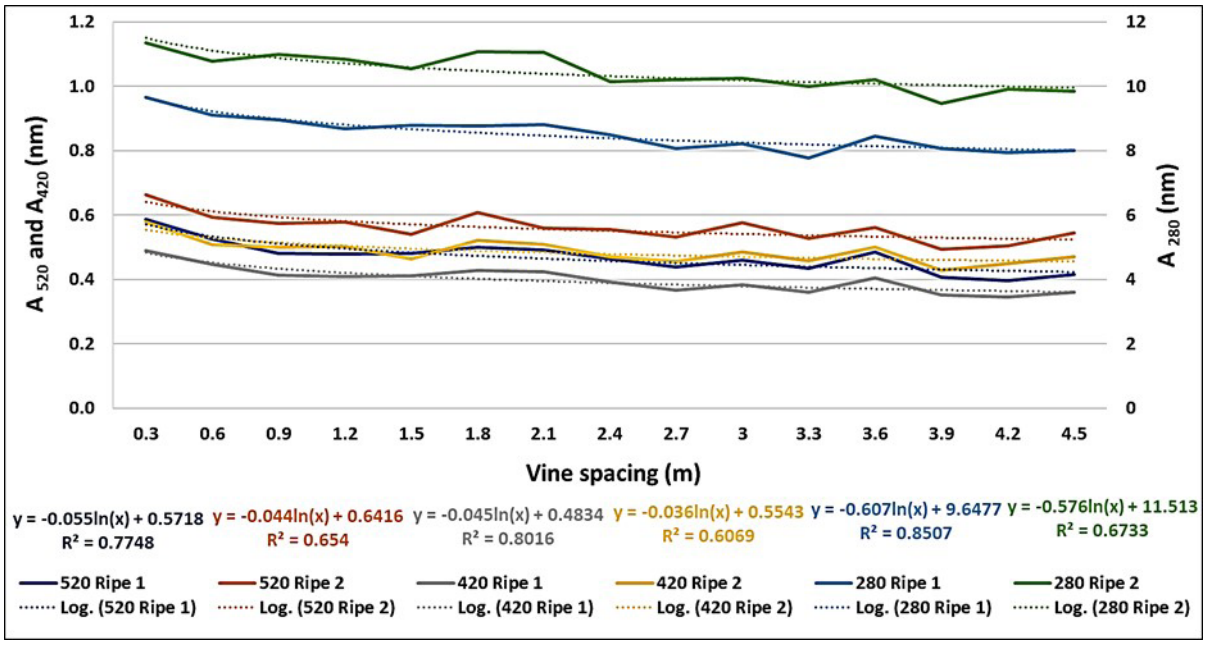


FIGURE 4. Wine total anthocyanin and phenolic composition at two grape ripeness levels of NNE–SSW oriented Shiraz/101-14 Mgt, planted to different vine spacings, Breede River Valley, Robertson, South Africa (average of 2015–2019 seasons).

The wine volatile composition of the different plant spacing treatments displays components that were consistently found during all seasons (Table 2). It includes only some of the (primarily yeast-derived) metabolites formed during the winemaking process, namely fatty acids, esters, higher alcohols, and a C6-alcohol. This serves as a demonstration of the wine aroma modifications that may occur with grape ripeness level and plant spacing changes. Although the substantial role of other odorants, such as monoterpenes, C_{13}-norisoprenoids, methoxypyrazines, etc., in the wine olfactogram is acknowledged (Escudero *et al*., 2007; Slaghenaufi *et al*., 2022), they were not included in this study. Within the fatty acid group, acetic and octanoic acids increased, whereas butyric, iso-butyric, propionic and iso-valeric acids decreased, with an increase in grape ripeness level; the esters (diethyl succinate and ethyl lactate) also decreased. Except for propanol and butanol, higher alcohols, as well as the C6 compound hexanol, increased from the first to the second ripeness level. Considering the aroma descriptors normally associated with these compounds (Aznar *et al*., 2001; Escudero *et al*., 2007), but acknowledging the individual odour activity values, volatile group vectors, chemical interactions, and complexity of a wine medium that may change with grape composition and under different winemaking conditions (Van Schalkwyk *et al*., 1995; López *et al*., 1999; Ferreira *et al*., 2007; Guidoni and Hunter, 2012), riper grapes in this study seemed generally to have led to wines with less sharp, acidic, sweaty, fatty, buttery, cheesy, and fruity odours. The aroma became less medicinal, but more alcoholic and harsher with a flowery, rose-like flavour, yet may have been perceived as grassy and green. There seemed to be a marked shift in wine aroma with grape ripening. This description, although far from completely elaborated, points to the interaction of the different impact factors at the vineyard level, altering the grape composition foundation of the wine. Altogether, grape ripeness level showed a marked impact on the presence of practically all analysed volatile compounds. This is in line with previous findings on the significance of grape ripening dynamics in changing wine style (Hunter *et al.*, 2004a; Nadal and Hunter, 2007; Hunter *et al.*, 2014a; Hunter *et al.*, 2017). No prominent changes were observed between vine spacing treatments. The latter is surprising, as it is clear that vine spacing affected many vegetative and reproductive growth balances, physiological and related processes, and the microclimate environment, which suggest that modification to the aroma profile would be eminent. However, this is confirmation of the many impacting and counteracting factors at the vineyard level that annually compound into the eventual grape and wine composition.

4. Wine sensory quality

The wine sensory descriptors colour intensity, overall aroma, fruity aroma, and tannin intensity were higher at the second ripeness level but showed a decreasing trend with wider spacing (Figure 6). Vegetative aroma and spiciness decreased from the lower to higher ripeness level, but the former was perceived as higher and the latter as unchanged with wider spacing. At the second harvest date, sensory intensity perception of pepper, jam and alcohol seemed higher and showed increasing trends with wider spacing. Despite the wine composition results, a clear decrease in overall wine quality with increasing spacing was perceived at both grape ripeness levels. Discriminating wines of the different vine spacing treatments would finally be difficult and mostly dependent on the recognition of very delicate

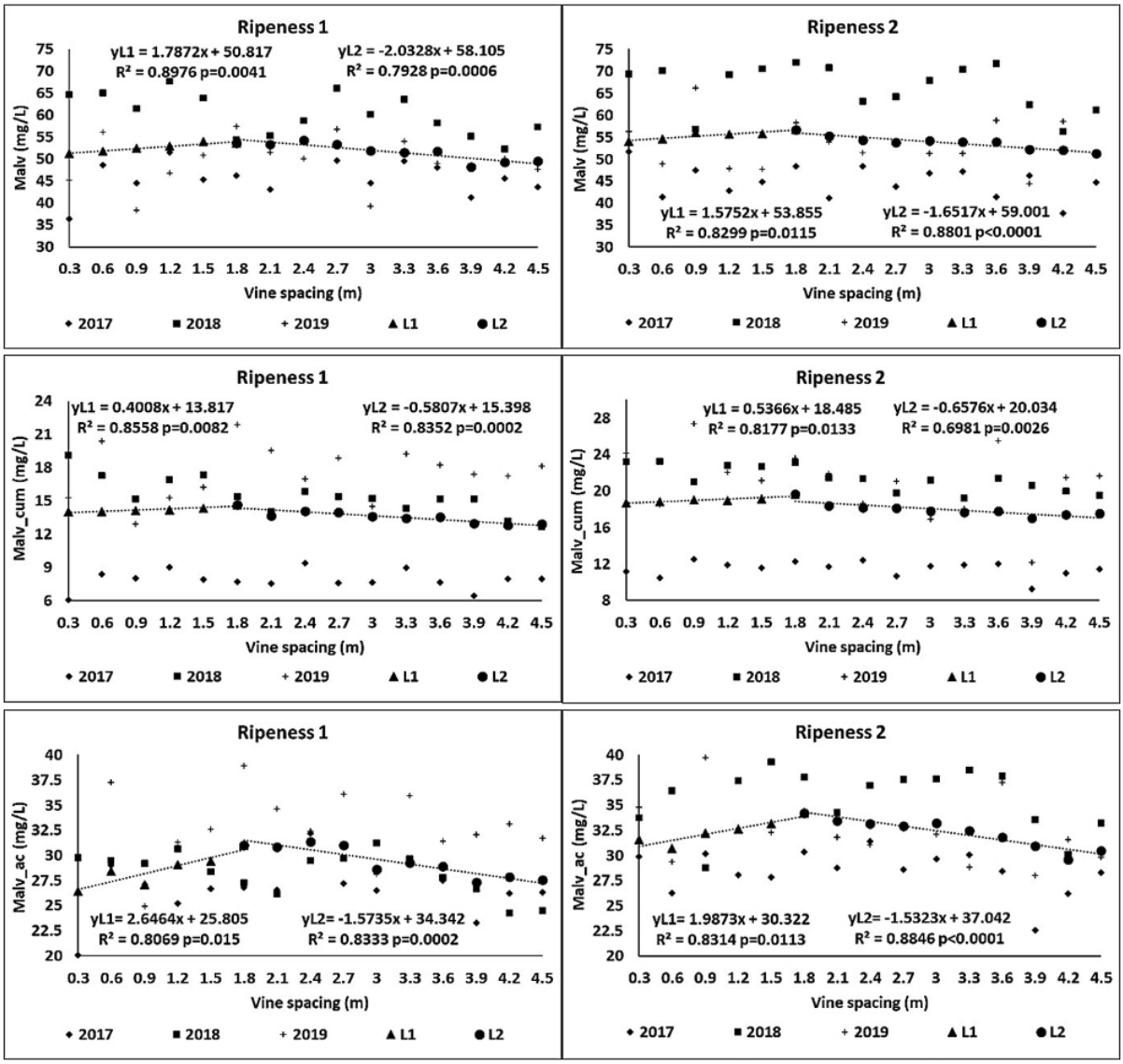


FIGURE 5. Polynomial linear trends of wine individual anthocyanin concentrations at two grape ripeness levels (Left – grape ripeness level 1; Right – grape ripeness level 2) of NNE–SSW oriented Shiraz/101-14 Mgt, Breede River Valley, Robertson, South Africa (2017–2019 seasons).

nuance shifts, as previously demonstrated under field conditions over many seasons (Guidoni and Hunter, 2012; Hunter *et al*., 2014a; Hunter *et al*., 2017). Major changes between neighbouring vine spacing treatments were not expected. Rather, an expectation of turning points instead of definite cut-off points seemed more realistic. Attempts at understanding the physicochemical effects of grapes on wine, together with the numerous secondary reactions during maceration, fermentation of the must, ageing of the wine, and beyond, are further complimented by the sensory knowledge and skills of expert wine panels. To make a meaningful contribution to expanding understanding and interpretation of the interactive impacting factors on end products, it is essential that sensory panels be sensibly selected and well-orientated.

5. General

In line with the growth parameters, grape and wine quality mostly appeared to reach an optimum of around 1.8 m vine spacing under the conditions of this experiment. Differential soil type, water, and nutrition needs per spacing group seemed evident. Wider-spaced vines indicated a requirement for richer/more fertile, accessible edaphic conditions in comparison to narrower-spaced vines to maintain growth balances, canopy microclimate, surface valorisation, and sustainability, confirming the dictating effect of soil conditions on plant spacing. It may be possible to push the vine spacing by one or two increments below or beyond the optimum under certain conditions (soil and climatic, e.g., richer or poorer; warmer or cooler). This should however be managed very carefully to prevent disharmony that may develop between the available space for vine development, growth, microclimate, yield obtained per surface area, grape and wine composition, and sensory quality of the wine.

The results confirm the significant role of terroir conditions and cultivation practices that alter the canopy and root system, on grape composition and wine quality.

TABLE 2. Wine volatile composition at two grape ripeness levels of NNE–SSW oriented Shiraz/101-14 Mgt, planted to different vine spacings, Breede River Valley, Robertson, South Africa (2015–2019 seasons).

Vine spacing (m)	Fatty acid (mg/L)						Ester (mg/L)		Higher alcohol (mg/L)					C6-alcohol (mg/L)
	Acetic acid	Butyric acid	iso-Butyric acid	Octanoic acid	Propionic acid	iso-Valeric acid	Diethyl succinate	Ethyl lactate	iso-Amyl-alcohol	1- Butanol	iso-Butanol	2-Phenyl ethanol	1-Propanol	1-Hexanol
Ripeness 1														
0.3	49.85	0.53	1.53	0.11	6.36	1.31	2.79	26.85	140.61	1.16	24.16	28.04	60.11	1.12
0.6	47.93	0.54	1.38	0.10	5.21	1.32	2.68	29.21	145.85	1.32	24.10	28.88	60.33	1.12
0.9	54.33	0.54	1.54	0.09	5.96	1.83	2.80	28.49	148.85	1.33	26.24	28.89	60.21	1.17
1.2	50.20	0.53	1.54	0.11	5.82	1.45	2.67	28.39	144.66	1.26	27.37	27.95	59.62	1.10
1.5	57.40	0.52	1.38	0.10	5.61	1.50	2.53	29.25	132.57	1.20	24.57	28.32	62.33	1.06
1.8	51.10	0.52	1.47	0.11	6.34	1.27	2.91	27.84	139.77	1.21	24.98	28.13	59.66	1.17
2.1	51.44	0.54	1.42	0.12	5.44	1.54	2.83	28.04	146.55	1.37	24.93	28.51	58.23	1.17
2.4	53.65	0.50	1.56	0.13	5.68	1.36	3.30	27.82	139.79	1.23	25.89	27.61	54.91	1.16
2.7	53.34	0.55	1.44	0.12	6.03	1.63	2.78	28.06	143.62	1.38	24.58	28.50	61.59	1.14
3.0	56.19	0.59	1.56	0.13	6.53	1.46	2.84	30.37	139.17	1.27	26.32	27.82	56.19	1.11
3.3	47.29	0.55	1.51	0.13	5.69	1.95	2.76	29.70	145.72	1.39	25.10	28.88	62.48	1.16
3.6	51.26	0.61	1.50	0.13	5.46	1.57	2.81	29.82	158.10	1.44	26.98	28.84	62.62	1.28
3.9	49.68	0.54	1.51	0.13	4.64	1.50	2.71	28.04	141.97	1.31	24.98	28.84	59.61	1.12
4.2	52.35	0.53	1.51	0.12	5.67	1.69	2.61	29.77	147.37	1.35	25.81	29.16	57.58	1.14
4.5	52.78	0.64	1.51	0.08	6.02	1.73	2.60	26.50	138.68	1.37	25.63	29.25	64.23	1.16
LSD (p = 0.05)	3.97	0.09	0.22	0.14	1.02	0.51	0.48	2.60	18.21	0.16	2.58	3.13	5.20	0.13
Ripeness 2														
0.3	57.32	0.21	1.38	0.28	3.31	1.27	2.47	26.75	179.79	1.45	26.77	28.24	63.63	1.29
0.6	56.23	0.21	1.35	0.26	3.67	1.10	2.15	27.03	179.77	1.58	27.66	31.10	61.81	1.26
0.9	58.87	0.25	1.29	0.21	3.26	0.98	2.59	28.59	200.36	1.50	30.69	31.12	57.53	1.42
1.2	56.03	0.20	1.50	0.32	2.80	1.23	2.37	27.33	180.01	1.46	27.70	29.54	54.19	1.31
1.5	56.42	0.18	1.50	0.31	4.06	1.34	2.51	24.38	174.20	1.43	26.87	31.54	59.72	1.31
1.8	55.75	0.21	1.43	0.24	3.06	1.00	2.37	27.12	192.16	1.47	27.14	31.85	59.19	1.24
2.1	52.01	0.21	1.52	0.31	3.20	0.93	2.70	27.69	195.23	1.52	29.81	29.15	59.04	1.36
2.4	53.03	0.22	1.38	0.26	2.82	1.03	2.03	25.28	185.37	1.42	25.77	24.92	58.12	1.26
2.7	59.30	0.24	1.43	0.29	3.06	1.51	2.35	28.81	180.68	1.63	29.18	29.80	59.36	1.35
3.0	58.07	0.24	1.59	0.34	3.28	0.96	2.42	27.64	186.92	1.54	28.00	29.41	60.64	1.33
3.3	57.22	0.19	1.33	0.27	3.59	1.27	2.36	24.73	173.28	1.41	27.09	29.70	49.59	1.28
3.6	61.12	0.22	1.36	0.32	4.23	1.22	2.52	26.06	178.51	1.57	27.40	31.45	51.15	1.35
3.9	57.11	0.23	1.43	0.29	3.75	1.03	2.51	26.95	194.70	1.57	28.34	31.41	51.59	1.35
4.2	55.18	0.19	1.37	0.24	2.79	0.97	2.61	25.85	174.46	1.36	23.58	29.88	50.78	1.25
4.5	58.70	0.23	1.35	0.26	3.44	1.14	2.55	25.42	169.21	1.48	24.62	30.95	53.26	1.32
LSD (p = 0.05)	6.87	0.05	0.20	0.09	0.92	0.49	0.39	3.68	19.60	0.19	3.64	3.18	5.63	0.10

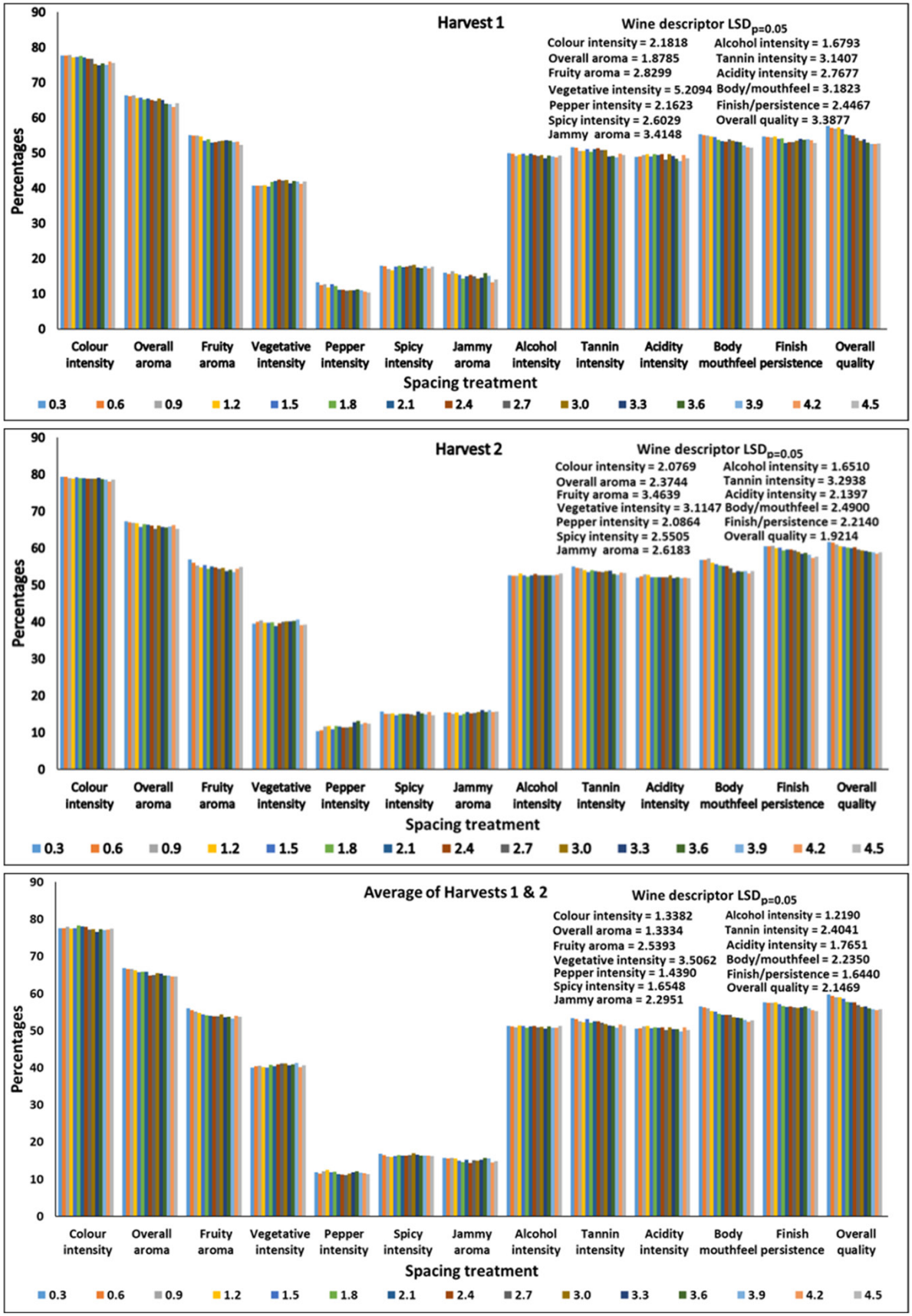


FIGURE 6. Sensory quality (descriptor response and profile) of wines made from grapes harvested at two ripeness levels from Shiraz/101-14 Mgt, planted to different vine spacing, Breede River Valley, Robertson, South Africa (average of 2015–2019 vintages).

Considering data collected since vineyard establishment over a period of more than 10 years in this study and those previously reported in accompanying studies (Hunter and Volschenk, 2024a; Hunter and Volschenk, 2024b), gradual changes in the root system, vegetative and reproductive growth balances, canopy microclimate, and physiological dynamics appeared from narrow to wide vine spacing. This led to wider-spaced vines having, amongst others, lower leaf:fruit ratios that not only caused non-linear, disharmony between increasing spacing and the growth, canopy microclimate and yield return of the vines but also delayed grape ripening. This was reflected in the grape and wine composition and wine sensory profiles of the different spacing treatments.

CONCLUSION

Grapevine growing and grape composition are infinitely affected by cultivation and environmental conditions, such as edaphic and climatic factors. The choice and execution of practices may lead to positive or negative mitigation of the array of terroir conditions that are normally encountered by the plant. Vine spacing per se essentially addresses the potential growth development of the grapevine within an allocated space, the aim being to optimise space utilisation with realisation in yield, and grape (and wine) quality objectives and longevity of the plant, to satisfy sustainable, valorised farming. This requires a whole-plant approach in which both abiotic and biotic factors need to be considered. The study demonstrated the major effect that essential cultivation practices, in this case vine spacing, may have on an already complex soil–vineyard–atmosphere unit. The natural controlling effect of the soil and root system on the physiological processes of the whole vine and the plant interactions with the respective below- and aboveground environment spheres (atmosphere, geosphere, hydrosphere, biosphere, anthroposphere) were manifested at growth, production, as well as grape and wine quality levels. Together with the companion papers, this comprehensive study provided much-needed guidance on the choice of plant spacing under the different conditions commonly encountered by producers in grapevine growing and winemaking.

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