

# The impact of grape ripeness level on berry and wine composition and potential wine style of *Vitis vinifera* L. cv. Pinotage

by

**Etienne Louis Adriaan Terblanche**



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Department of Viticulture and Oenology, Faculty of  
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*Supervisor:* Dr. Jakobus Johannes Hunter

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## Declaration

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## Summary

The understanding of grapevine reaction to its environment and resulting grape composition, wine composition and sensory profile forms the foundation from which wine producers make product-based/market-related, downstream cultivation decisions. Yet, surprisingly limited information is available to producers with regards to the expected changes in wine style linked to a range of harvest dates, including early or late/delayed harvest. Therefore the main objective of this descriptive study was to create a reference base to better assist practical harvest decision making. The native South African *Vitis vinifera* L. cv. Pinotage was studied due to its early and rapid sugar accumulation, analogous to the predicted grapevine response due to climate warming. In this field study, the extent of grape- and wine composition and sensory profile changes for cv. Pinotage was assessed at five (R1-R5) ripeness levels that were defined by sugar level/Brix (ca. 21, 23, 25, 27 & 29 °B) over three vintages (2015-17) and two sites planted to Pinotage/140 Ruggeri (A) and Pinotage/1103 Paulsen (B), under Mediterranean conditions (unirrigated), Western Cape, South Africa. The unirrigated grapevines grafted to drought resistant rootstocks adapted well (at physiological level) to low seasonal rainfall and high mean temperatures. Despite canopy deterioration/senescence during late ripening, the spectrum of ripeness levels (R1-R5) was completed within 21 days. Berry sizes remained constant from R1-R4 and accumulation or decrease of berry constituents was considered independent of the concentration effect by berry size reduction (increased skin:pulp ratio) as this only manifested at R5. Late season berry size reduction (dehydration) at R5 was linked to increases in primary metabolites (sugars and acids) and minerals (both phloem and xylem bound), but decreases in phenolic and anthocyanin contents. Concentration of berry phenolic compounds and anthocyanins increased from R1 to R4 (peak), before declining towards R5. Changes in grape aromatic profile were subtle and reaction of key components was significantly influenced by Vintage and Site. Results suggest a relatively minor shift in overall grape aroma profile per ripeness level during the compact harvest window (21 days). Even so, wine volatile profiles displayed significant definition regarding ripeness levels, showing distinctive changes for components of various volatile groups. Importantly, known impact odorants of Pinotage, such as ethyl octanoate (*sweet fruit/floral*), isoamyl acetate (*banana*), and  $\beta$ -damascenone (*prune*), displayed ripeness level related changes. As such, these shifts in aroma profile were also displayed by descriptors and intensities thereof in sensory analyses. Moreover, particularly taste/palate descriptors, including acidity, body, astringency, alcohol and concentration, which were well associated with basic fruit chemistry (sugar, titratable acidity and pH), displayed a controlling effect on sensory profile. This exhaustive grape compositional field study placed ripening related changes in context to those determined/affected by the environment (Vintage/Climate) and Site (Soil & Genotype). It points to the negative effects of extended hang time and provides much needed insight into wine compositional changes during rapid sugar accumulation. Novel sensory information regarding potential wine styles, as differentiated by ripeness level, was generated. This can easily be utilised by producers to achieve the desired product outcome.

## Opsomming

Ons begrip van die wingerd se reaksie op omgewingsfaktore en die daaropvolgende druif- en wynsamestelling en sensoriese profiel vorm die grondslag waarop wynprodusente hul produkgebaseerde/markverwante verbouingsbesluite maak. Ondanks bogenoemde is daar verbasend min inligting vir produsente beskikbaar ten opsigte van verwagte verandering in wynstyl gekoppel aan rypheidsvlakke. Die hoofdoelstelling van hierdie beskrywende studie was dus om 'n verwysingsbron daar te stel om produsente rakende oesbesluite behulpsaam te wees. *Vitis vinifera* L. cv. Pinotage, 'n Suid Afrikaanse kultivar, is gebruik in die studie as gevolg van sy vroeë en vinnige suiker-akkumulering, analoog aan die verwagte wingerdreaksie as gevolg van klimaatsverwarming. In hierdie veldstudie is die omvang van druif- en wynsamestelling veranderinge asook die sensoriese profiel veranderinge van cv. Pinotage by vyf (R1-R5) rypheidsgrade, soos gedefinieer deur suikervlakke/Brix (ca. 21, 23, 25, 27 & 29 °B), gemeet oor drie oesjare (2015-17) en op twee liggings met Pinotage/140 Ruggeri (A) en Pinotage/1130 Paulsen (B), onder Mediterreense klimaatsomstandighede (nie-besproei) in die Wes-Kaap, Suid Afrika. Die onbesproeide wingerd wat op droogtebestande onderstokke geënt is, het goed (op 'n fisiologiese vlak) by lae seisoenale reënval en hoë gemiddelde temperature aangepas. Desondanks die agteruitgang/veroudering van die lower tydens laat rypwording, is die volle spektrum rypheidsvlakke (R1-R5) binne 21 dae voltooi. Korrelgrootte was deurgaans konstant by R1-R4 en verhoging of afname in korrelinhoud was dus grootliks onafhanklik van die konsentrasie-effek as gevolg van afname in korrelgrootte/dehidrasie (hoër dop:pulp verhouding), aangesien dit slegs by R5 voorgekom het. Die laat-seisoen afname in korrelgrootte (dehidrasie) by R5 het 'n verhoging in primêre metaboliete (suikers en sure) en minerale, maar verlaging in fenoliese en antosianien inhoud tot gevolg gehad. Die konsentrasie fenoliese verbindings en antosianiene het van R1-R4 (piek) verhoog, voordat dit by R5 afgeneem het. Veranderinge in die aromatiese profiel van die druive was subtiel en die reaksie van sleutelkomponente is merkbaar deur Oesjaar en Ligging beïnvloed. Resultate dui op 'n relatiewe lae verskuiwing in die totale druifaroma profiel per rypheidsgraad gedurende die kompakte oestydperk (21 dae). Bogenoemde in ag genome, het die vlugtige komponente (aroma) van die wyn duidelike definisie ten opsigte van rypheidsvlakke getoon, met onderskeibare veranderinge vir komponente van verskillende chemiese groepe. Dis belangrik om daarop te let dat bekende impak geure van Pinotage, soos byvoorbeeld isoamiel asetaat (piesang), etiel oktanoaat (soet vrugtig/blomme) en  $\beta$ -damasenoen (pruim), rypheidsvlakverwante veranderinge getoon het. Hierdie veranderinge in die aromaprofiel is ook deur geurbeskrywende terme en hul intensiteit in sensoriese analyses, getoon. Daarbenewens speel veral smaakbeskrywende terme, insluitende suurheid, volheid, vrankheid, alkohol en konsentrasie, wat ooreenstem met basiese vrug-chemie (suiker, titreerbare suur en pH) 'n dikterende rol in die algehele sensoriese profiel. Hierdie omvattende druifsamestelling veldstudie plaas rypwordingverwante veranderinge in konteks met dié wat deur die omgewing (Oesjaar/Klimaat) en Ligging (Grond & Genotipe) teweeggebring/beïnvloed word. Dit wys op die negatiewe effek van 'n verlengde druive hangtyd en verskaf noodsaaklike insigte rakende wynsamestelling veranderinge gedurende vinnige suiker akkumulering. Nuwe en eiesoortige inligting ten opsigte van potensiële wynstyle, soos geonderskei deur verskillende rypheidsvlakke, is ingewin. Hierdie inligting kan maklik deur produsente benut word om die gewenste produkdoelwit te bereik.

This dissertation is dedicated to my wife Lizanda and baby daughter, Elmie who was born on the 8<sup>th</sup> of January 2019.

## Biographical sketch

Etienne Louis Adriaan Terblanche, was born in Paarl, Western Cape, South Africa, on 17 March 1986. He received his secondary schooling at Jan van Riebeeck High School in Cape Town before enrolling for a B.Sc. (Viticulture and Oenology) degree at Stellenbosch University in 2005. After graduation in 2008, he continued his studies in the field of Viticulture and obtained a B.Sc. Hons (Viticulture) from Stellenbosch University in 2009. He then completed his M.Sc. in Viticulture and Oenology (Vinifera Euromaster EMAVE) titled “Aroma precursors in *Vitis vinifera* L. cv. Viognier as influenced by training system and grapevine water status” at Montpellier SupAgro, France, and ISA, Lisbon, Portugal, in 2012. Subsequent to his return to South Africa, he joined Delheim Wine Estate in Stellenbosch where he worked as viticulturist, before joining Vinpro as viticultural consultant late in 2018.

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## Preface

This dissertation is presented as a compilation of seven chapters. Each chapter, is introduced separately and is written according to the style of the South African Journal of Enology and Viticulture.

**Chapter 1**      **General Introduction and Project aims**

**Chapter 2**      **Literature review**

- a. Links between grape berry ripening and wine composition
- b. *Vitis vinifera* L. cv. Pinotage

**Chapter 3**      **Research results**

Genotype x Environment x Ripeness level interaction: Effects on physiology, vegetative and yield components of unirrigated *Vitis vinifera* L. cv. Pinotage

**Chapter 4**      **Research results**

Berry composition of *Vitis vinifera* L. cv. Pinotage as related to grape ripeness level

**Chapter 5**      **Research results**

Wine composition of *Vitis vinifera* L. cv. Pinotage as related to grape ripeness level

**Chapter 6**      **Research results**

Wine sensory profile of *Vitis vinifera* L. cv. Pinotage as modulated by grape ripeness level

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# Chapter 1

## General Introduction and Project aims

### 1.1 INTRODUCTION

Grape berries undergo a sequence of complex processes during ripening (Coombe, 1976), which are thought to be evolutionary traits aimed at improving animal attraction and palatability of fruit to ensure seed dispersal and species survival (Giovannoni, 2001). So too does the outcome of these ripening processes dictate many critical wine qualitative attributes (Adams, 2006; Conde *et al.*, 2007; Bindon *et al.*, 2014). There is general consensus among producers and researchers alike that wines from earlier harvested grapes can be dominated by vegetative flavours, while later harvested grapes display greater fruit intensity (Berg & Ough, 1977; Du Plessis & Van Rooyen, 1982; Van Rooyen *et al.*, 1984). However, more recent studies (Hunter *et al.*, 2004; Nadal *et al.*, 2004; Heymann *et al.*, 2013; Bindon *et al.*, 2014) have pointed towards greater complexity between these two opposites, suggesting the potential of stylistic modulation of wine sensory profiles along a ripeness gradient (Hunter *et al.*, 2004; Hunter & Volschenk, 2018). From a practical point of view, this creates an opportunity for wine producers to alter wine composition through the choice of harvest date. However, the scope of these potential changes due to ripeness level remains largely undocumented (Heymann *et al.*, 2013).

Harvest decisions have been/and are still largely based on measurement of grape sugar content as an indicator of potential wine alcohol degree. Studies suggesting improved indicators, such as ratios of sugar/acid, that are better related to overall wine composition have been put forward (Du Plessis & Van Rooyen, 1982; Van Rooyen *et al.*, 1984; Hunter *et al.*, 2004), but have not been adopted widely. Nonetheless, commercial ripeness levels in terms sugar content have increased dramatically over time (Heymann *et al.*, 2013). For instance, recommended sugar levels at harvest first published for cv. Pinotage were 20 – 23 °B and harvest occurred during late February and early March in Stellenbosch, South Africa (Kriel, 1983; De Villiers & Theron, 1987). However, recent years have seen significant change, as commercial ripeness levels for Pinotage are currently 24 - 26 °B and now occur during late January and early February (Marais *et al.*, 2001; Van Schalkwyk & Schmidt, 2009). This shift, seen in many wine regions of the world, have been attributed to improved viticultural practices, improved plant material as well as changed consumer preferences (Heymann *et al.*, 2013; Van Leeuwen *et al.*, 2013). Moreover trends have also been specifically linked to modern era wine producers deliberately picking at late maturities in order to avoid green flavours and mitigate berry heterogeneity (also brought

about by injudicious vineyard practices) to achieve ‘phenolic’ maturity (Hunter *et al.*, 2010; Kontoudakis *et al.*, 2011; Gil *et al.*, 2012; Bindon *et al.*, 2013). However, these factors are all considered secondary to climatic variables as affected by climate change (warming) (Jones *et al.*, 2005). Research on temperature related effects indicates that increases in temperatures will certainly affect berry metabolism, as has been reported for a variety of primary and secondary metabolites (Ruffner *et al.*, 1976; Kliewer, 1977; Bergqvist *et al.*, 2001; Spayd *et al.*, 2002), essentially conceding to the possible detrimental effects of high temperatures on secondary metabolite accumulation (Tarara *et al.*, 2008). In addition, changes manifested by increases in mean temperatures will also alter overall grapevine response, leading to earlier grapevine phenology, rapid sugar accumulation and shortened harvest windows, further exasperated by decreased water resource availability for agriculture (Petrie & Sadras, 2008; Webb *et al.*, 2012; Hannah *et al.*, 2013; Sadras & Moran, 2013). The predicted changes will force producers to make harvest decisions with a smaller margin for error as shortened harvest windows will allow for less time to react and will likely create logistical challenges regarding fruit processing. Improved knowledge of the relationship between compositional changes in grape berries and wine sensory attributes will aid producers to remain competitive amidst changing climate and market related needs.

In the context of this, the effect of ripeness level was studied using local variety *Vitis vinifera* L. cv. Pinotage, which is both an early variety and displays rapid maturation similar to expected response due to climate change. Pinotage is well adapted to South African conditions (Mediterranean) and its popularity has risen dramatically in terms of vineyard plantings in the last decade (SAWIS, 2017). Vineyards are often cultivated without irrigation, which is also representative of the probable future situation. Moreover, due to the practical outlook of the study, it was undertaken in a mature commercial vineyard, cultivated unirrigated, and planted to two drought tolerant rootstocks (Ruggeri 140 and Paulsen 1103). The use of rootstocks have been proposed as a viticultural practice to better adapt to soil and climate conditions (Carbonneau, 1985; Southey, 1992; Hunter *et al.*, 2010). Some rootstocks provide increased drought tolerance and have been suggested as an important part of adaptation strategy to climate change (Serra *et al.*, 2014). These details aimed at providing a novel perspective on ripeness related changes, including the measurements from the various known influence spheres, including climate, site/soil, vineyard, and fruit and wine composition, to finally assess wine sensory attributes.

## 1.2 PROJECT AIMS

The goal of this descriptive study was to create sufficient context that would enable accurate reasoning of changes (grape/wine composition) that occur during ripening. This would improve current viticultural knowledge (cause and effect) regarding changes in potential wine style of *Vitis vinifera* L. cv. Pinotage. A field-based study pertaining multiple vintages and more than one measurement site allows for the collection of a large data set, which would include much of the variability encountered in practical viticulture. Although direct extrapolation of findings to other varieties and regions would not be possible, it would certainly provide an informative base for extrapolative ideas and future studies to build on. Finally, the study endeavoured to propose simplified practical guidelines, based on exhaustive scientific research, to producers with regard to potential wine style modification related to ripeness level.

To achieve the above, the following project aims were set:

- a) The field-based characterisation of the grapevines (physiological, vegetative and reproductive growth, berry micro-climate) and growing conditions (climate, soil) during ripening and the time leading up to the ripening period. As an additional layer, the characterisation of two drought resistant rootstocks in combination with cv. Pinotage would provide much needed knowledge of future warmer, drier, more extreme growing conditions.
- b) The detailed characterisation of berry and wine composition during ripening to provide insight into the causes of sensory effects in relation to ripeness level as well as effects of growing conditions and vineyard reaction.
- c) The characterisation of wine sensory profile changes in relation to ripeness level, by employing multiple sensory evaluation techniques in order to corroborate findings and develop a universally relevant sensory vocabulary for Pinotage.
- d) The utilisation of vineyard and grape compositional data in combination with the sensory descriptions to put forward useful practical guidelines regarding the effects of ripeness level on potential wine style.

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## Chapter 2

### LITERATURE REVIEW

#### A. Links between grape berry ripening and wine composition

##### 2.1 INTRODUCTION

Grapes (from *Vitis spp.*) are non-climacteric fleshy fruit, which undergo a sequence of complex processes during ripening (Coombe, 1976). The outcome of these ripening processes dictates many critical wine qualitative attributes (Coombe & McCarthy, 2000; Conde *et al.*, 2007; Bindon *et al.*, 2014). Grape berries can be divided into three main parts, namely the skin (exocarp), pulp (mesocarp) and seeds (surrounded by endocarp) (Coombe, 1992; Ollat *et al.*, 2002). Individual grape berries are attached to the stem (rachis) by the pedicel to collectively form a grape bunch. Berries develop heterogeneously and can differ in size (Melo *et al.*, 2015) and stage of maturity on a single bunch (Fournand *et al.*, 2006; Kontoudakis *et al.*, 2011).

Grape berry development follows a double sigmoid curve and can be divided into three stages (Coombe, 1976). Stage I commences after the fertilisation of the inflorescence and ends just prior to véraison; imported carbohydrates are used for seed development, cell division and cell enlargement (Coombe, 1992). At the same time the synthesis and accumulation of the organic acids, tartaric- and malic acid (Iland & Coombe, 1988), and tannins and hydroxycinnamates occur (Kennedy *et al.*, 2002). Pre-véraison berries are also characterised by the presence of plant hormones, auxins, cytokinins and gibberellins (Conde *et al.*, 2007). Gibberellins are linked to cell enlargement and showed a positive correlation to berry seed number, thereby exogenous application has been successfully used to increase berry size in seedless table grapes (Retamales *et al.*, 1995). Conversely, cytokinins and auxins are thought to impact berry development through protein synthesis and displays high concentrations in early berry development, before declining at Stage II (Davies *et al.*, 1997). Stage II signifies a short lag phase, before stage III that coincides with véraison, commences. At véraison, berries initiate softening and sugars, anthocyanins and aroma components are accumulated, whereas malic acid is metabolised (Ojeda *et al.*, 2002; Castellarin, Pfeiffer, *et al.*, 2007; Castellarin *et al.*, 2016). Increases in plant growth regulators abscisic acid (ABA), ethylene and brassinosteroids are associated with the onset of ripening post-véraison. Specifically ABA has been reported to positively influence the accumulation of berry sugar and phenolic compounds during the post-véraison stage (Kuhn *et al.*, 2014).

The various processes involved in grape ripening and the accumulation of secondary metabolites are highly dependent on environmental factors (Jackson & Lombard, 1993; Koundouras *et al.*, 2006), which in turn affect wine composition and sensory properties (Bindon *et al.*, 2013, 2014). Accordingly, the availability of water has been shown to directly influence grape composition (Castellarin, *et al.*, 2007a; Deluc *et al.*, 2009), but also indirectly, through altered berry size (Ojeda *et al.*, 2002) and bunch microclimate (Smart, 1985; Hunter, 2000; Santos *et al.*, 2003). Considering the expected positive relationship between increased bunch exposure and a rise in berry temperature (Bergqvist *et al.*, 2001), it is hard to separate the effects of sunlight and temperature in field conditions (Spayd *et al.*, 2002). Generally, studies conducted in warm climates have conceded negative effects of high berry temperatures on secondary metabolite accumulation (Bergqvist *et al.*, 2001; Spayd *et al.*, 2002; Reshef *et al.*, 2017). Moreover, predictions in the context of global warming have warned of concurrent advanced grapevine phenology and earlier ripening (Petrie & Sadras, 2008), compressed harvest windows (Webb *et al.*, 2012) and drought conditions, that may impact the viticultural suitability of various current winegrowing regions (Hannah *et al.*, 2013). While grapevines are thought to show resilience regarding changes in yield (Sadras *et al.*, 2017), the modulation of berry metabolism leading to altered wine composition is a major concern. Therefore studies elucidating the interactions between environment, berry ripening and composition (Dai *et al.*, 2011) play an important part in developing future adaptive strategies (van Leeuwen *et al.*, 2013; Bigard *et al.*, 2018).

This review aims to compile the relevant information regarding the changes in the grape berry during ripening that impact wine qualitative attributes. A detailed understanding of the dynamics of critical components during grape ripening is crucial as viticulturists aim to improve wine quality and differentiate various wine styles in the face of challenging climate changes that may be characterised by a shorter harvest window.

## 2.2 DISCUSSION

### 2.2.1 Role of water in grape berries and wine

Water is the most abundant constituent of both grape must and wine and acts as solvent for many minerals and primary and secondary metabolites. In most wine producing countries it is illegal to add water to dilute/alter wine and must, with the exception of the USA. Adding water to wine would essentially dilute constituents, resulting in a less flavoursome product if not coupled with other concentration techniques (Harbertson *et al.*, 2009). However, water via natural precipitation and/or irrigation is an essential element in grapevine cultivation. In fact, water is considered a major driver in grapevine reaction (Deloire *et al.*, 2004) with a multifaceted

influence on ripening (Chaves *et al.*, 2010) and decisive impact on grape composition (Etchebarne *et al.*, 2009; Intrigliolo *et al.*, 2012).

In grapevines, water is absorbed from the soil matrix mainly via fine root hairs and transported through the root system and grapevine aerial parts to the berry (Deloire *et al.*, 2004; Chaves *et al.*, 2010). In the pre-véraison period (Stage I and II), water flux to the berry is supplied via the xylem and the berry is considered sensitive to water deficit (Ojeda *et al.*, 2001). Direct decreases in xylem flux cause decreases in mesocarp cell turgor and inhibit green berry growth (Thomas *et al.*, 2006). In the post-véraison period (Stage III) active influx of water and products of photosynthesis (mainly sugars) is via the phloem and water deficit may act on berry size indirectly through a decrease in photosynthesis (Wang *et al.*, 2003), inhibiting the enlargement of mesocarp cells (Roby *et al.*, 2004). Throughout grape berry growth and ripening transpiration constitutes the major fraction of water lost by the berry (Coombe, 1992), with small losses due to xylem backflow been proposed (Etchebarne *et al.*, 2009; Tilbrook & Tyerman, 2009). Increased transpiration is linked to environmental conditions such as elevated temperatures, light incidence and evaporative demand by high vapour pressure deficits (Ollat *et al.*, 2002). Thereby the net water content in the ripening berry can be defined as the flux into the berry from the xylem (pre-véraison) and phloem (post-véraison) minus transpiration and possible xylem backflow. In the latter stages of ripening when berry size has reached a maximum and xylem and phloem vessels become progressively blocked, berry dehydration takes place due to continued transpiration (Coombe & McCarthy, 2000). This net water loss is due to the isolation of the berry from vascular transport pathways and leads to shrinking of the berry and constituent concentration (Hunter *et al.*, 2014; Carlomagno *et al.*, 2018).

Considering that many wine qualitative components such as tannins, anthocyanins and aroma compounds and their precursors are primarily located in the berry exocarp (skin), changes in skin:pulp ratio has been shown to influence grape composition (Coombe & McCarthy, 2000; Ojeda *et al.*, 2001; Kennedy *et al.*, 2002). Both pre- and post-véraison (Ojeda *et al.*, 2002; Roby *et al.*, 2004) water deficits have shown to impact berry size and increase skin:pulp ratios, while late ripening deficits leading to berry dehydration can also lead to increased berry constituent levels (Carlomagno *et al.*, 2018). At the same time, water deficits during ripening have been shown to directly influence grape composition on biosynthetic level through up regulation of anthocyanin metabolism (Castellarin, *et al.*, 2007a), also up regulating ABA, isoprenoid and carotenoid metabolism, thereby increasing secondary metabolite production (Deluc *et al.*, 2009). This could also be attributed to increased grape berry sunlight interception, as a result of decreased grapevine growth (Santos *et al.*, 2003; Keller *et al.*, 2016). Therefore, altering *e.g.* flavonoid metabolism that is known to be sensitive to changes in light and concomitant

temperatures (Downey *et al.*, 2006). For instance, flavonol glucoside concentrations in grape berries were positively correlated with increased sunlight interception (Haselgrove *et al.*, 2000; Spayd *et al.*, 2002). Moreover, the accumulation of *e.g.* anthocyanins showed greater dependency on temperature conditions than on sunlight interception (Morrison & Noble, 1990; Downey *et al.*, 2006), displaying both synergistic and antagonistic interactions with temperature and sunlight (Tarara *et al.*, 2008). Ultimately, inter-dependent reactions such as the above highlight the multi-dimensional effect of grapevine water status on grape/wine composition. This necessitates a well-developed understanding of grapevine reaction in order to manage plant water status to achieve the desired wine goals.

### **2.2.2 Role of sugar in grape berries and wine**

Sucrose is produced through photosynthesis in the mesophyll cells of mature leaves and is the principal sugar translocated to the grape berry (sink) (Coombe, 1992; Hunter & Ruffner, 2001). Sucrose is loaded into the phloem vessels of source cells via sieve element companion cell complexes (se/cc) by either a symplastic or (most likely) apoplastic mechanism. Moreover, sucrose, as the principal osmotic component of the phloem, is thought to drive the translocation of all the other phloem-bound constituents through mass-flow along the osmotic gradient. Upon its arrival in the berry phloem, sucrose may be unloaded by different pathways; prior to véraison, most of the imported sucrose is metabolised by the green berry and phloem unloading occurs via the symplastic route (Ollat *et al.*, 2002). However, after véraison the unloaded sugars are stored in the vacuoles of berry mesocarp cells which become progressively isolated from the se/cc complex enabling phloem unloading despite the high concentration of sugars at the sink (Hunter & Ruffner, 2001; Wang *et al.*, 2003). Once sucrose is unloaded from the se/cc complexes into the apoplastic area (sink) (Zhang *et al.*, 2006), cell wall invertase cleaves sucrose into glucose and fructose. The cleaved products are then transported across the plasma membrane and tonoplast into the vacuole with the aid of monosaccharide transporters. Some sucrose may also be cleaved by sucrose synthase in the cytosol or be taken up directly by disaccharide transporters into the vacuole and cleaved there (Agasse *et al.*, 2009; Lecourieux *et al.*, 2014). The concurrent up regulation of aquaporins and sugar transporters in the plasma membrane and tonoplast during ripening supports the hypothesis that sugar and water transport are linked (Fouquet *et al.*, 2008; Dai *et al.*, 2011; Kuhn *et al.*, 2014). Sucrose loading continues throughout ripening until phloem flow becomes interrupted by loss in cell membrane integrity/vitality terminating the compartmentalisation of glucose and fructose and thereby nullifying the osmotic gradient of the sink (Rogiers *et al.*, 2017).

The fermentation of sugars present in the grape berry (glucose and fructose) and conversion to ethanol (EtOH), CO<sub>2</sub> and heat by the *Saccharomyces cerevisiae* yeast is a key process in wine production. As grape berries accumulate sugar during ripening, wine potential EtOH content increases ( $\approx 17\text{g/L}$  per 1% alc v/v). If the goal is to produce naturally sweet wines, the fermentation can be stopped before all the sugar is consumed by the yeast, *e.g.* by removing yeast cells (filtration or centrifugation), addition of anti-microbial agents (such as SO<sub>2</sub>) or by fortification (addition of EtOH) (Ribéreau-Gayon *et al.*, 2006b). Ethanol toxicity is primarily associated with the damage of cell membranes and transport systems (Matallana & Aranda, 2017). Excessively high must sugar content associated with advanced grape maturity, firstly incurs increased osmotic stress to yeast cells and secondly allows for yeast EtOH toxicity to occur before the sugar is consumed completely. The unfermented sugars (mainly fructose) may facilitate the growth of wine spoilage yeasts and bacteria (Ribéreau-Gayon *et al.*, 2006b). In addition to fructose, wines also contain small amounts of other sugars, such as arabinose, xylose, ribose, rhamnose, and galactose (Vidal *et al.*, 2003). The residual sugars in dry wines ( $< 5\text{g/L}$ ) have a significant impact on sensory properties. Apart from sweetness *per se*, residual sugars in wine may increase wine body, mouthfeel and viscosity, while it can also negate astringency (Brandão *et al.*, 2017). Because of its critical sensorial impact, wine sugar content is often used as stylistic denominator ranging from dry ( $< 5\text{g/L}$ ) to semi-sweet (5 – 30 g/L) and sweet ( $> 30\text{g/L}$ ) wines (SAWIS, 2017). Equally, EtOH has been reported to increase wine sensory properties, such as body, sweetness (Zamora *et al.*, 2006), viscosity and hotness (Gawel *et al.*, 2007) and may also reduce the perception of astringency (Gawel, 1998) and increase bitterness (Fischer & Noble, 1994). In addition, high EtOH concentrations decrease the headspace concentration and partitioning of volatile compounds in model wines (Villamor *et al.*, 2013), which may result in a reduction of perceived aromas (Escudero *et al.*, 2007; Golnder *et al.*, 2009). Interestingly, in studies where unripe grapes were chaptalised (sugar added to must to increase wine EtOH content) to a similar level as found in ripe grapes, the additional EtOH improved the sensory properties of the unripe wines (Casassa *et al.*, 2013; Sherman *et al.*, 2017). These wines displayed greater levels of sweetness, mouth-feel and viscosity than unchaptalised wines (Casassa *et al.*, 2013). Sherman *et al.* (2017) postulated that wine EtOH content *per se* had a greater influence on wine sensory attributes than grape ripeness level.

### **2.2.3 Role of minerals in grape berries and wine**

Potassium (K<sup>+</sup>) is the most abundant mineral in grape must and wine and has a decisive impact on wine quality (Mpelasoka *et al.*, 2003). Excessive amounts of K<sup>+</sup> in must and wines lead to high wine pH, through the exchange of K<sup>+</sup> with hydrogen (H<sup>+</sup>) from free tartaric acid and

subsequent precipitation of insoluble potassium bi-tartrate and di-tartrate. Thereby increasing wine pH and lowering the must/wine free-acid content. Low acid/high pH musts are susceptible to oxidative and microbial spoilage, because of the decreased molecular sulphur dioxide fraction (added preservative) and improved wine conditions for microbial growth. In growing conditions prone to high  $K^+$  must content, pH is lowered by costly tartaric acid additions. Further precipitation of the added tartaric acid due to high must  $K^+$  is common and increases pH correction costs. Increased wine pH also decreases red wine colour stability, as a result of reduced presence of the red flavilium cation form of anthocyanins (Adams, 2006). Anthocyanins are located in the berry skin where  $K^+$  concentration is also present in greater amounts than in the pulp. Colour intensity is thus already affected inside the intact berry skin. Extraction of coloured anthocyanins during red wine making coincides with further leaching of skin  $K^+$  to the must and pH increases. Finally, wines characterised by high pH exhibit 'flat' and excessively 'round' tastes when compared with wines with lower pH (Fischer & Noble, 1994). In the light of the significant role of grape  $K^+$  content on wine quality, understanding the dynamics of grape berry potassium accumulation is paramount in the attempt to mitigate the negative effects related to excessive concentrations.

#### *2.2.3.1 Potassium accumulation during ripening*

Notwithstanding the negative effects of excessive  $K^+$  content on wine quality, potassium is an essential, highly mobile macronutrient in plants and has a strong role in regulating cell membrane potential mediating the uptake of other ions and sugars (Rogiers *et al.*, 2017). Potassium plays a key role in grapevine functioning through its involvement in plant signalling, osmoregulation, maintaining cytoplasmic pH, enzyme activation, and protein and starch synthesis. At whole plant level, it is associated with photosynthesis, plant water relations, turgor maintenance and phloem transport (Mpelasoka *et al.*, 2003).

Potassium is taken up from the soil via the roots through root cell membrane transporters and channel proteins. Transport of highly mobile  $K^+$  to shoots, leaves, storage organs and grape berries is principally affected via the phloem (Keller, 2015). Remobilisation of  $K^+$  from storage organs to fruit has been shown (Conradie, 1981), but to date these mechanisms are poorly understood (Rogiers *et al.*, 2006).

In conjunction with sugar accumulation (Fig 1),  $K^+$  accumulates rapidly in grape berries during ripening (Ollat *et al.*, 2002). However, prior to véraison the accumulation rate of  $K^+$  into berries is relatively slow (Ollat & Gaudillère, 1996) at approximately 20-40 mg per day (Rogiers *et al.*, 2006) and then accelerates 2-4 fold post-véraison (Rogiers *et al.*, 2017) to reach a maximum at maximum berry weight (Esteban *et al.*, 1999). Berry pedicel girdling studies confirmed that the

phloem is the major route for  $K^+$  import into the berry post-véraison, coinciding with the shift from xylem to phloem dominated supply of water to the berry (Coombe & McCarthy, 2000). Studies detailing post-véraison Grenache and Shiraz berry  $K^+$  compartmentalisation found 59-60% of total berry  $K^+$  accumulates in the mesocarp, 32-37% in the exocarp and 3-6% in the seed (Rogiers *et al.*, 2006; Etchebarne *et al.*, 2009). Moreover,  $K^+$  concentrations in the exocarp/skin were found to be 2-6 fold higher than in the mesocarp/pulp (Iland & Coombe, 1988). During late ripening  $K^+$  accumulation slows with corresponding cessation of phloem functioning. This is followed by the breakdown of mesophyll membranes terminating compartmentalisation and filling the apoplastic space with sugars, thereby nullifying the osmotic gradient and arresting  $K^+$  accumulation. Potassium concentration can then only increase through berry dehydration (Carlomagno *et al.*, 2018).

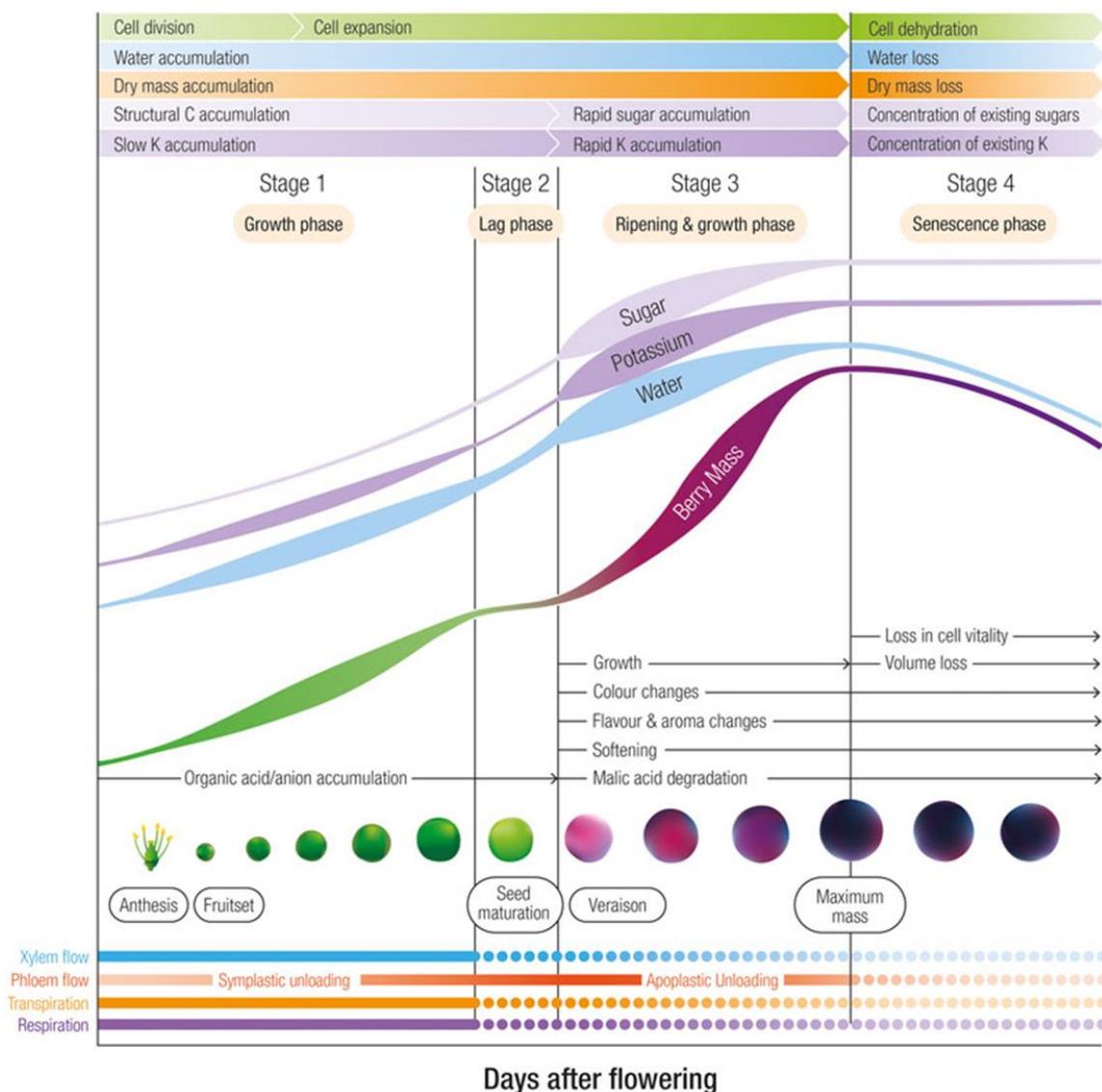


Figure 1. Summary of the developmental stages of berry growth, specifically indicating the accumulation of sugar, potassium and water in context of other important plant regulatory processes (taken from Rogiers *et al.*, 2017).

### 2.2.3.2 Importance of other minerals

Other minerals, such as nitrogen, calcium and magnesium, together with minimal amounts of sodium, phosphorous, zinc and manganese are also present in grape berries (Conradie, 1981; Rogiers *et al.*, 2006). Broadly speaking, minerals can be divided into two groups regarding their accumulation during ripening (1) minerals that continue to accumulate throughout berry growth and (2) minerals that accumulate mostly prior to véraison. The first group, including potassium (as discussed above), sulphur, phosphorus, boron, iron and copper are considered phloem mobile and rates of accumulation are consistent with increased phloem flow after véraison. Therefore, apart from the content available for uptake from the soil, the functioning of the phloem and grape water relations after véraison all impact on the accumulation of these minerals (Etchebarne *et al.*, 2009). Although these minerals are present in small quantities, they may impact grapevines and resulting wines. Especially nitrogen, in the form of amino acids plays an important part in must composition, as it is essential for yeast growth, fermentation kinetics and flavour metabolism (Bell & Henscke, 2005). The major volatile compounds derived from sugar and amino acid metabolism include; higher alcohols, esters, carbonyl compounds, volatile fatty acids and sulphur-related compounds that decisively impact wine aroma (Swiegers & Pretorius, 2007). Low amounts of yeast assimilable nitrogen leads to stuck and sluggish fermentation, which negatively impacts wine quality (Bisson & Butzke, 2000). The major amino acids found in grape musts are arginine (yeast assimilable) and proline (not yeast assimilable), and relative ratios differ considerably among different varieties (Garde-Cerdán *et al.*, 2009). Amino acids accumulate in grape berries during ripening and are often at their peak at commercial ripeness levels *e.g.* 25°B for Grenache before declining in later ripeness levels > 25°B (Garde-Cerdán *et al.*, 2018). Thereby ripeness level will in combination with variety and growing conditions also impact the nitrogen content of the must which will affect yeast metabolism and wine aromatic characteristics.

Other minerals such as magnesium is an important component of chlorophyll and its absence can impact photosynthesis negatively. Grapes are generally poor in phosphorous, iron and copper. However, unusual high amounts of these compounds may influence wine quality through *e.g.* protein haze formation ( $\text{Fe}^{2+}$ ) and the oxidation of positive thiol-containing aroma compounds ( $\text{Cu}^{2+}$ ) (Ribéreau-Gayon *et al.*, 2006a).

The second group of minerals consisting of calcium, manganese and zinc generally accumulate only prior to véraison, and berry import is restricted to xylem functioning (Rogiers *et al.*, 2006), thereby the accumulation of these minerals would be directly impacted by pre-véraison water relations, and concentration during ripening subject to the impact of the import of water to the

berry during the post-véraison period (Esteban *et al.*, 1999). Calcium has a significant role in strengthening grape berry cell walls, providing resistance against *Botrytis cinerea* infection (Hocking *et al.*, 2016). In grape must, it can also increase wine pH by combining with tartaric acid and precipitating as  $\text{Ca}^{2+}$  tartrate (Ribéreau-Gayon *et al.*, 2006a).

## 2.2.4 Role of organic acids in grape berries and wine

The presence of relatively high concentrations of organic acids in grape must and wine is a distinctive attribute of the produce of *Vitis vinifera*. Of the organic acids, primarily tartaric and malic acids impart important sensory characteristics to wines; high levels are associated with tart, hard and sour tastes, while low levels produce flat, dull, soapy tastes (Fischer & Noble, 1994; Demiglio & Pickering, 2008; Fontoin *et al.*, 2008). The presence of free organic acids in combination with the quantity of  $\text{K}^+$  present impacts wine pH. As discussed, wine pH dictates many wine reactions, colour stability, molecular  $\text{SO}_2$  content, inhibits microbial growth of spoilage yeasts and bacteria, and affects hydrolysis of aroma components from glycoside precursors during ageing (Francis *et al.*, 1992; Ribéreau-Gayon *et al.*, 2006b; Loscos *et al.*, 2009). In addition to tartaric and malic acid, oxalic, citric, fumaric, succinic, shikimic and acetic acids occur either irregularly or only in trace amounts (Hunter *et al.*, 1991).

### 2.2.4.1 Dynamics of tartaric acid metabolism during ripening

Tartaric acid is the most abundant and strongest acid present in grape musts/wine and constitutes 60 - 70% of all titratable acidity in wine. Therefore it is the main contributor to lowering wine pH and precipitation with  $\text{K}^+$  and  $\text{Ca}^{2+}$  to form  $\text{K}^+$  bi-tartrate and di-tartrate and  $\text{Ca}^{2+}$  tartrate crystals. Through this action the content of tartaric acid decreases during the fermentation and maturation to up to 2/3 of its original content.

Contrary to most organic acids found in grapes, the metabolic origin of tartaric acid falls outside the oxidative metabolism of sugars (Loewus & Stafford, 1958). Tartaric acid biosynthesis is thought to initiate from possible precursors, hexose, pretaric- and L-ascorbic acid and can follow one of two pathways in grape berries and leaves (DeBolt *et al.*, 2004). Generally in plants of the *Geraniaceae*, cleavage of a six-carbon intermediate between either position C2/C3 or C4/C5, depending on the plant species, yields oxalic acid and L-threonate, which is then converted to tartaric acid in the leaves (Hale, 1962). However Loewus (1999) proposed a direct pathway to predominate in the *Vitaceae*, yielding tartaric acid and a two-carbon compound, possibly glycol-aldehyde. Studies by DeBolt *et al.* (2006) identified the gene and characterised the coded enzyme responsible for the proposed rate limiting step in tartaric acid biosynthesis from ascorbic acid. Concluding that L-idonate dehydrogenase (L-IdnDH) catalyses the conversion of L-idonate

to 5-keto D-gluconic acid. In grape berries, tartaric acid biosynthesis occurs exclusively from anthesis to véraison (Coombe & McCarthy, 2000; DeBolt *et al.*, 2006). Tartaric acid accumulates in the vacuoles of mesocarp cells at the periphery of the grape berry and diminishes in concentration as the berry size increases post-véraison due to imports of sugar and water (Iland & Coombe, 1988; Coombe, 1992). Although absolute grape berry tartaric content remains stable post-véraison, previous reports have shown increases in grape berry pH in the latter ripening stages due to tartaric precipitation with  $K^+$  (Ollat *et al.*, 2002). Grape berry tartaric content has also been shown to be dependent on berry size, modulated by irrigation, often displaying greater per berry content in irrigated treatments, but lower concentration than non-irrigated treatments (Esteban *et al.*, 1999). In addition, decreased sunlight interception has shown to decrease berry size and modulate berry tartaric and oxalic acid content, showing diminished tartaric acid contents for small berries completely shaded. This has led to the hypothesis that organic acids and in particular tartaric acid may act on the osmotic potential of the grape berry (DeBolt *et al.*, 2008).

#### 2.2.4.2 Dynamics of malic acid metabolism during ripening

Malic acid confers a green apple-like taste to grape must and wines, in red wines malic acid is predominantly completely consumed during malo-lactic fermentation (Volschenk & Van Vuuren, 2006). Malo-lactic fermentation involves the conversion of malic acid to lactic acid, reducing the tartness of the wines, while also imparting complexity of flavour. This is often also done as precaution prior to bottling to prevent malo-lactic fermentation in bottle (Ribéreau-Gayon *et al.*, 2006b).

Unlike many other fruits, grapes do not contain large amounts of citric acid, and the large quantity of tartaric acid present in the fruit is not used in primary metabolic pathways. Therefore malic acid is the only organic acid in high quantities that is actively metabolised throughout ripening of grapes (Sweetman *et al.*, 2009). The net loss of grape berry malic acid is due to metabolic degradation during ripening, which occurs after an earlier period of net accumulation (Coombe, 1976). Pre-véraison grapes accumulate malic acid mostly through the metabolism of sugars that have been translocated to the berry, but also potentially through fruit photosynthesis (Hale, 1962). The switch from net accumulation to degradation of malate occurs just before véraison, when malic acid content is at its highest (Ruffner *et al.*, 1976). Previous works suggest that  $\beta$ -carboxylation of phosphoenolpyruvate is the most important pathway involved in malic acid synthesis (Hunter & Ruffner, 2001; Sweetman *et al.*, 2009). The enzyme, phosphoenolpyruvate carboxylase catalyses the step leading to the formation of oxaloacetate, which is then reduced to malate by a cytosolic malate dehydrogenase (Ruffner & Kliewer, 1975).

On the contrary, levels of malic acid in harvested fruit may be largely determined by the rate of degradation during ripening. Results presented in molecular studies suggest the involvement of pyruvate in the degradation of malic acid in grape berries, as the expression of enzymes involved in pyruvate metabolism was generally favoured during ripening (Deluc *et al.*, 2007, 2009; Sweetman *et al.*, 2009). In particular, cytosolic NADP-malic enzyme is considered to play a key role in the regulation of malate breakdown catalysing the oxidative decarboxylation of malic acid to pyruvate and CO<sub>2</sub> (Ruffner & Kliewer, 1975; Ruffner *et al.*, 1984). An additional breakdown pathway involves the diffusion of malic acid into the mitochondria and its subsequent degradation by a mitochondrial malate dehydrogenase forming oxaloacetate or, alternatively, its oxidation to pyruvate through the action of a mitochondrial NAD-malic enzyme. This effectively implies that cytosolic and mitochondrial isoforms of malate dehydrogenase could participate in both malate synthesis and catabolism in response to metabolic changes occurring during grape development (Sweetman *et al.*, 2009).

Grapes cultivated in cool conditions generally display greater concentrations of malic acid during ripening than grapes cultivated in warm conditions (Jackson & Lombard, 1993). Equally on a microclimatic level, grapes receiving less sunlight exposure exhibit greater malic content than those from more exposed microclimatic conditions (Hunter, Volschenk, *et al.*, 2004). This negative correlation between temperature and malic acid levels is due to the effect of temperature on the balance between malic acid synthesis and catabolism (Ruffner *et al.*, 1984). The negative correlation between mitochondrial malate dehydrogenase activity and malate concentration during ripening supports the notion that mitochondrial malate dehydrogenase participates in malate degradation in response to increased temperatures. In contrast, the cytosolic isoform of malate dehydrogenase functions at lower temperatures, reducing oxaloacetate to malate (synthesis). Moreover, the temperature sensitivity of phosphoenolpyruvate carboxylase and malic enzyme was elucidated by Lakso & Kliewer (1975), showing that the activity of malic enzyme increases with increasing temperature up to 46°C, indicating high thermal stability. In contrast, phosphoenolpyruvate carboxylase has a temperature maximum of *c.a* 38°C, with a rapid heat inactivation above this temperature. The differential temperature responses of these anabolic and catabolic enzymes are responsible for malic acid levels in grape berries, with proposed optimal temperature ranges for malic acid accumulation identified as 25-30 °C and maintenance 20-25 °C (Lakso & Kliewer, 1975). Physiological data show that the decrease in malic acid during maturation is the result of reduced malate synthesis in combination with an accelerated catabolic rate (Ruffner & Kliewer, 1975; Ruffner *et al.*, 1984; Conde *et al.*, 2007; Sweetman *et al.*, 2009).

### 2.2.5 Role of phenolic compounds in grape berries and wine

Phenolic compounds are major contributors to the distinctive attributes of wine, this large group of compounds impact wine on various sensory levels, including visual, taste and tactile influences (Kennedy, 2008). The predominant fractions of grape phenolics are odourless. However grape isolates have confirmed the presence of small amounts of volatile phenols released from glycosidically bound precursors during ripening that may contribute clove, smoky, and spicy aromas to wines (Yuan & Qian, 2016a, 2016b).

Odourless anthocyanins and flavonols are pigmented compounds and represent the phenolic groups that influence the appearance of wines. Flavonols are yellow pigments which are present in far lesser amounts than red pigmented anthocyanins in red grapes and wines; however they can significantly contribute to wine colour in anthocyanin co-pigmentation reactions (Downey *et al.*, 2006). As the major class of pigmented phenolics, anthocyanins impart colour to wines, while involvement in taste have been linked to their presence by increasing extraction of flavan-3-ols during winemaking (Kilmister *et al.*, 2014). Flavan-3-ol monomers (catechin and epicatechin) are identified by their contribution to mouth feel (tactile) properties in wines, they can combine to form oligomers and polymers called condensed tannins. Tannins (proanthocyanidins) are linked to the perception of astringency in red wines (Gawel, 1998). Astringency is a complex descriptor as its intensity and duration can be influenced by a variety of factors in combination with tannins. The –OH groups from tannin molecules bind to salivary proteins, inducing precipitation and causing a loss of oral lubrication, which triggers a drying sensation on the palate (Fischer & Noble, 1994; Gawel, 1998). Apart from astringency, tannins can also impart bitterness, which is a taste sensation which increases for lower molecular weight tannins (Arnold *et al.*, 1980; Noble, 1995). Generally speaking, excessive astringency and bitterness may impact wine quality negatively, while the same holds true for inadequate levels. Considering tannin perception in total, the astringency of tannins also displays a distinct temporal aspect to their perception, the astringency of a wine can linger beyond that of other components, which is generally considered undesirable (Noble, 1995). In addition, the sensorial impact of tannins seems to be more complex than simple differing intensities of astringency/bitterness as Gawel *et al.* (2007) correlated a range of mouth feel/texture descriptors to wine tannin content. From further investigations, it is clear that the perception of astringency in wine could be influenced by many components in wine, including ethanol (Fontoin *et al.*, 2008; Casassa *et al.*, 2013), acidity (Fischer & Noble, 1994), viscosity (Gawel *et al.*, 2007) sugars (Vidal *et al.*, 2003) and anthocyanins (Vidal *et al.*, 2004; Kilmister *et al.*, 2014). In the light of this, it is clear that in addition to possible changes in phenolic profile during ripening,

various components (which can also be modulated by ripeness level) prove to influence tannin perception. In a third dimension, human response to astringency and bitterness is highly varied, making it very difficult to conduct studies assessing these attributes in a reliable manner (Fischer *et al.*, 1994). This indicates towards the challenges regarding interpretation of quantitative tannin measures, without sound sensorial proofing and emphasises the need for continued research in this area.

#### 2.2.5.1 Anthocyanin accumulation during ripening

Anthocyanins (red pigments) accumulate in the vacuoles of grape berry skins with the onset of véraison, and are products of the phenyl-propanoid pathway (Fig 2), differing from other flavonoids in the final step, where cyanidin is converted to anthocyanin *via* the enzyme anthocyanin-3-O-glucosyltransferase (Bogs *et al.*, 2007). Differences in hydroxylation on the B-ring produces five distinct anthocyanin-3-O-glucosides, which include delphinidin- (Delf), cyanidin- (Cya), petunidin- (Pet), peonidin- (Peo), and malvidin- (Malv). Malvidin-monoglucosides are the most prevalent and exclusive anthocyanin forms present in *Vitis vinifera* varieties, while di-glucosides are present in non-*vinifera Vitaceae spp.* In addition to free monoglucosides, most varieties produce acetyl-, p-coumaryl- and to lesser extent caffeoyl-glucoside derivatives (Fong *et al.*, 1971; Roggero *et al.*, 1986). Essentially, this leads to significant varietal variation in anthocyanin acylation, which produces unique anthocyanin profiles for different varieties (De Villiers *et al.*, 2004; Guidoni & Hunter, 2012). Anthocyanins increase during ripening to reach a maximum at harvest (24-25°B) (Ribéreau-Gayon, 1971; Holt *et al.*, 2010) or decline just prior to harvest and/or during over-ripening (Roggero *et al.*, 1986). Increases during ripening of methoxylated forms (Malv, Peo and Pet) have been reported while p-coumaryl glucoside peaks early and decreases during late ripening (Fournand *et al.*, 2006). Understanding ripeness related changes under field conditions is challenging as anthocyanins and upstream metabolism are highly responsive to cultural conditions (Reshef *et al.*, 2017). Fruit sunlight exposure was found to modulate the composition of anthocyanins, increasing the proportion of acylated and coumarylated forms, (Downey *et al.*, 2006) as well as Malv (Tarara *et al.*, 2008) and its derivatives. In addition, high night temperatures compared to low night temperatures reduced the proportion of Delf, Cya and Pet (Mori *et al.*, 2005). In the context of general environmental reactions moderate increases in sunlight and temperatures increased anthocyanin potential, but antagonistic effects are prevalent in cases of excessive exposure and berry temperatures, which can mitigate ripening effects (Spayd *et al.*, 2002; Castellarin *et al.*, 2007b; Tarara *et al.*, 2008).

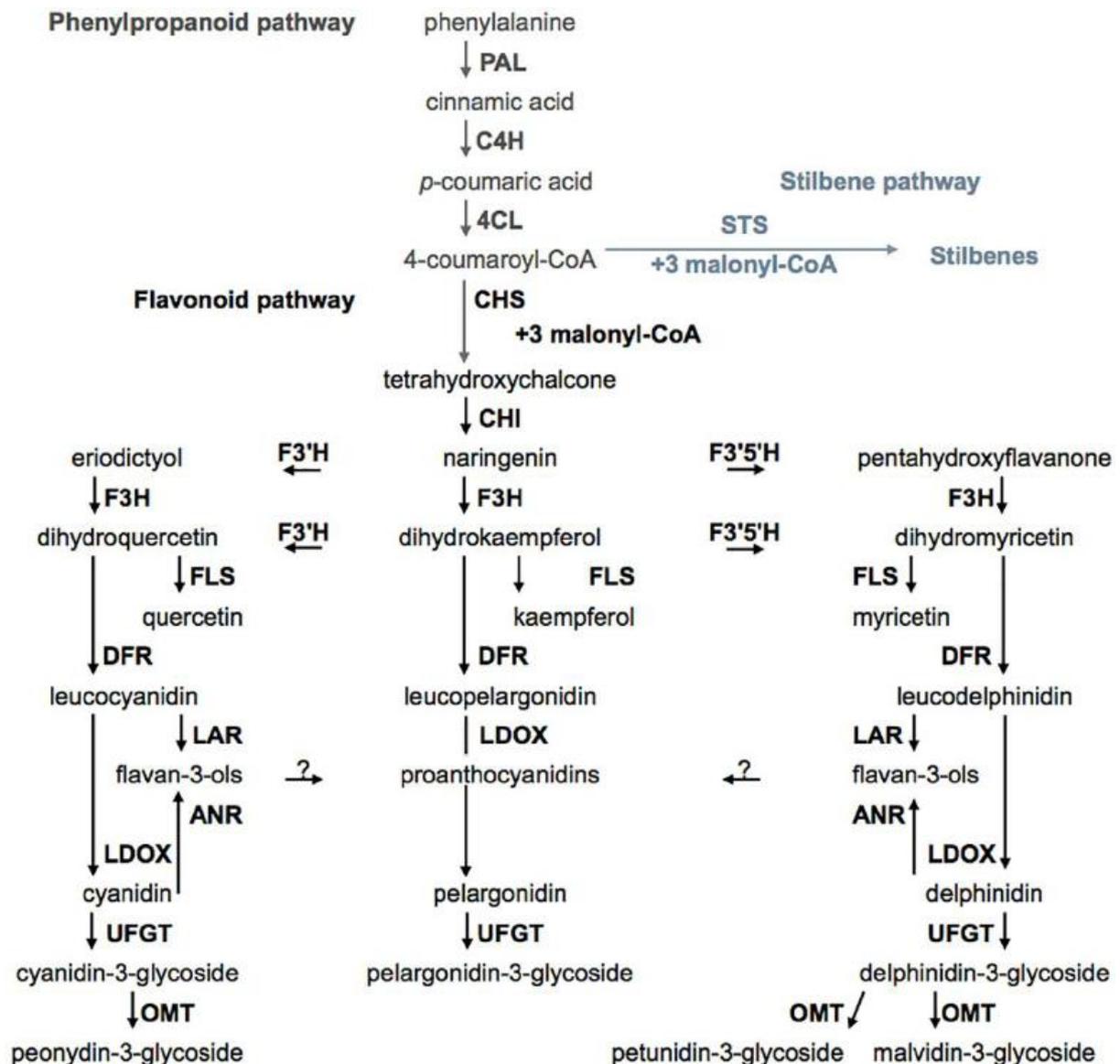


Figure 2. Biosynthetic pathways of grape berry secondary compounds. Phenylalanine ammonia lyase (PAL), cinnamate-4-hydroxylase (C4H), 4-coumaroyl:CoA-ligase (4CL), stilbene synthase (STS), chalcone synthase (CHS), chalcone isomerase (CHI), flavonoid 3'-hydroxylase (F3'H), flavonoid 3',5'-hydroxylase (F3'5'H), flavanone-3-hydroxylase (F3H), flavonol synthase (FLS), dihydroflavonol reductase (DFR), leucoanthocyanidin reductase (LAR), anthocyanidin reductase (ANR), leucoanthocyanidin dioxygenase (LDOX), dihydroflavonol 4-reductase (DFR), flavonoid glucosyltransferase (UGFT), O -methyltransferase (OMT) (taken from Teixeira *et al.*, 2013)

#### 2.2.5.2 Proanthocyanidin accumulation during ripening

Biosynthesis of tannin subunits originate from phenylalanine and follow the phenylpropanoid pathway to form polymers of flavan-3-ols (proanthocyanidins) (Bogs *et al.*, 2007). The concentration of grape proanthocyanidins increases throughout berry development (Kennedy, 2008). Starting at fruit set, tannins and hydroxycinnamic acids increase until véraison. Tannins are located in the skin, pulp and seed of the berry (Adams, 2006). The relative contributions of the different berry parts has become a subject of intense research (Downey *et al.*, 2003; Roby & Matthews, 2004), as tannin localisation seems to have a distinctive effect on tannin properties,

rate of accumulation, extraction during winemaking and final wine sensory properties (Harbertson *et al.*, 2002; Kennedy *et al.*, 2002). Post-véraison changes in total skin tannin content are generally minor on a biosynthetic level (Harbertson *et al.*, 2002), and final concentration is largely dependent on changes in berry fresh mass, during this period (Ojeda *et al.*, 2002; Roby *et al.*, 2004). However, skin tannins show a definitive tendency to increase in polymerisation during ripening (Kennedy *et al.*, 2002), for instance; increasing in mean degree of polymerisation (mDP) from 7.3 in green berries, to 11.3 in red berries just after véraison to 27 in Shiraz berries at harvest (Kennedy *et al.*, 2001). On the other hand, seed tannin content decreases rapidly after the onset of véraison (Kennedy *et al.*, 2000). However the timing of this decline is highly vintage dependent (Harbertson *et al.*, 2002). This decline is accompanied by colour changes that occur in the seed (green to brown) and has been linked to the oxidation of tannins present in the coat of the seed (Kennedy *et al.*, 2000). In addition, Downey *et al.* (2003) attributed the ripening related decline in flavan-3-ols from seeds to increased covalent bonds of extension units, making them unavailable for extraction during winemaking. Considering the whole berry, the general consensus is that mDP increases during ripening (Kennedy *et al.*, 2002; Downey *et al.*, 2003; Fournand *et al.*, 2006). These increases in mDP have been implicated with a decrease in wine astringency (Gawel, 1998; Vidal *et al.*, 2002; Monagas *et al.*, 2005; Kennedy, 2008). This is thought to be brought about by a decrease in highly astringent low molecular weight monomeric (low mDP) content of seeds (Harbertson *et al.*, 2002). Thereby, Ristic *et al.* (2010) could link higher grape skin tannin and anthocyanin content and lower seed tannin with increased wine sensory quality. However, seed tannin generally displayed poor relationship to wine tannin in the same study, highlighting the complex interaction during extraction (Bindon *et al.*, 2017). From the research gathered in different growing conditions, changes in biosynthesis and accumulation are also highly reactive to growing conditions (Ojeda *et al.*, 2002; Cadot *et al.*, 2006; Downey *et al.*, 2006; DeBolt *et al.*, 2008). In addition, due to the large structural variation, measurement of proanthocyanidins has proven to be highly subject to analytical methods, and much work is needed in order to fully elucidate the myriad of possible components.

## **2.2.6 Role of aroma precursors in grape berries and wine**

### *2.2.6.1 Varietal aroma*

Aroma can originate from many sources during the winemaking process. Sources include grape metabolism, pre-fermentative reactions, fermentation products, and changes during wine ageing (Ribéreau-Gayon *et al.*, 2006a; Robinson *et al.*, 2014). However, the source donating the distinctiveness of the variety lies undoubtedly in the aromatic (free and bound) composition of the grapes (Bravdo, 2001) as they form the boundaries within which all the other processes may

act. Based on their relative importance to varietal aroma, aroma compounds may be classified as impacting, contributing or insignificant (Jackson, 2014). Impacting compounds are associated with the distinctive varietal aroma of wines. Contributing compounds generally add to the complexity of wine, but do not directly impact on varietal distinctiveness. Nevertheless, a major part of aromatic compounds found in grapes are often encountered below perception thresholds (insignificant), yet they appear to play a vital role in overall wine perception (Jackson & Lombard, 1993; Jackson, 2014; Robinson *et al.*, 2014)

The most widely studied impacting compounds in *Vitis vinifera L.* belong to the terpene family. These compounds are responsible for the characteristic aroma of the “Muscat” varieties. They are also present in lower amounts in other varieties such as Syrah and Cabernet Sauvignon, yet still have an impacting contribution (Marais, 1983; Bureau, Baumes, *et al.*, 2000; Ribéreau-Gayon *et al.*, 2006b). Other compounds impacting varietal aroma include norisoprenoids, methoxypyrazines and thiols. Norisoprenoids are formed from the breakdown of carotenoids and are present in grapes in a glycoside form (Razungles *et al.*, 1993; Yuan & Qian, 2016a). The norisoprenoids like  $\alpha$ - and  $\beta$ -ionone (violet character) and  $\beta$ -damascenone (red fruit/ tobacco) and its derivatives are thought to contribute distinctive characteristics to wines of Syrah and Cabernet Sauvignon (Pineau *et al.*, 2007) and have been isolated in wines of Pinotage (Waldner & Marais, 2002; Weldegergis *et al.*, 2011). Methoxypyrazines are nitrogen-containing compounds present in grapes as volatiles and do not undergo modification during winemaking (Lacey *et al.*, 1991; Romero *et al.*, 2006). They contribute the grassy, herbaceous character synonymous with varieties such as Cabernet Sauvignon and Merlot (Lacey *et al.*, 1991; Roujou de Boubée *et al.*, 2000). The thiols, such as 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA) are sulphur containing volatiles and contribute distinctive *tropical* and *passion fruit* characteristics to Sauvignon blanc, Colombar and Chenin blanc (Du Plessis & Augustyn, 1981; Tominaga *et al.*, 1998). These compounds are thought to be formed by yeasts from S-cysteine conjugates found in grapes (Tominaga *et al.*, 1998, 2006), but have recently also been shown to be produced from C6-alcohol and aldehyde precursors, also present in grapes (Harsch *et al.*, 2013).

The C6-alcohols and aldehydes (*herbaceous*) and aromatic alcohols (*fruity*) are also present in high concentration in most varieties in glycosylated forms. However, the perception thresholds of these components are also relatively high and these compounds are thought to contribute to the complexity and background of wine aroma, rather than induce distinctive varietal specific aroma perception (Bakker & Clarke, 2012; Jackson, 2014).

### 2.2.6.2 C6 Compounds

C6 compounds (aldehydes and alcohols), also known as green leaf volatiles (GLV) are associated with the herbaceous and grassy characteristics of wines (Dubois, 1994). These aromas are considered as detrimental to perceived wine quality if they are present in quantities above the perception threshold (Bakker & Clarke, 2012). They are found in high concentrations as glycosides in the skin and solid parts of the grape berry (Gomez *et al.*, 1994). Importantly, the C6-aldehydes and alcohols derive from the oxidation of grape polyunsaturated fatty acids (C18), such as oleic, linoleic and linolenic acid initiated by the lipoxygenase pathway when berries are crushed (Fig 3). Four enzymatic activities are sequentially initiated during this pathway. Firstly, an acyl-hydrolase frees the fatty acids with 18 carbon atoms from membrane lipids. Next, a lipoxygenase catalyses the fixation of oxygen and finally the peroxides obtained are spilt into C6 aldehydes. Some of these may be further reduced to their corresponding alcohols, by an alcohol dehydrogenase. However the C6-aldehydes are the dominant form present in grapes. By this action, grape processing techniques allowing for the increased mechanical breakdown of grape solids generally lead to increased C6-induced herbaceous characters in wine (Coelho *et al.*, 2006; Hendrickson *et al.*, 2016). Ripeness level has been shown to affect concentrations of C6 compounds, which was generally associated with wines made from unripe grapes (Dubois, 1994; Ribéreau-Gayon *et al.*, 2006a). Recent works have shown distinctive evolution in C6-aldehydes and alcohols for different varieties during ripening (Gomez *et al.*, 1994; Vilanova *et al.*, 2012; Bindon *et al.*, 2013), for instance aldehydes were the major C6 constituent found in Riesling, while alcohols dominated the C6 component in Cabernet Sauvignon (Kalua & Boss, 2010). This has important sensory implications as C6 aldehydes have significantly lower perception thresholds than C6 alcohols (Bakker & Clarke, 2012). Increases in C6-compounds from véraison to harvest have been recorded in many growing conditions (Canuti *et al.*, 2009; Rocha *et al.*, 2010; Vilanova *et al.*, 2012), however, detailed information regarding these components during late stage maturity is sparse (Bindon *et al.*, 2014). The C6 aldehydes are generally the dominant form during berry maturation, which can readily be reduced by dehydrogenase activity to corresponding alcohols. These alcohols have also been implicated as precursors to hexyl acetate (*fruity*) and thiols (3MH) (*tropical*) during fermentation. Thereby the prominence of herbaceous aromas in early maturity, especially in the case of low MP content in warm climates (Romero *et al.*, 2006) could also be due to a lack of other aromatics, rather than a decrease in C6 compounds.

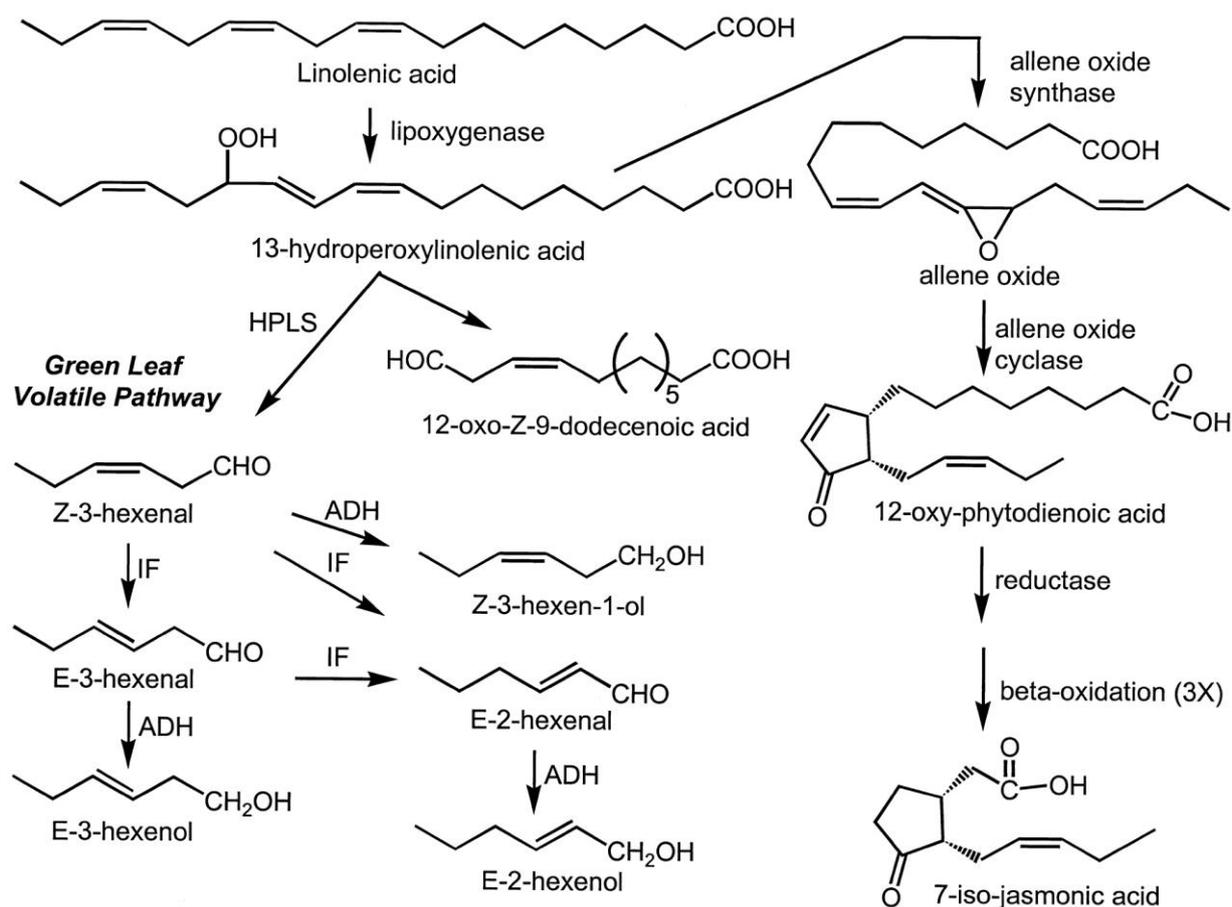


Figure 3. Intermediates in the metabolic conversion of linolenic acid to jasmonic acid and a series of hexenyl volatiles catalysed by the enzymes hydroperoxide lyase (HPLS), isomerisation factor (IF) and alcohol dehydrogenase (ADH) (taken from Blee, 1998)

### 2.2.6.3 Methoxypyrazines

Methoxypyrazines (MP) are heterocyclic nitrogen compounds that originate from the metabolism of amino acids in grape berries (Lacey *et al.*, 1991). Their aroma contribution is usually herbaceous, and have been described as bell pepper, asparagus and tomato leaf (Buttery *et al.*, 1969; Roujou de Boubee *et al.*, 2000). The main MP found in grapes and wines are 3-ethyl-2-methoxypyrazine (ETMP), 3-sec-butyl-2-methoxypyrazine (SBMP), 3-isopropyl-methoxypyrazine (IPMP) and 3-isobutyl-2-methoxypyrazine (IBMP), which is most common (Augustyn *et al.*, 1982; Allen *et al.*, 1991). These compounds are present in grapes in a potent (perception threshold 2 ng/L) odorous, free form and do not undergo modification during wine production (Sala *et al.*, 2004). This has led to significant correlations between grape and wine MP content as well as sound correlations between wine MP content and sensory perception of vegetative characters (Allen *et al.*, 1995). However in low concentrations MP may contribute positively to the complexity of wines (Robinson *et al.*, 2014).

The content of IBMP, SBMP and IPMP generally increase during the pre-véraison period, although previous studies indicated unique accumulation patterns for different MP derivatives

(Allen *et al.*, 1991; Lacey *et al.*, 1991; Hashizume & Samuta, 1999). In Cabernet Sauvignon the content of IPMP peaked and started to decline before véraison, while the IBMP peak coincided with véraison and it only started to decline thereafter. The formation of IBMP during grape berry development has been shown to be a result of methylation of 3-isobutyl-2-hydroxypyrazine (IBHP), and suggestions for its decrease are related to both photo-degradation and enzymatic de-methylation. The overall response of MP's to ripening levels suggest a rapid decrease post véraison, very similar to the response seen in malic acid. High correlations between the decrease of malic acid and the decrease of IBMP have been reported over a range of cultivation and climatic conditions, and it has been implemented as practical indicator for determining ripeness levels (Allen *et al.*, 1995; Roujou de Boubée *et al.*, 2000; Romero *et al.*, 2006). Increased sunlight exposure of grape berries have been reported to reduce IBMP levels at harvest (Hashizume & Samuta, 1999; Marais *et al.*, 1999), although there are some contrasting results presented in literature (Ryona *et al.*, 2010). Sunlight exposure seems to have two opposing effects on the concentration of MP in grapes; (1) by promoting the formation of MP in immature grapes and (2) reducing the content of MP in ripening grapes by either de-methylation or photo-degradation. Thus, final grape MP concentrations represent the net result of the anabolic and catabolic processes.

#### 2.2.6.4 Monoterpenes

Terpenes are one of the most important groups of aroma compounds present in grapes, must and wine (Fig 4). The volatile compounds of this family are mainly related to the monoterpenes (10C) and sesquiterpenes (15C) which are made up out of 5C isoprene units (Ribéreau-Gayon *et al.*, 2006a). Monoterpenes can be divided into three distinctive categories. First are the free aroma compounds, characterised by compounds of low perception thresholds. This category is dominated by linalool, nerol and geraniol and some furan and pyran forms of linalool oxides (Luan *et al.*, 2006; Ilc *et al.*, 2016). However, in some cases depending on factors such as climatic conditions and cultivation practices, additional compounds such as  $\alpha$ -terpineol, citronellol, hotrienol, nerol-oxide and many other oxides may also be present in grapes (Mateo & Jiménez, 2000). Oxides such as linalool- and nerol-oxide tend to have relatively small impact on wine aroma despite their high perception thresholds (Ribéreau-Gayon *et al.*, 2006a), while rose-oxide (more odoriferous) has shown to play an important role in wines made from Gewürtztraminer (Guth, 1997). Second are the polyols (diols and triols), or polyhydroxylated forms of monoterpenes. These compounds are not highly odoriferous, but can be hydrolysed to more aromatic compounds, such as for example hotrienol and nerol-oxide from 3,7-dimethylocta-1,5-dien-3,7-diol (Wilson *et al.*, 1984; Strauss *et al.*, 1987). The third category is

the glycosidically bound fraction of monoterpenes, which are the most abundant forms of monoterpenes in grapes (Marais, 1983). Monoterpenes are bound to sugars to form glycosides. In grapevines glycosides occur mainly in the diglycoside form, while in most other plants monoglycosides are more common (Ribéreau-Gayon *et al.*, 2006a). In spite of a large number of studies characterising the terpene content of different varieties (Marais, 1983; Williams *et al.*, 1995; Ribéreau-Gayon *et al.*, 2006a) and their distribution within the berry (Gomez *et al.*, 1994) as well as the effect of cultivation practices on fruit terpene content (Reynolds *et al.*, 1996; Marais *et al.*, 1999) relatively little is known regarding their biosynthesis. Molecular level studies indicated that monoterpene biosynthesis occurred *via* the mevalonic acid pathway (MVA) in grape berries from flowering up until ripening (Schwab *et al.*, 2008; Kuhn *et al.*, 2014; Robinson *et al.*, 2014). In addition, studies have concluded that the biosynthesis of monoterpenes in *Vitis vinifera* occurs almost exclusively *via* a novel 1-deoxy-d-xylulose 5-phosphate/2C-methyl-d-erythritol 4-phosphate (DOXP/MEP) pathway in both grape berries and leaves (Luan & Wüst, 2002). Furthermore, oxidative metabolism of monoterpenes in grape berries to form polyols (diols) has proven to be related to enzymatic oxygenation reactions rather than direct photo-oxygenation (Luan *et al.*, 2004). The enzymatic oxidative modification of monoterpenes, such as geraniol and linalool, in Morio Muscat seem to be related to ripening stage (Luan *et al.*, 2006). In the case of geraniol, glycosylation seems to remain constant throughout ripening, while there is a sharp increase in oxidative activity and the end of ripening, leading to the production of potent odorants such as rose-oxide (Luan *et al.*, 2005), supporting the notion that aromatic potential is linked to ripening stage (Wilson *et al.*, 1984). Linalool displayed the opposite activity, possessing significant oxygenase activity early in the ripening stage, which then deteriorated as ripening progressed, while glycosylation remained constant throughout ripening (Luan *et al.*, 2006).

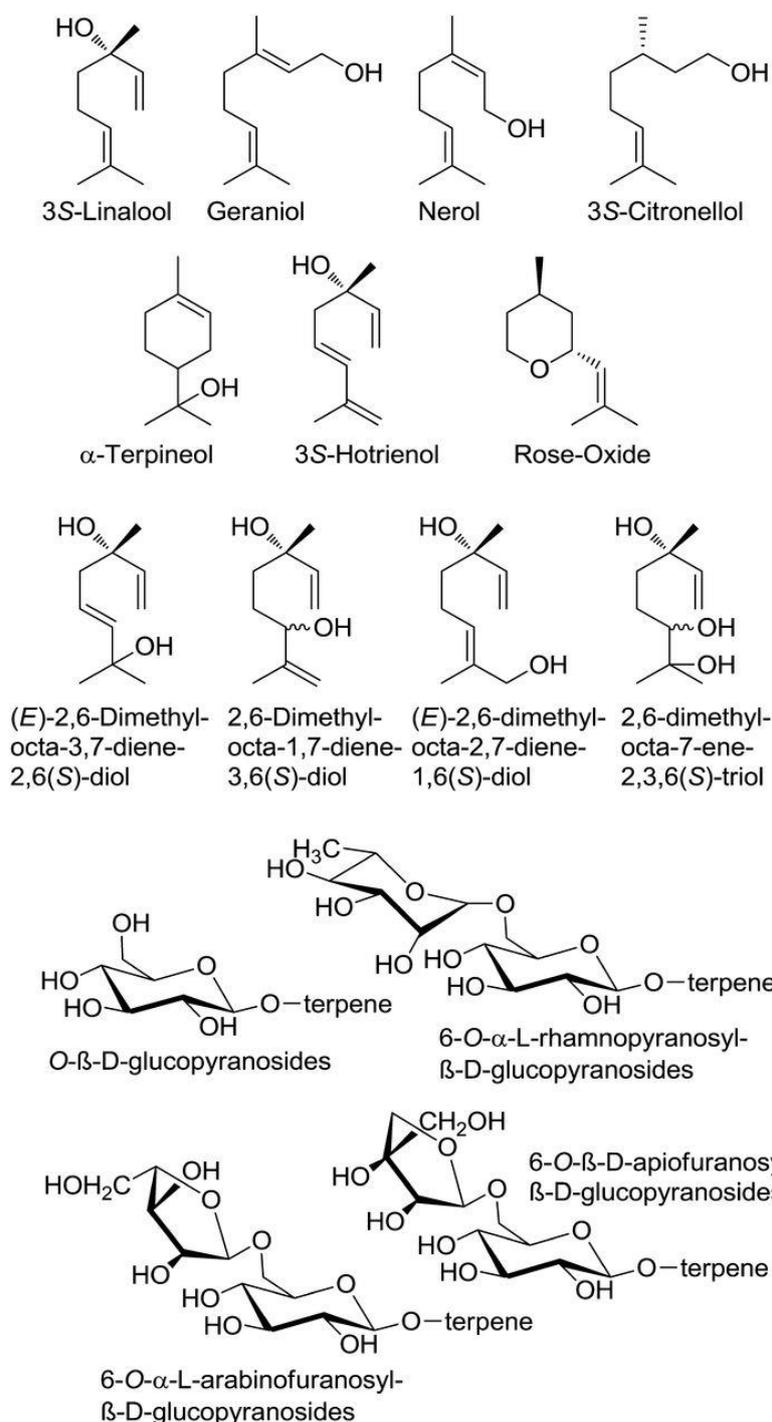


Figure 4. Structural formulae of selected monoterpenes and their glycosylated conjugates found in grapes and wines (taken from Bönisch *et al.*, 2014)

#### 2.2.6.5 C13- Norisoprenoids

The C13-norisoprenoids (floral aroma) represents an important part of the volatile compounds of non-floral grapes, such as Cabernet Sauvignon (Waldner & Marais, 2002; Pineau *et al.*, 2007; Bindon *et al.*, 2013), Syrah (Ristic *et al.*, 2010), Chenin blanc (Marais *et al.*, 1992) Pinot noir (Fang & Qian, 2006) and Pinotage (Waldner & Marais, 2002; Weldegergis *et al.*, 2011). Formation of C13-norisoprenoids in the grape berry involves carotenoid breakdown (Razungles *et al.*, 1993; Yuan & Qian, 2016a), primarily located in the berry exocarp (Marais *et al.*, 1992;

Gomez *et al.*, 1994). The most important of these compounds are  $\alpha$ - and  $\beta$ -ionone (violet character) and  $\beta$ -damascenone (red fruit/tobacco), directly impacting wine aroma, but also known for synergistic actions, increasing the perception of certain esters and masking green aromas imposed by IBMP on wine aroma (Escudero *et al.*, 2004, 2007; Pineau *et al.*, 2007).

It was originally proposed that carotenoids could be degraded by chemical, photochemical, and oxidase-coupled mechanisms (Kanasawud & Crouzet, 1990). In recent years, more studies supported the hypothesis of the involvement of a region-specific oxygenase in the formation of C13-norisoprenoids (Baumes *et al.*, 2002; Mathieu *et al.*, 2005). Although the enzymatic systems involved have not yet been fully discovered, a family of carotenoid cleavage dioxygenase enzymes has been implicated in production of plant apo-carotenoids, e.g. C13-norisoprenoids (Young *et al.*, 2012). A carotenoid cleavage dioxygenase capable of producing C13-norisoprenoids from lutein and zeaxanthin (VvCCD1) was cloned from grapes by Mathieu *et al.*, (2005). They also reported that the expression of VvCCD1 increased at véraison, although there was a 1–2 week lag between increased transcript expression and a significant increase in glycosylated C13-norisoprenoids. Overall, C13-norisoprenoids tended to increase in grape berries during ripening (Razungles *et al.*, 1993; Yuan & Qian, 2016a) and resulting wines (Fang & Qian, 2006; Bindon *et al.*, 2013). In addition, increased sunlight exposure of berries is thought to increase carotenoid breakdown and thereby also the presence of C13-norisoprenoids (Ristic *et al.*, 2007, 2010). However this has been shown to be more consistent in cool ripening conditions, and detrimental effects of high temperatures, high levels of sunlight exposure, induced by different growing conditions, on norisoprenoid accumulation have also been reported (Marais *et al.*, 1992; Oliveira *et al.*, 2003; Koundouras *et al.*, 2006; Ristic *et al.*, 2007, 2010).

#### 2.2.6.6 Benzoids/Higher alcohols

Aromatic alcohols are present as glycosides in grapes; the most prominent are benzyl alcohol and 2-phenylethanol (rose) (Ribéreau-Gayon *et al.*, 2006a; Kalua & Boss, 2009; Vilanova *et al.*, 2012), while phenyl-acetaldehyde is also present exclusively in grapes (Ilc *et al.*, 2016). These compounds are associated with fruity aromas and can contribute to the overall complexity of wines, but exhibit very high sensory thresholds (Bakker & Clarke, 2012). Glycosides of aromatic alcohols increase during ripening and seem to follow a similar evolution than monoterpenes during ripening (Coelho *et al.*, 2006; Kalua & Boss, 2010; Rocha *et al.*, 2010). Gomez *et al.* (1994) found the majority of the glycosides of aromatic alcohols in the grape skin, while the ratio between free and bound aromatic alcohols remained unchanged between the various parts of the grape berry. Importantly, aromatic alcohols are also formed in large quantities as fermentation by-product (Jackson, 2014; Robinson *et al.*, 2014; De-la-Fuente-Blanco *et al.*, 2016). Thus, the

specific contribution of grape derived glycosides to eventual wine aroma is difficult to determine. In the view of their relatively high perception threshold (Ribéreau-Gayon *et al.*, 2006a), it is conceivable that grape derived aromatic alcohol glycosides are limited to a supplementary role to the already positive impact of aromatic alcohols produced during fermentation (Boss *et al.*, 2018).

### 2.3 OVERALL GRAPE COMPOSITION

In light of the decisive impact of ripeness level on grape composition (Marais, 1983; Coombe & McCarthy, 2000; Coelho *et al.*, 2006; Conde *et al.*, 2007, Bindon *et al.*, 2013), grape/wine compositional studies investigating the effects of viticultural practices cannot afford to overlook the importance thereof, during the interpretation of results. Likewise, due to the fact that grapevines show high reactivity and plasticity with regard to growing conditions and cultivation/viticultural practices (Koundouras *et al.*, 2006; Hunter *et al.*, 2010) ripeness level related studies should undoubtedly acknowledge the possible effects of *terroir*. Modern understanding of grape composition involves the recognition that it is an integrative result of genotype x environment and viticultural practice (including ripeness level) interaction (Hunter *et al.*, 2014). Of these effects genotype is certainly the most predictable, genotype may refer to variety/cultivar (scion), but can also refer to variety of rootstock and the combination thereof with the scion. Rootstocks are known to impart certain characteristics to the scion (Southey, 1992), which will affect vigour, yield and adaptability to soil conditions (Hunter *et al.*, 2010; Serra *et al.*, 2014). This can be utilised as cultivation choice by the producers in combinations with viticultural practices, such as pruning and trellis system and row orientation (Hunter & Volschenk, 2018). These cultivation practices may act to influence berry bunch microclimate and bunch exposure. The development of aroma precursors in many varieties have been positively influenced by increased sunlight exposure of the berries (Marais *et al.*, 1992; Zoecklein *et al.*, 1998; Bureau *et al.*, 2000a; Ristic *et al.*, 2010). However, the response has been different regarding different growing climates. In cooler climates exposed berries consistently produced fruit with greater aromatic potential than shaded berries (Marais *et al.*, 1992; Reynolds *et al.*, 1996a; Reynolds *et al.*, 1996b), whereas in warmer climates, the highest aromatic potential was achieved in partially shaded berries (Zoecklein *et al.*, 1998; Bureau *et al.*, 2000b). Considering the expected positive relationship between increased bunch exposure and a rise in berry temperature (Smart, 1985; Bergqvist *et al.*, 2001) it is hard to separate the effects of sunlight and temperature under field conditions (Spayd *et al.*, 2002). Studies conducted in warm climates have conceded negative effects of high berry temperatures on secondary metabolite accumulation (Spayd *et al.*, 2002; Downey *et al.*, 2006; Tarara *et al.*, 2008; Reshef *et al.*, 2017)

and have shown that the effects of sunlight are heavily dependent upon the extent to which berry temperature is elevated as a result of increased sunlight (Bergqvist *et al.*, 2001). This dictating effect induced by climate is very important regarding expected changes due to global warming. Despite this, the evolution of compounds along the ripening period certainly presents producers with the opportunity to dictate wine composition within the boundaries set by the relevant local climatic conditions and cultural practices.

## 2.4 CONCLUSIONS

Wine is an infinitely complex matrix, with a multitude of factors influencing the composition and final characteristic attributes. While much is known, the sheer magnitude of compounds with its varied, amplifying, masking, synergistic and antagonistic properties in wine, poses a major challenge in the understanding of the effect of individual compounds and their impact on the collective matrix. Nonetheless, it is clear that the process of ripening impacts key components (Fig 4). From the literature, including the molecular detail of biosynthesis of primary and secondary metabolites (not discussed here), berry development and maturation follow consistent compositional trends defined by genotype and dictated by environmental factors. This poses a major opportunity as it allows the natural manipulation of the product (wine) through choice, often cost free (depending on berry weight), and further allows the producer to invariably adapt to a changing climate. Powerful statistics and analytical methods allow for an ever increasing amount of variables to be assessed, which will aid the global understanding of this complex matrix. Field proofing of hypotheses is essential before results are passed down to producers.

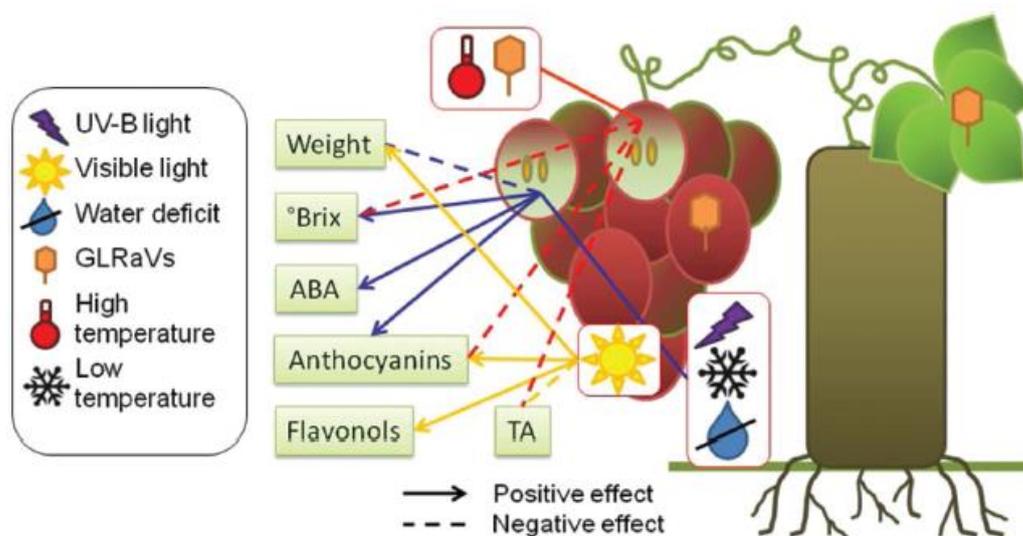


Figure 5. Environmental cues affecting typical biochemical parameters associated with berry ripening in coloured grapes. Boxes with a red outline enclose environmental signals with similar effects on the indicated parameters, with the exception of UV-B light, which does not enhance ABA content in berries. Arrows and dashed lines indicate positive and negative effects on the indicated ripening parameters, respectively. TA, titrable acidity (taken from Kuhn *et al.*, 2014).

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## **B. *Vitis vinifera* L. cv. Pinotage**

### 2.6 INTRODUCTION

Pinotage was developed by Prof. A.I. Perold of Stellenbosch University in 1925, when he successfully crossed *Vitis vinifera* L. cv. Pinot noir and *Vitis vinifera* L. cv. Cinsaut (Addendum A). Since its development and commercialisation in the 1950's, Pinotage has seen a steady acceptance by the South African wine industry and by 1979 there were 660 639 Pinotage vines planted in the Cape. Demand for the variety increased when wine export sanctions were lifted (1994), paving the way for a unique South African offering. Currently, there are 20 814 088 Pinotage vines planted in South Africa, amounting to 6979 ha of vineyard, thereby ranking 3rd with regard to total vineyard area planted to red varieties (1<sup>st</sup> Cabernet Sauvignon – 10360 ha, 2<sup>nd</sup> Shiraz/Syrah – 9735 ha) (SAWIS, 2017). Plantings have also spread across the world as recognition of Pinotage wine quality has grown. The variety can now be found in Australia, Brazil, Canada, France, New Zealand, Switzerland and the United States of America (OIV, 2017).

Despite the recent popularity of Pinotage, varietal specific information available on scientific platforms for Pinotage is limited and research results have largely been confined to practical publications. A search conducted for publications on a well-known scientific database; Web of Science™ (September 2018) with “Pinotage” in the topic field; yielded 75 results (Fig 1), only Agiorgitiko, the signature red variety from Greece (Koundouras *et al.*, 2006), yielded less publications among the eight unique red varieties included in the search. Varietal specific scientific information is essential to improve the understanding, cultivation and ultimately the competitiveness of any grape variety. Here, varietal specific information available for Pinotage is summarised and shortcomings pointed out. Up to date context for interpreting results from studies conducted with Pinotage is provided.

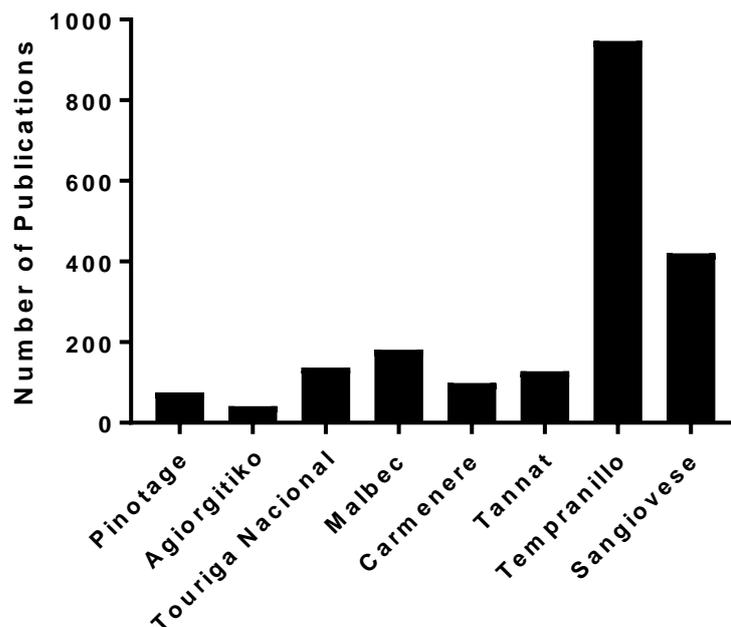


Figure 1. Search conducted on scientific database Web of Science™ (September 2018), specifying signature *Vitis vinifera* L. cultivars from various countries.

## 2.7 VITICULTURE

### 2.7.1 Ampelographic information

*Phenology* Budding 2/9 - 13/9

Flowering 20/10 - 10/11

Ripening 20/2 – 3/3 (20 -22°B)

Data from Stellenbosch 1970 – 1981 (De Villiers & Theron, 1987)

*Shoot tips* Felt-like and white.

*Leaves* Large, longitudinal, five-lobed, dark green, leathery appearance and crimped. Lateral sinuses medium to deep, teeth often occur in the sinus. Petiole sinus lyre-shaped and narrow. Cobweb-like trichome (hair) configuration on ventral side of leaf blade, teeth convex, broad and blunt.

*Bunch* Relatively small and conical, medium to compact.

*Berries* Small, oval, dark coloured with tough, thick skins. Soft, juicy pulp (Goussard, 2009).

*Growth* Field observations conducted at the University of Stellenbosch have categorised Pinotage as a variety with moderate vigour and yield potential (10 – 15 t/ha) and upright growth habit. Moreover, Pinotage is not excessively sensitive to major fungal diseases such as *Oidium spp* (powdery mildew) and *Plasmopara spp.* (downy mildew). In contrast, it is considered less resistant to *Botrytis cinerea*

than Cabernet Sauvignon, but generally no serious problems are encountered due to its early ripening, occurring well before the autumn rains (Goussard, 2009).

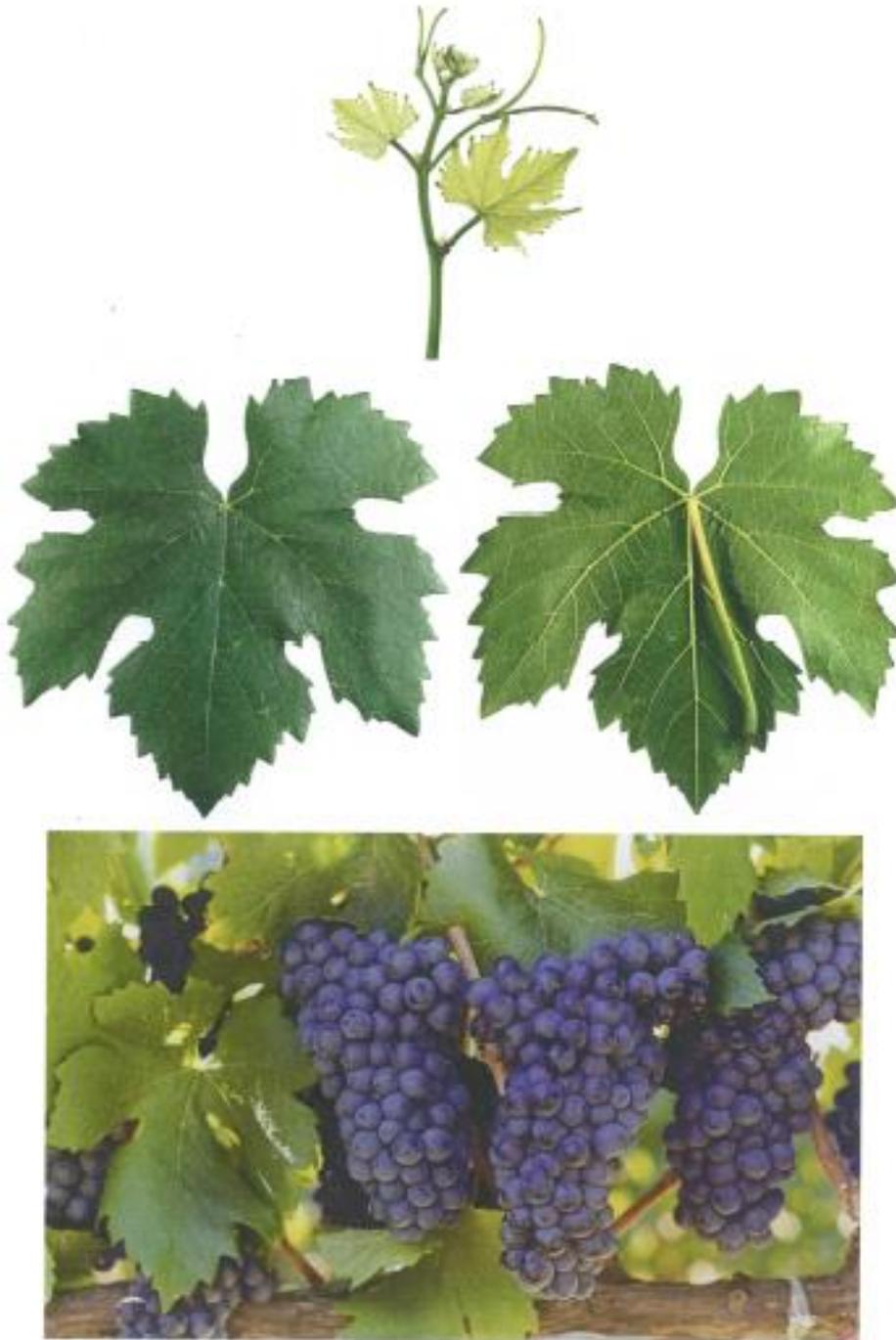


Figure 2. Shoot tip (top), leaf blade (middle) and bunches (bottom) of *Vitis vinifera* L. cv. Pinotage (Goussard, 2009).

## 2.7.2 Cultivation

Genotype has been shown to have a major impact on the agronomic performance of grapevines and therefore the use of clones has become an important part of modern viticulture (Jackson & Lombard, 1993). In a comparative study (Kriel, 1983), seven clonal selections (grafted to Jacquez rootstock) were evaluated (1970 – 1981) on the basis of shoot mass, sugar content, acid concentration, occurrence of viruses and *Botrytis cinerea* resistance. Three clones were selected (PI 45, PI 48 and PI 50) and made available to industry. Since then PI 50 has been removed due to virus infection of the source material and have been replaced by field selections PI 6 and PI 7 (Table 1). Due to its youth, Pinotage clonal material is limited. However, recent advances in understanding the genetic make-up of Pinotage will prove valuable to fast track breeding programs. The sequencing of both the genome and transcriptome of Pinotage have given insights into stress response networks and will aid the selection process for better adapted plants for future climate challenges (Coetzee, 2018).

Table 1. Commercially available Pinotage clones in 2017.

Clones	Origin	Year Selected	Viticulture Remarks	Wine Remarks
PI 45	Vititec/Bellevue	1972	Average yield and vigour	Good quality
PI 48	Vititec/Slaley	1972	Average yield and vigour	Good quality, widely planted
PI 50	Meerendal	1976	Discontinued due to virus infection of source material	
PI 6	Kanonkop	1996	Average yield and vigour	Good quality
PI 7	Warwick	1996	Average yield and vigour	Good quality

Traditionally, Pinotage has been cultivated as a bushvine (gobelet) in South Africa, with local presupposition that it produces wines of higher quality when cultivated in this way. A long term investigation into the impact of vine training (bushvine versus VSP) on Pinotage wine quality displayed no consistent trends regarding this perceived relationship (Van Schalkwyk & Schmidt, 2009a, 2009b, 2009c, 2009d), rather pointing to vineyard site, vintage, ripeness level, grapevine water status and bunch microclimate as complex drivers to wine quality. This is consistent with reports for many other varieties and growing conditions (Smart, 1985; Jackson & Lombard, 1993; Hunter, 2000; Hunter *et al.*, 2004, 2010). Practices such as regulated deficit irrigation (Myburgh, 2011; Serra Stepke, 2014) and canopy management have been shown to improve bunch microclimate (Du Toit, 2003) and subsequent grape composition and wine quality of Pinotage. The negative impact of management practices which are prone to deliver fruit with heterogeneous maturity, sunburn, extensive shrivelling and general over-ripeness have been

documented on a practical level (Du Toit, personal communication, 2003; Van Schalkwyk & Schmidt, 2009b).

### 2.7.3 Ripening

Pinotage is an early ripening variety, with a relatively short growth cycle, ripening up to eight weeks before Cabernet Sauvignon in Stellenbosch, and often avoids several heat waves by being harvested before the warmest month (February) of the season (De Villiers & Theron, 1987; Van Schalkwyk & Schmidt, 2009a). This has led to the suggestion that mean January temperatures are used to assess ripening conditions of potential Pinotage vineyard sites, rather than mean February, which is the local norm for red varieties grown in the Western Cape (Van Schalkwyk & Schmidt, 2009a).

Due to its rapid sugar accumulation, and disadvantages associated with over-ripeness, determining the correct ripeness level is considered a pressure point in the process of obtaining a high quality product. Research regarding Pinotage ripeness levels was first published by Du Plessis & Van Rooyen (1982) in a study aiming to determine correlations between classical ripeness indices, TSS ( $^{\circ}$ Brix), TA (Titratable acidity) and pH, and wine quality. They proposed a maturity index of  $^{\circ}$ Brix:TA ratio and identified a value of 3.9 as “optimum ripeness” for highest perceived wine quality (Fig 3). Subsequent work using multifactorial analysis proposed an additional maturity index, namely sugar content ( $^{\circ}$ B) x pH and values ranging between 85 and 95 were proposed for highest quality (Van Rooyen *et al.*, 1984).

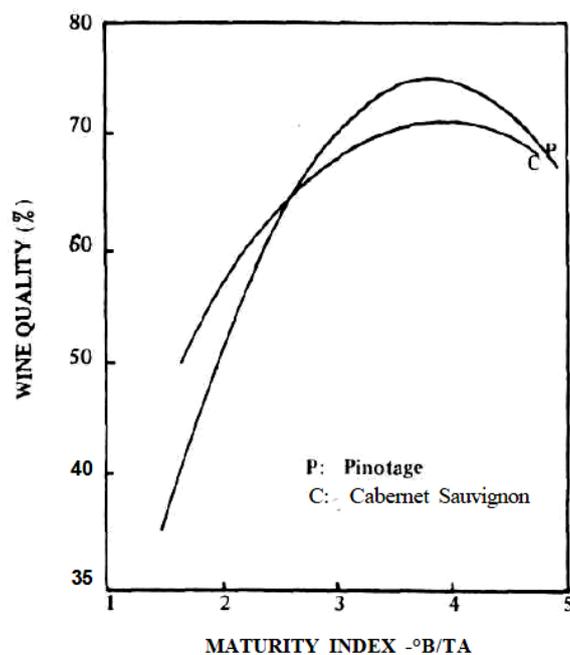


Figure 3. The relationship between perceived wine quality and the Maturity index  $^{\circ}$ B:TA for Pinotage and Cabernet Sauvignon grown in Stellenbosch, South Africa (taken from Du Plessis & Van Rooyen, 1982).

Apart from the classical indices and ratios thereof, grape colour/pigmentation was also identified as potential ripeness level indicator for Pinotage (Marais *et al.*, 2001). In later studies it was proposed only as a supplementary indicator as correlations were not consistent due to the comparatively high values obtained in Pinotage (Marais & October, 2005). Early studies (Marais *et al.*, 1979; Du Plessis & Van Rooyen, 1982; Van Rooyen *et al.*, 1984) assessed a wide range of ripeness levels in their pioneering exploratory works. However, modern era Pinotage producers necessitate more detail, as winemakers cite picking date as one of the major determinants of quality (Du Toit, 2003). Yet, as with many other varieties the complex nature of the grape ripening process has proven difficult to unpack and universal relationships with basic indices such as pH, TA and TSS unsuccessful. This has seen a major drive in grape compositional analyses in the search for improved ripening indicators.

Currently, grape compositional published information available for Pinotage is limited to pH, TA and TSS levels and must nitrogen content (Marais *et al.*, 2001). Ough & Kriel (1985) reported Pinotage as having the highest must ammonium concentrations (148 mg/L) of nine varieties studied in the Stellenbosch area. Inherent high natural levels of nitrogen can be considered a positive in terms of yeast nutrition, development of intense fermentation aroma (esters and higher alcohols) and efficient malo-lactic fermentation. However, there are also implications of excessive nitrogen levels, especially in finished wines, where high nitrogen levels increase the possibility of bacterial spoilage leading to off-flavours, acroline (bitter taste) production and even the occurrence of carcinogenic ethyl carbamate (Joubert, 1980).

## 2.8 OENOLOGY

### 2.8.1 Varietal Aroma

Varietal aroma is considered an essential contributor of wine *typicity*, and the understanding of the drivers of varietal aroma will surely allow for the successful development of viticultural or oenological practices to further improve varietal wine quality (Ribéreau-Gayon *et al.*, 2006). Initial studies were aimed at understanding Pinotage varietal aroma in relation with others; Pinotage was distinguished successfully from Cabernet Sauvignon on the basis of fermentation by-products: ethyl acetate, isoamyl acetate, ethyl decanoate and hexanol contents (Marais *et al.*, 1981). In a more detailed study; Pinotage wines differed from other wines (Cabernet Sauvignon, Merlot and Shiraz from various regions) due to significantly lower levels of 2-phenyl ethanol, isobutyric acid, isobutanol, methanol, iso-valeric acid, butanol, diethyl succinate, hexanol and isoamyl alcohol. However, the Pinotage wines had the highest concentration of butyric acid, acetic acid, octanoic acid, propanol, hexanoic acid, ethyl hexanoate, ethyl octanoate, ethyl lactate

and isoamyl acetate (Louw *et al.*, 2010). Isoamyl acetate, together with hexyl acetate and ethyl octanoate, have already been confirmed as important contributors to the aroma profile of young Pinotage wines (Marais *et al.*, 1979; Van Wyk *et al.*, 1979), providing ample evidence of the precursors to varietal character related to genotype specific grape composition (Ribéreau-Gayon *et al.*, 2006).

Indeed, a key attribute to Pinotage varietal aroma was reported to be related to the relatively high free ammonium and amino acid levels present in Pinotage musts compared to other varieties (Ough & Kriel, 1985). Joubert (1980) confirmed the relationship between high must nitrogen levels and the formation of the typical Pinotage fermentation aroma, displaying increased isoamyl acetate content with increases in must nitrogen content, decreased fermentation temperature, increased maceration and selected *Saccharomyces spp.* strains. However, excessively high concentrations of isoamyl acetate were shown to be unpleasant and shifted wine aroma from fruity to an acetone/chemical type of aroma. Isoamyl acetate is readily hydrolysed in an acidic medium (wine) to a non-odorous form in a temperature dependent reaction. Higher fermentation and wine storage temperatures result in increased hydrolysis of acetate esters and the rapid disappearance of the typical banana aroma (Marais, 2003).

Apart from fermentation derived components, grape components in the form of norisoprenoids were also thought to play a role in the typical berry/plum Pinotage aroma. Both  $\beta$ -damascenone and  $\beta$ -ionone were isolated in Pinotage wines at concentrations that exceed their sensory threshold values in water (Waldner & Marais, 2002).  $\beta$ -damascenone is widely considered to be present in most red wines and is an important aromatic driver in both Pinot noir (Yuan & Qian, 2016) and Shiraz wines (Mayr *et al.*, 2014). It is considered a compound that can have distinctive impact on aroma, but requires large changes in concentration to increase intensity (Escudero *et al.*, 2004). In addition,  $\beta$ -damascenone in low concentrations have been shown to mask green flavours of methoxypyrazines and increase fruit aromas, through a synergistic effect with other components (Pineau *et al.*, 2007). In a comprehensive study of Pinotage wine volatile composition, Weldegergis *et al.* (2011) elucidated 206 volatile components (from 9 commercial Pinotage wines), ranging from esters, alcohols, aldehydes, ketones, acids, acetals, furans and lactones, to sulphur compounds, nitrogen compounds, terpenes, hydrocarbons, volatile phenols and pyrans, illustrating the complex nature of components that make up the Pinotage varietal aroma. Therefore, a series of compounds is most likely responsible for the varietal aroma of Pinotage. This is supported by the wide array of sensory characteristics linked to Pinotage, as described in the official Pinotage aroma wheel (Fig 4) (Marais & Jolly, 2004). The aroma wheel provides both broad guidelines (inner wheel) and detailed descriptors (outer wheel), which aid the assessment of Pinotage wines. This has been a valuable contribution to conduct and compare

sensory evaluations in a structured manner. While a substantial body of research has been produced regarding the fermentation components related to young Pinotage wines, links to sensory attributes remain largely unresolved. Additional compositional-sensorial studies are needed in order to supplement the current reference base. This will allow rigorous evaluation of viticultural and oenological practices aimed at improving wine quality and sustainability of Pinotage.

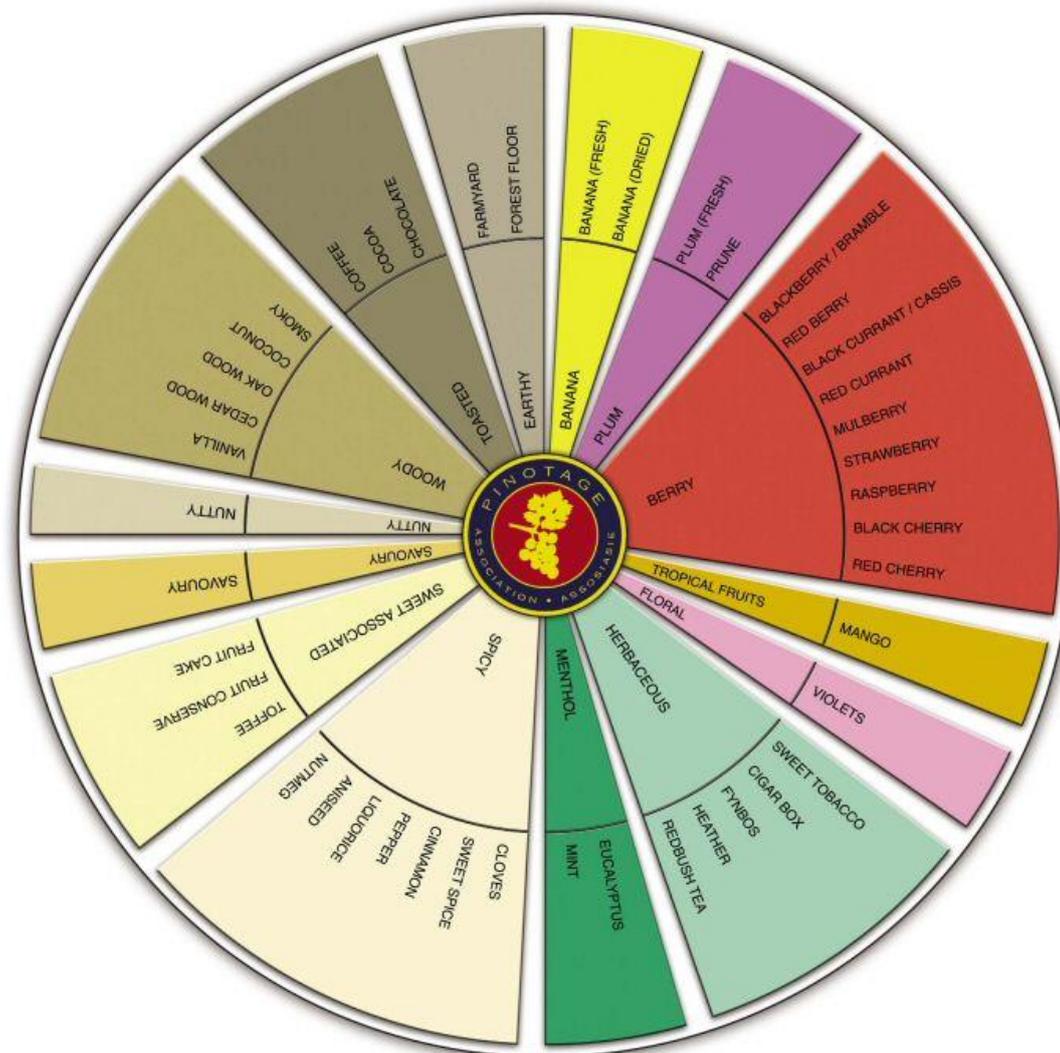


Figure 4. Pinotage aroma wheel, indicating two levels of aroma descriptions, the inner level with broad descriptors and the outer wheel with detailed descriptors (Marais & Jolly, 2004).

### 2.8.2 Phenolic composition

Grape derived phenolic compounds play a critical role in wine quality. Phenolic compounds primarily impart wine astringency and colour of red wines, while flavonoids also play a role in colour stability during ageing (Downey *et al.*, 2006). Recent medical research has attributed various health benefits of red wine largely to the anti-oxidant role of wine phenolic compounds

(Guilford & Pezzuto, 2011). A study investigating the anti-oxidant capacity of wines made from five red varieties grown in South Africa, confirmed that Pinotage exhibited equal measure in anti-oxidant capacity to the other wines examined (De Beer *et al.*, 2003). Moreover, comparative studies conducted on individual polyphenols of South African Pinotage, Cabernet Sauvignon and Shiraz wines ascribed differential polyphenol profiles to varietal (genotype) impact and climatic conditions (Rossouw & Marais, 2004). Here, Pinotage displayed considerably higher concentrations of malvidin-3-O-glucoside, procyanidin B1 and caftaric acid than Shiraz and Cabernet Sauvignon. In addition, Van der Merwe *et al.* (2012) reported Pinotage having the highest content of polymeric phenols, flavanols, tannins and free anthocyanins when compared to Shiraz, Merlot and Cabernet Sauvignon wines grown in similar conditions. Cooler growing conditions as well as vine structure promoting greater fruit exposure, favoured the occurrence of phenolic compounds in a study where wine phenolic content were evaluated from various climatic regions in South Africa (De Beer *et al.*, 2006). These important findings have all contributed to aid a possible authenticating of Pinotage wines according to a typical phenolic profile. In addition, the abundant phenolic content found in Pinotage wines in numerous studies confirm not only its unique profile, but also highlight the importance of these components in wine quality.

## 2.9 CONCLUSIVE REMARKS

*Vitis vinifera* L. cv. Pinotage is a crossing of Pinot noir x Cinsaut made in South Africa in 1925. Since then it has seen a steady acceptance in the South African wine industry. Currently, it ranks third regarding total area planted to red varieties in South Africa with 6979 ha. Pinotage is an early ripening variety, characterised by rapid sugar accumulation and relatively high must nitrogen content. Varietal aromas of young wines have thus far been linked to various esters (isoamyl acetate, ethyl octanoate and hexyl acetate) and grape derived norisoprenoids ( $\beta$ -damascenone and  $\beta$ -ionone). Pinotage wines are rich in phenolic compounds (proanthocyanidins) and anthocyanins (malvidin-3-O-glucoside), producing deeply coloured and structured wines. Pinotage wines have been successfully discriminated from other varietals on the basis of unique compositional profiles and sensory characteristics. However, many gaps remain in terms of understanding the impact of various viticultural and oenological practices on wine sensory characteristics. Further research will be important to successfully navigate the rigors of a competitive market amidst a changing and challenging climate.

A substantial body of local research has contributed to the success and continual improvement in cultivation and production of this relatively young variety, yet much remains unknown. Continuous research is necessary to ensure its commercial success. Further compositional studies

linking grape/wine composition and wine sensory attributes will improve the assessment of viticultural and oenological practices. The impact of ripeness level appears to be a crucial pressure point in the production of high quality Pinotage. Finally, the ability to adapt wine styles according to growing conditions and market needs, using well researched viticultural and oenological practices, would ensure the continued international competitiveness of this unique South African variety.

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## 2.12 ADDENDUM A

### **Pinotage Discovery**

Pinotage was developed by Prof. A.I. Perold of the University of Stellenbosch in 1925, when he successfully crossed *Vitis vinifera* L. cv. Pinot noir and *Vitis vinifera* L. cv. Cinsaut. However, the Pinotage anecdote was not without a twist of fate. In fact, it was lecturer C. Niehaus and Prof. C.J. Theron, Perold's successors at the University of Stellenbosch who managed to rescue the mere four seedlings produced by the crossing in 1925, after re-discovering the abandoned seedlings in the garden of Prof. Perold's former residence at the University's Welgevallen Experimental grounds. After salvaging the seedlings, Prof. Theron propagated and proceeded to graft the material in an experimental vineyard at nearby Elsenburg Agricultural College in 1935. Here, he and Perold soon realised its viticultural potential and selected the best lineage of the four crossings. At this stage they were simply known as Pinot Fin x Hermitage crossings (1 - 4), Hermitage being the colloquial term used for Cinsaut at the time, and hence the conjunctive name of "Pino-tage" was born. Realising its potential for making ripe, structured and deep coloured wine, oenology lecturer C.T. de Waal produced the first wine of note in small casks at the Elsenburg College Experimental Winery in 1941 and two years thereafter the first commercial planting (Myrtle Grove, Sir Lowry's Pass) ensued. As a result of the early successes, a number of commercial plantings followed in the early 1950's, most notably at Kanonkop and Bellevue estates in Stellenbosch. Interestingly, both of these plantings are still producing wines in 2018. Finally the first bottling, designating Pinotage as varietal on the label, came in 1961, with the 1959 Lanzerac Pinotage produced from fruit bought from Bellevue Estate by Stellenbosch Farmers Winery.

## Chapter 3

### **Genotype x Environment x Ripeness level interaction: Effects on physiology, vegetative and yield components of unirrigated *Vitis vinifera* L. cv. Pinotage**

#### 3.1 ABSTRACT

The scion/rootstock combination (genotype) is the interface between growing conditions and product (grape/wine) and proved to have a profound impact on grapevine reaction. Selection thereof has become a major qualitative denominator as well as the focus of attempts to navigate the negative effects of climate change. To supplement the limited pool of varietal specific information, the response of unirrigated Pinotage/140 Ruggeri and Pinotage/1103 Paulsen was monitored under Mediterranean conditions (Western Cape, South Africa), for three consecutive seasons (2015-2017) over a range of ripeness levels from 21-29°B. Climatic conditions in 2015 (665 mm annual rainfall) were moderate, while 2016 (368 mm) and 2017 (486 mm) were exceptionally dry and warm, with average maximum temperatures during the month of ripening which were higher in 2016 (+4.66°C) and 2017 (+0.77°C) compared to long term means. Consequently, grapevines experienced higher ( $-0.6 > \Psi_{PD} > -0.8$  MPa) post-véraison water deficit in 2016 and 2017. Nonetheless, grapevines demonstrated effective adaptive behaviour, effective stomatal regulation and late season recuperation in the drier conditions. Generally, higher seasonal water deficits reduced leaf area (LA) (m<sup>2</sup>/vine) development, principally by reducing secondary LA. Leaf senescence during the ripening phase also reduced LA and increased light interception in the bunch zone. Due to comparable pre-véraison water deficits over vintages, berry weights at ripeness levels of 21-27°B remained stable, whereas a decrease in berry weight by berry dehydration was significant at the last ripeness level. Within the scope of this study, the early and rapid maturing Pinotage/140 Ru and Pinotage/1103 P grapevines displayed robust plant responses amidst strenuous climatic conditions.

#### 3.2 INTRODUCTION

The prevailing growing conditions, together with the viticulturists' interventions both long- and short term, give rise to multifaceted grapevine physiological responses, which in turn affect growth, yield and grape and wine quality (Hunter *et al.*, 2010).

Managing plant water status has long been recognised as critical, due to its extensive effect on growth and productivity of grapevines. Plant water availability drives growth through carbon assimilation *via* photosynthesis (Chaves, 1991) and cell enlargement (Cosgrove, 1993).

Grapevines aim to maintain cell turgor through various adaptive processes that include stomatal control of transpiration (Hsiao, 1973), cell wall elasticity (Patakas & Noitsakis, 1997) and embolism and recuperation of hydraulic vessels (Zufferey *et al.*, 2011).

At whole-plant level, these responses are incorporated into mechanisms of drought tolerance or avoidance (Lovisolo *et al.*, 2010; Medrano *et al.*, 2017). Grapevines modify evaporative surface through differential canopy development, reduced shoot length, and leaf number and size (Matthews *et al.*, 1987; Hunter *et al.*, 2014), and particularly suppressed secondary leaf area development (Lebon *et al.*, 2006). Under severe water deficit conditions, leaf senescence may occur, leading to partial or complete loss of leaf area. Root system functioning plays a central role in grapevine drought tolerance by optimising absorptive (root) surface to available soil volume. Effective root systems buffer grapevines against dry conditions (Hunter & Myburgh, 2001). Root/shoot signalling *via* abscisic acid (ABA) has been shown to be significant in regulating the photosynthetic response, inducing tight stomatal regulation at whole plant level (Stoll *et al.*, 2000; Lovisolo *et al.*, 2010). On a practical level, vineyard management practices can take advantage of these plant responses to optimise source/sink relationships to benefit fruit development (Hunter *et al.*, 2014), improve canopy efficiency, maximise sunlight interception, increase photosynthetic activity (Escalona *et al.*, 2003), and enhance bunch microclimate (Santos *et al.*, 2003; Keller *et al.*, 2016), with major consequences for ripening, and berry and wine composition (Smart *et al.*, 1990; Koundouras *et al.*, 2006; Hunter *et al.*, 2014).

Grape berry development follows a double sigmoid curve and can be divided into three stages (Coombe, 1976). Stage I commences after the fertilisation of the inflorescence and ends just prior to véraison. Imported carbohydrates are used for seed development, cell division and cell enlargement and the synthesis of organic acids (Coombe, 1992; Conde *et al.*, 2007). At this stage, water flux to the berry is supplied *via* the xylem and the berry is considered sensitive to water deficit (Ojeda *et al.*, 2001). Direct decreases in xylem flux cause decreases in mesocarp cell turgor and inhibit berry growth (Thomas *et al.*, 2006). Pre-véraison berries are characterised by the presence of developmental hormones; auxin, cytokinins and gibberellins, primarily produced in the seed (Conde *et al.*, 2007). It has been hypothesised that signalling *via* ABA could act (under water deficit conditions) to limit cell division and therefore berry size during the pre-véraison period (Chaves *et al.*, 2010). Stage II signifies a short lag phase and soon after, stage III (coinciding with véraison) commences. At véraison berries initiate softening, sugars and anthocyanins accumulate, and malic acid is metabolised (Ojeda *et al.*, 2002; Castellarin *et al.*, 2007, 2016). At this stage water, minerals and sugars are mainly supplied *via* the phloem and berry size increase is driven by cell expansion up to the point where maximum berry weight is reached (Coombe, 1992). During the active influx of solutes to the berry, berry size may be

influenced by the rate of influx to the berry, which is dictated by the availability of photosynthetic products and grapevine water status (Wang *et al.*, 2003). After this berry dehydration coincides with progressively hindered phloem flux in the latter ripening stages up to a point where it is finally blocked (Coombe & McCarthy, 2000). The continuation of berry transpiration and isolation of the berry from vascular transport pathways, thus lead to shrinking of the berry and solute concentration (Hunter *et al.*, 2014; Carlomagno *et al.*, 2018).

Among genotypes (scion), grapevines display differential performance in drying conditions (Soar *et al.*, 2006a) *vis à vis* photosynthesis, stomatal conductance and water use efficiency (WUE) (Bota *et al.*, 2001; Schultz, 2003; Soar *et al.*, 2006a), resulting in variable growth (Gómez-del-Campo *et al.*, 2002), yield and grape composition (Intrigliolo *et al.*, 2012). However, at a physiological level, variation in photosynthetic efficiency seems to be minor (Bota *et al.*, 2001), suggesting differences in WUE are centred on hydraulic conductivity and stomatal regulation (Escalona *et al.*, 1999). Initial studies grouped genotypes in either isohydric (tightly regulated/‘pessimistic’) or anisohydric (‘optimistic’) stomatal behaviour patterns (Schultz, 2003), yet subsequent work have demonstrated conflicting results (Soar *et al.*, 2006a), suggesting non-strict responses (Lovisolo *et al.*, 2010), although different varieties cultivated in analogous conditions produce unique responses (Chaves *et al.*, 2010). Likely, the influence of rootstock and prevailing conditions play an important role in Genotype x Environment responses and should not be overlooked when assessing the scion physiology (Soar *et al.*, 2006b). Rootstocks (American *Vitis spp.* with resistance to *Phylloxera*) are known to confer other qualitative traits to the scion (Conradie, 1983). An extensive study (multi-regional and multi seasonal) by Southey (1992) investigating rootstocks under field conditions revealed the critical importance of choice of rootstock in overall grapevine reaction, proposing a rootstock selection criteria based on resistance to pests and diseases, propagation affinity, vigour and grape quality and soil conditions. Drought tolerance of certain rootstocks have shown to be an important criterium to buffer dry conditions (Carbonneau, 1985; Southey & Jooste, 1991; Serra *et al.*, 2014). In particular, rootstocks with *V. Berlandieri* and *V. Rupestris* parentage, such as 110 Richter and 140 Ruggeri and later 1103 Paulsen, were identified as being drought resistant under Mediterranean conditions (Southey, 1992). Rootstocks interact with the scion to alter hydraulic conductance and root/shoot signalling (Iacono *et al.*, 1998; Soar *et al.*, 2006b) as well as leaf morphology, including stomata size and density (Serra *et al.*, 2017).

Despite many works detailing scion/rootstock response (Carbonneau, 1985; Southey, 1992; Lovisolo *et al.*, 2010; Hunter *et al.*, 2014), the governing effect of growing conditions on grapevine reaction remains an important consideration. Particularly the impact of temperature as mediating factor in grapevine growth is critical (Kliewer, 1977), as grapevine metabolic (Lakso

& Kliewer, 1975; Ruffner *et al.*, 1976; Dai *et al.*, 2011) and physiological (Escalona *et al.*, 1999; Sadras *et al.*, 2012; Sadras & Moran, 2013) processes are controlled within temperature ranges (Hunter & Bonnardot, 2011) and determine grapevine canopy development. In addition, the gradual depletion of soil water during the growing season, which is dictated by rainfall/irrigation, soil water holding capacity, root functioning and demand from the canopy (due to size and atmospheric conditions like temperature/humidity) acts to modulate grapevine growth, canopy development and functioning (Hunter, 2000; Hunter & Myburgh, 2001; Hunter *et al.*, 2010, 2014). Canopy functioning essentially dictates grapevine capacity to produce and ripen its crop (Buttrose, 1966) and is certainly dynamic in terms of changing conditions (Escalona *et al.*, 1999), but is also subject to ageing (decline in functioning), which will influence grape yield components and composition depending on ripeness level (Hunter *et al.*, 2014). Moreover, canopy characteristics will largely determine bunch microclimate, which will have an effect on secondary metabolism and resulting grape composition (Smart, 1985; Hunter, 2000). In the light of the critical effect of prevailing conditions, the *terroir* effect is unavoidable in field conditions. This is supported by the dominant effect of season and locality seen in grapevine physiological field experiments (Hunter *et al.*, 2004; Koundouras *et al.*, 2006). To our knowledge the systematic reporting of Genotype x Environment x Ripeness level response for the important local South African variety Pinotage has not yet been produced.

The aim of this descriptive study was to characterise grapevine physiological, vegetative and reproductive responses in relation to environmental conditions, featuring changes in grape maturation in order to create a relevant varietal-specific base of understanding. This study is followed by a sequence of related studies, detailing the impact of grape ripeness level in combination with seasonal effects on grape composition, wine composition and wine sensory attributes. This will provide sound context to more accurately reason and assess vine performance and grape/wine qualitative potential, with reference to future dryer and more extreme conditions.

### 3.3 MATERIALS AND METHODS

#### 3.3.1 Experimental Vineyard

The experiment was carried out during three consecutive seasons (2015, 2016 and 2017) in a unirrigated *Vitis vinifera L.* cv. Pinotage vineyard, grafted onto two known drought tolerant rootstocks (*Vitis Berlandieri* x *Vitis rupestris*), namely Pinotage (clone PI 50A)/140 Ruggeri (clone RU 354B) planted in 1997 and Pinotage (clone PI 48A)/1103 Paulsen (clone PS 28A) planted in 1999. The vineyard is located at the University of Stellenbosch Welgevallen Experimental Farm in Stellenbosch, Western Cape, South Africa. The area is under the influence

of a Mediterranean climate, characterised by winter rainfall and warm and dry summers. Vines are orientated in a North–South direction and the vineyard locality (approx. 200 m altitude) is characterised by a slope (3°) with a north western aspect. The vines are spaced 2.75 m x 1 m (3636 vines/ha) and the soil classified as Oakleaf soil form originating from weathered granite (Soil Classification Working Group, 1991). Vines are trained to a uni-lateral cordon and pruned to five, two-bud spurs per plant. Foliage was managed by a vertical shoot positioned trellis system with three sets of movable foliage wires. Standard canopy management practices were applied during the growth season, including early suckering (the judicious removal of shoots not allocated during pruning), vertical positioning of shoots, and topping once shoots passed 30 cm of the top foliage wire. An annual cover crop of *Triticale spp.* was sown in autumn and controlled with herbicide before budburst, to ensure a mulch layer within the work row.

### 3.3.2 Measurements and analyses

Climatic data on a meso-climate scale was obtained from temperature logger (CR200 – Campbell Scientific) located on the Welgevallen Experiment farm. Soil cores were collected at two depths (0 – 30 cm and 30 – 60 cm) to determine soil physical and chemical properties. Five shoots (including bunches) per experimental plot of 30 vines were sampled, in order to determine total leaf area, primary and secondary leaf area, number and mass of primary leaves, number and mass of secondary leaves, number of secondary shoots, shoot lengths, bunch mass, and berry mass and volume. Leaf area was determined by means of destructive measurement and captured using a LICOR Model 3100 area meter. Light intensity in the bunch zone of the canopy (east facing side) was measured (five measurements per plot) during mid-morning by means of a LICOR Line Quantum Sensor and expressed as a percentage of ambient light level determined in the vine row. Photosynthetic activity ( $P_n$ ) as well as transpiration ( $E$ ) of fully expanded leaves on primary shoots in the basal part of the canopy was measured (three leaves per plot) during mid-morning using a closed system LI-6400 portable photosynthesis meter (LI-COR Biosciences, Nebraska, USA), with light source set at  $2000 \mu\text{mol m}^{-2}\text{s}^{-1}$ . Pre-dawn leaf water potential of mature leaves was determined (five leaves per plot) before sunrise using a pressure chamber (Scholander *et al.*, 1965). All seasonal measurements were completed on a single day and followed a bi-weekly frequency, from flowering to the last harvest date. During winter, after the completion of the growth cycle, pruning weight per vine was determined for all the grapevines in the experimental plot.

### 3.3.3 Harvesting sampling

Grapes were harvested at five sequential harvest dates coinciding with approximate total soluble solids/degree Brix of 21, 23, 25, 27 and 29, respectively. At every harvest date, yield per vine was measured and 12 bunches were randomly selected for analysis. After bunch mass was determined, all the berries were separated from the rachis (keeping the pedicel intact). From a sub-sample of 200 randomly selected berries, berry volume and weight were recorded. The remaining berry sample was either immediately crushed for compositional analyses or frozen at  $-80\text{ }^{\circ}\text{C}$  for analysis at a later stage.

### 3.3.4 Experimental layout

Vineyards grafted onto the two rootstocks were located directly adjacent to one another. Considering the variables [clone (A-PI 50 vs B-PI 48), age (A-1997 vs B-1999) and slope (A-Top vs B-Bottom)] of the experimental vineyard, rootstock was not considered a treatment as such, but rather the combination of rootstock and site, therefore resulting in two measurement sites: Site A (Pinotage/140 Ruggeri) and Site B (Pinotage/1103 Paulsen). Experimental plots were assigned by a randomised block design, selecting vine parcels from an area within the vineyard which displayed homogeneous vigour. Experimental plots consisted of 30 vines each and were replicated five times per ripeness level for each site.

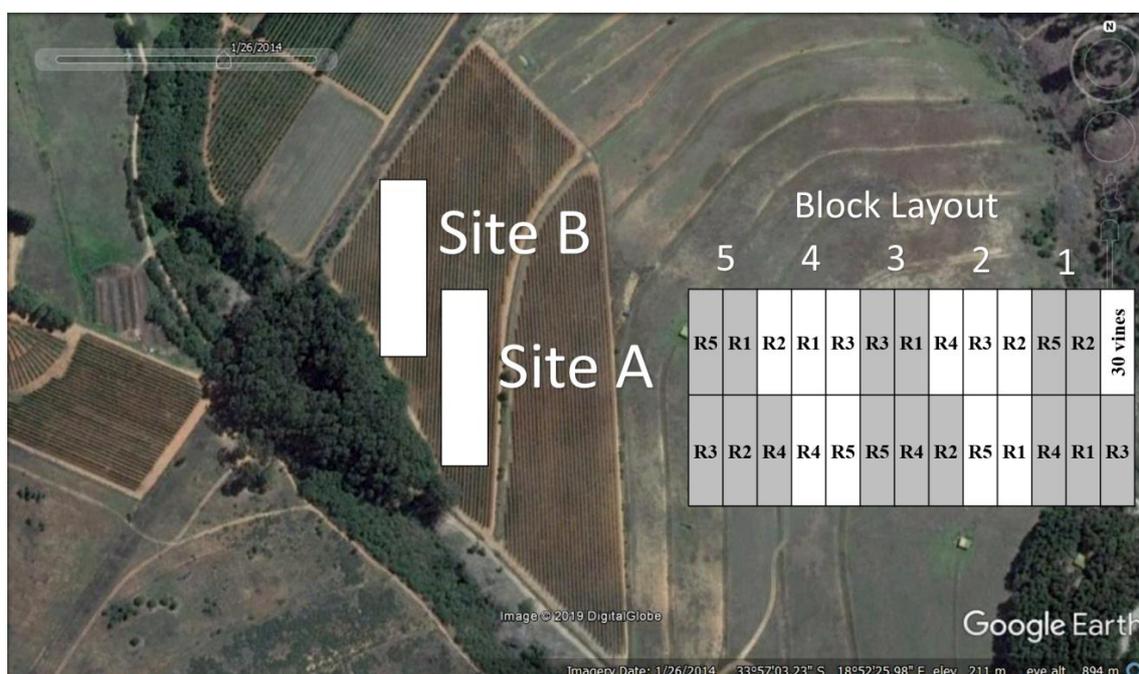


Figure 1. Experimental layout at Stellenbosch University Welgevallen experimental farm, indicating both measurement sites (A & B) and block design with five repetitions (blocks 1 -5) and five ripeness levels (R1-R5).

### 3.3.5 Statistical analyses

Analysis of variance (ANOVA) on the data was performed using statistical software, SAS version 9.2 (SAS Institute Inc., USA), and normality of data was evaluated using the Shapiro-Wilk test. To compare means, the Student's t-test for Least Significant Differences was calculated at the 5% significance level.

## 3.4 RESULTS AND DISCUSSION

### 3.4.1 Climatic conditions

The three season experimental period was characterised by a moderate season (2015), followed by two extremely dry seasons in 2016 and 2017 (Table 1). Dispersed summer rainfall occurred during all three growing seasons. The 2015 season received 30% less rainfall during the growing season; the 2016, 64% less and 2017, 50% less compared to the long-term mean. Regarding annual rainfall, 2015 received 32 mm more; the 2016, 247 mm less and 2017, 147 mm less than the long-term mean. The dry conditions in 2016 were amplified by high temperatures, reflected by the highest total seasonal growing degree days as well as temperatures (Tmax and Tmin) during January. This is the month in which Pinotage generally ripens (Van Schalkwyk & Schmidt, 2009). Upon closer investigation, 2016 displayed more frequent heat wave events (consecutive days of high temperatures) compared to the 2015 and 2017 vintages (Fig 2a, 2b & 2c). In conjunction with high temperatures and very low rainfall, evaporative demand was also high. As a whole, the contrast created by 2015, displaying moderate climatic conditions, versus 2016 and 2017, exhibiting warmer and drier conditions with higher day-night amplitudes (+ 3°C), provided relevant context to study the reaction of Pinotage grapevines to possible future warmer and dryer, more extreme conditions.

Table 1. Climatic parameters for Stellenbosch for the growing seasons 2015 to 2017, compared to long term means.

Climatic Parameter	2015	2016	2017	Long term Mean*
ETo (mm) <sup>1</sup>	780	875	904	835
Rainfall (mm) <sup>2</sup>	134	69	97	191
Annual Rainfall (mm) <sup>3</sup>	665	386	486	633
GDD <sup>4</sup>	1682	1757	1571	1627
Ave Tmax Jan (°C)	29.88	34.09	30.16	29.43
Ave Tmin Jan (°C)	16.03	16.66	13.11	14.68

\*Long term mean from Stellenbosch (Elsenburg) weather station (data represents 01-09-1995 to 01-09-2014)<sup>1</sup> Reference pan evapotranspiration (1 Sept – 1 March)<sup>2</sup> Rainfall seasonal (1 Sept – 1 March)<sup>3</sup> Annual Rainfall (Harvest to Harvest)<sup>4</sup> Growing Degree Days = Sum of mean daily temperature above 10°C (1 Sept – 1 March) † Presentation of climatic data was adapted from Winkler (1974) to the growth cycle of Pinotage (1 Sept – 1 March).

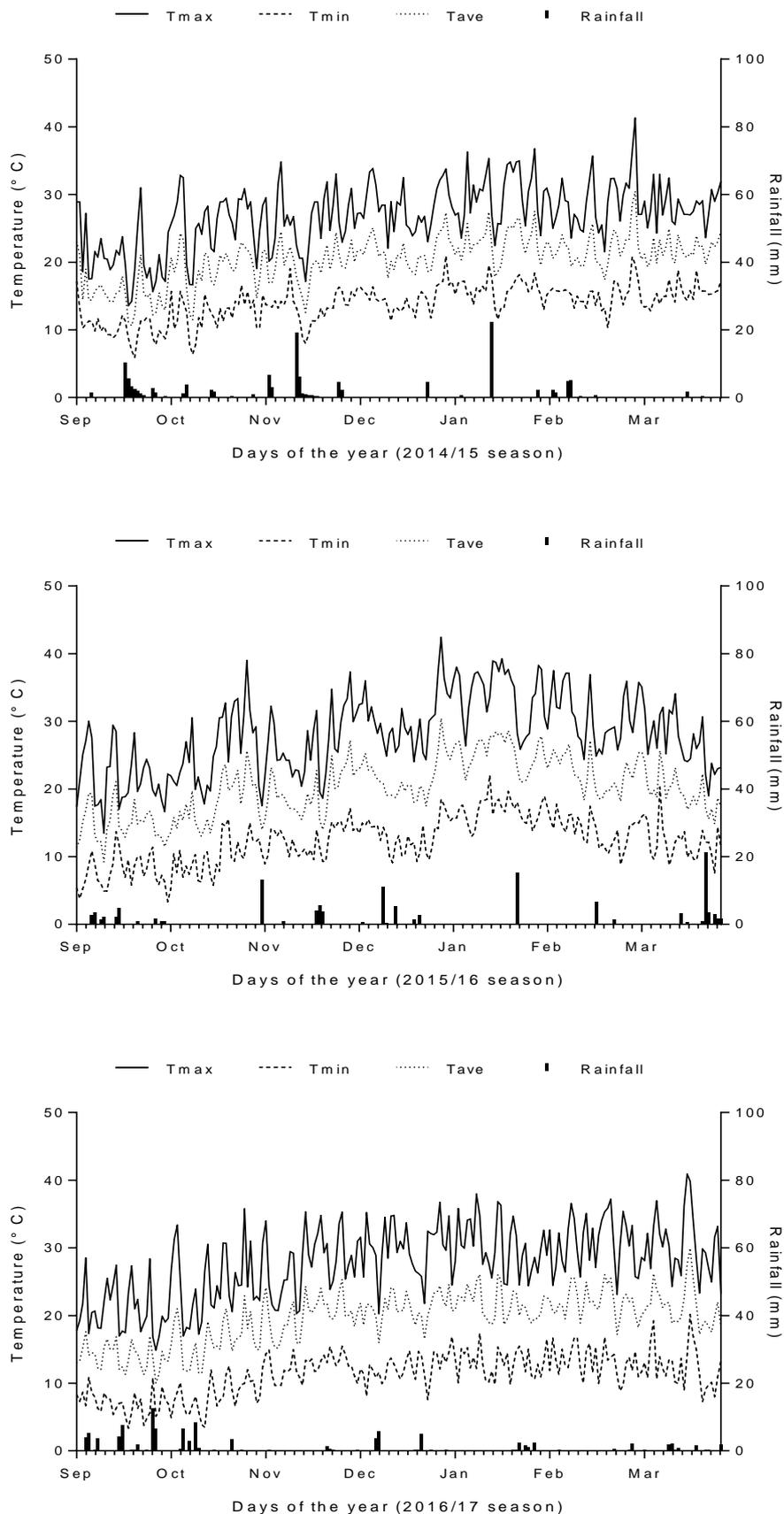


Figure 2. Daily temperature and rainfall measurements for the 2014/15(top), 2015/16(middle) and 2016/17(bottom) growing seasons for Stellenbosch, Western Cape, South Africa.

### 3.4.2 Soil conditions

Soil physical characterisation was done prior to planting of the vineyard, the soil was classified on both sites as Oakleaf soil form (Soil Classification Working Group, 1991), belonging to the cumulic group, these soils are considered to be recent and often encountered at foothills, established from unconsolidated sediments (Fey, 2010). Soil chemical properties (Table 2) displayed values coherent with the highly weathered nature of the soils of the Western Cape (WC), South Africa (Fey, 2010). Most notably low pH, and free H<sup>+</sup>, although greater than minimum (pH 5.5) prescribed for vineyards in WC. Soil concentrations for Na, K, Ca and Mg showed trends of decrease with the increase in depth, in the topsoil (0 – 30cm) mineral concentrations were within prescribed thresholds (Saayman, 1981; Van Schoor *et al.*, 2000), while P was below threshold in the subsoil (30 – 60cm). High clay content (> 25%) and unrestricted soil depth (> 0.9m) as determined before planting, resulting in good water holding capacity made unirrigated grapevine cultivation possible on both sites.

Table 2. Soil chemical analysis for cv. Pinotage, Stellenbosch (2017, final year of study).

Site	Depth (cm)	pH (KCl)	H <sup>+</sup> (cmol /kg)	Resist (Ohm)	Gravel Vol (%)	P - Bray II (mg/kg)	Exchangeable Cations (cmol+/kg)			
							Na	K	Ca	Mg
Site A	0 - 30	6.02a	0.23b	2210b	2.2c	29.66a	0.078a	0.20a	2.49b	0.98a
	30 - 60	5.98a	0.25b	2060b	6.2bc	14.08b	0.084a	0.13b	2.50b	0.74b
Site B	0 - 30	5.70b	0.44a	2766a	18.2ab	29.51a	0.080a	0.14b	2.52ab	0.94a
	30 - 60	5.72b	0.35b	2396ab	24.6a	16.04b	0.078a	0.11b	2.78a	0.80b

Mean values within columns followed by the same letter do not differ significantly ( $p \leq 0.05$ )

Site	Depth (cm)	Texture	Soluble S (mg/kg)	Carbon (%)	T - Value (cmol /kg)	Acid Sat.	% Base Saturation			
							Na	K	Ca	Mg
Site A	0 - 30	Clay	12.08ab	0.59a	3.98ab	5.97c	1.97a	5.04a	62.32b	24.70a
	30 - 60	Clay	14.56a	0.40b	3.71b	6.80c	2.26a	3.71b	67.30a	19.94b
Site B	0 - 30	Clay	11.06b	0.61a	4.11a	11.30a	1.97a	3.48bc	60.38b	22.86a
	30 - 60	Clay	11.73ab	0.53a	4.12a	8.98b	1.94a	2.72c	67.00a	19.36b

Mean values within columns followed by the same letter do not differ significantly ( $p \leq 0.05$ )

### 3.4.3 Water Status

Predawn leaf water potential ( $\Psi_{PD}$ ) measured on both sites (Site A & Site B) showed clear seasonal trends regarding changes in vine water status (Fig 3). As expected for unirrigated vines in a low summer rainfall region,  $\Psi_{PD}$  values became progressively negative (higher water deficit) as vegetative and reproductive components developed and soil water content decreased. Moreover,  $\Psi_{PD}$  values were significantly lower in the latter parts (véraison to harvest) of the 2016 and 2017 seasons compared to those recorded in 2015 (Table 3). Consequently, in 2016 and 2017 water deficits on both sites ranged from ‘moderate to strong’ (-0.4 to -0.6 MPa) at pré-véraison and increased to ‘strong to severe’ (-0.6 to -0.8 MPa) from véraison until late in the ripening phase (Ojeda, 2007). Grapevines recovered to ‘moderate’ deficit levels at the last measurement date; late season recuperation is linked to the vascular detachment of sinks and less demanding climatic conditions at the end of the season (Hunter *et al.*, 2014; Carlomagno *et al.*, 2018). Interestingly, cv. Pinotage ripens substantially earlier (4 – 6 weeks) than e.g. Shiraz as in Hunter *et al.* (2014) and Carlomagno *et al.* (2018), shifting the period of recuperation to one of the warmest/driest months (February) of the season. In this case, despite continued climatic pressure, recuperation took place, further highlighting the responsiveness of the grapevine and the role of active sinks on the hydraulic state of the plant. In contrast, 2015 only displayed ‘moderate’ deficits at maximum throughout the season and did not demonstrate any late season recovery. Comparing the two site-rootstock combinations, the sequence and severity of water deficits were similar in all three seasons and the combination of site and rootstock did not impact significantly to change the reaction of the plants with the same scion and rootstock parentage. However, in general water deficits as represented by  $\Psi_{PD}$  demonstrated the impact of dryer conditions in 2016 and 2017 compared to 2015 on vine water status. Moreover, given the recovery, response of grapevines under strenuous conditions indicated that physiological limits were not yet surpassed, most probably aided by a well-developed root system (Hunter & Myburgh, 2001) and drought tolerance inferred to scion by the rootstocks (Serra *et al.*, 2014).

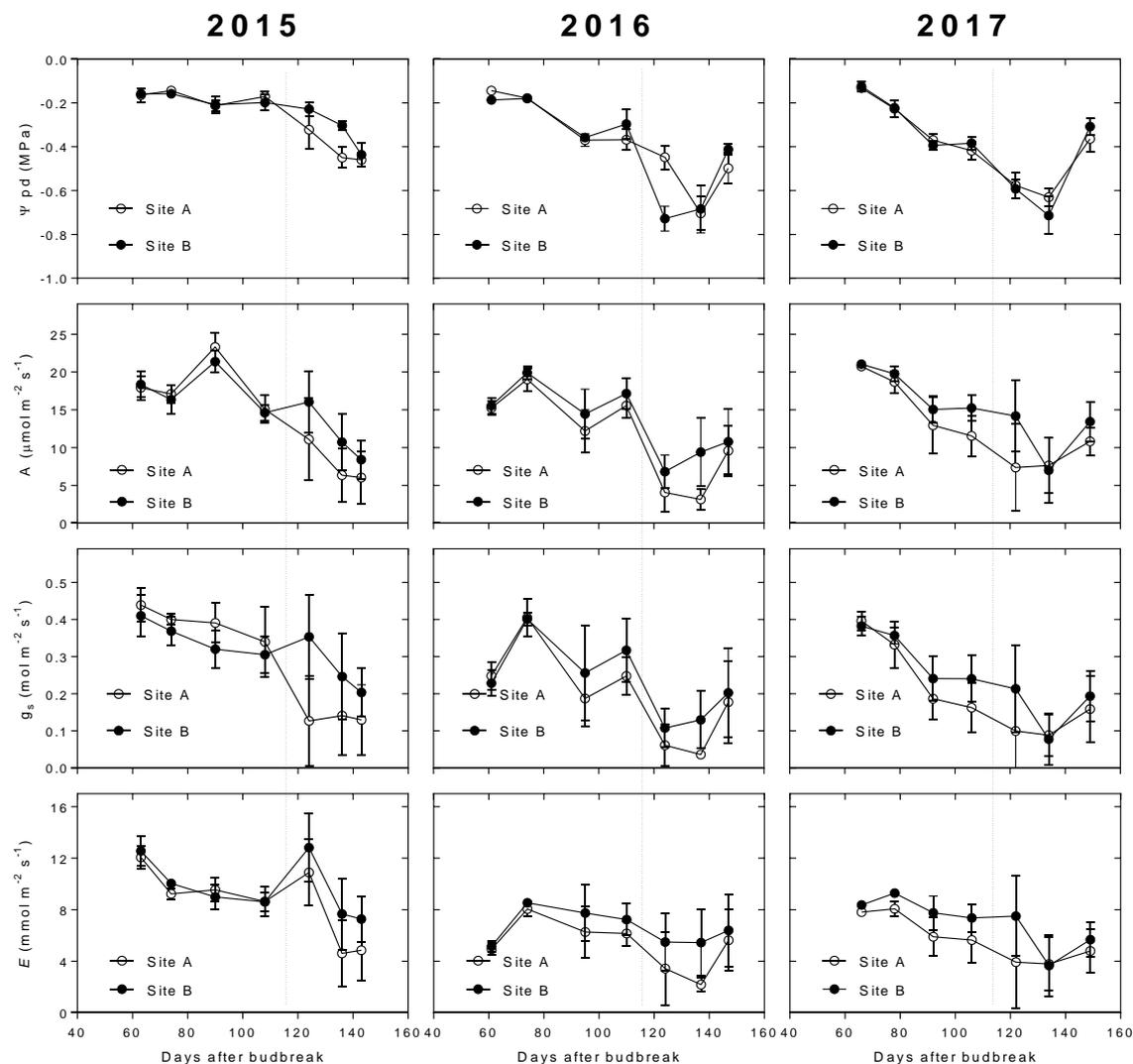


Figure 3. Predawn leaf water potential ( $\Psi_{pd}$ ), photosynthetic rate ( $A$ ), stomatal conductance ( $g_s$ ) and transpiration rate ( $E$ ) from flowering ( $\pm 60$  DAB) to final harvest ( $\pm 150$  DAB), for 2015 – 2017 in cv. Pinotage, Stellenbosch, South Africa (standard deviation confidence interval 95 %, --- indicates véraison).

### 3.4.4 Photosynthetic activity

For all three seasons, photosynthetic activity of basal primary leaves was maintained at moderate rates early season and declined substantially as the season progressed (Fig. 3). Photosynthetic rates were comparable and lower to those recorded by Hunter *et al.* (2014) for unirrigated Shiraz in Stellenbosch during several growing seasons using a similar protocol. The visible decline in photosynthetic activity in the pre-véraison stage was most probably due to grapevine water deficit (Table 3), mediated by tight stomatal regulation (Escalona *et al.*, 1999) and natural leaf senescence (particularly relevant as only primary basal leaves were measured) of the canopy during ripening (Hunter *et al.*, 1994). Moreover, interpreting lower photosynthetic rates during the measurement window based on the function of basal primary leaves is risky, as previous studies have shown grapevine adaptive strategies to include shifting of photosynthetic activity to

more responsive parts of the canopy (Medrano *et al.*, 2017), particularly younger secondary leaves. As expected, stomatal conductance and transpiration (Fig 3) declined in conjunction with photosynthetic rate, as grapevines favoured turgor maintenance over carbon assimilation and simultaneous water loss. Notably, values presented greater variability as environmental conditions became more stressful, especially during the ripening phase, and clearly point to different levels of adaptive capabilities of individual leaves/shoots/plants. Nonetheless, Pinotage grapevines on both sites displayed a level of maintenance physiological activity during the most extreme conditions (high temperature, high  $ET_0$  and low soil water availability) in the measurement window, e.g. the ripening phase in 2016 from 120 to 140 DAB (Fig 3).

Table 3. ANOVA results for grapevine physiological parameters for cv. Pinotage (Stellenbosch 2015-17)

	$\Psi_{Pd}$	A	gs	E	A/E	A/gs
Vintage	**	***	***	***	***	***
Site	<i>ns</i>	***	***	***	*	<i>ns</i>
DAB	***	***	***	***	***	***
Site x Vintage	***	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
DAB x Vintage	***	***	***	***	***	***
Site x DAB	***	***	**	***	*	***
Site x DAB x Vintage	***	**	**	**	**	<i>ns</i>

*ns* non-significant ; \*  $p \leq 0.05$  ; \*\*  $p \leq 0.01$  ; \*\*\*  $p \leq 0.001$ , Days after budbreak (DAB), Predawn leaf water potential ( $\Psi_{pd}$ ), photosynthetic rate (A), stomatal conductance (gs) and transpiration rate (E), and Instantaneous water-use efficiency (A/E) and Intrinsic water-use-efficiency (A/gs)

Adaptive behaviour, through tight stomatal regulation, was well demonstrated by the relationship between the stomatal conductance and  $\Psi_{PD}$  (Fig. 4), showing decreases in stomatal conductance as water deficit increased. A feature of drought resistance is the ability of plants to continue a level of carbon assimilation (photosynthetic activity) whilst minimising water loss (transpiration). Instantaneous water use efficiency (WUE), described as the ratio of leaf photosynthetic rate to leaf transpiration rate, displayed comparable trends for both sites during the measurement window (Fig. 5). Levels of instantaneous leaf WUE demonstrated associated increases and decreases with daily prevailing conditions within the season, although lower rates were more frequent during ripening, up to the point of late season recuperation. Moreover, in 2017 (dry season) grapevines maintained a relatively high WUE compared to that of 2015 (moderate season), further showing towards water status maintenance priority and increased WUE during times of increased water deficit. Considering intrinsic  $WUE_i$  (photosynthetic rate/stomatal conductance), a ratio which is often used to discern the differences in active stomatal regulation between genotypes (Schultz, 2003; Soar *et al.*, 2006; Chaves *et al.*, 2010), the values recorded over the three seasons were comparable for sites of different rootstocks.

Changes in  $WUE_i$ , particularly during the ripening phase (high water deficit) demonstrated grapevine adjustment to prevalent environmental conditions, through regulated stomatal aperture over a range of water potentials (Fig. 5).

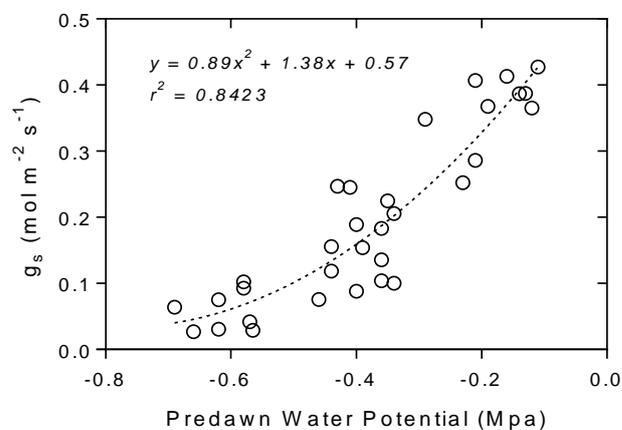


Figure 4. Relationship between stomatal conductance and predawn water potential for cv. Pinotage (Site A) during 2017 growing season.

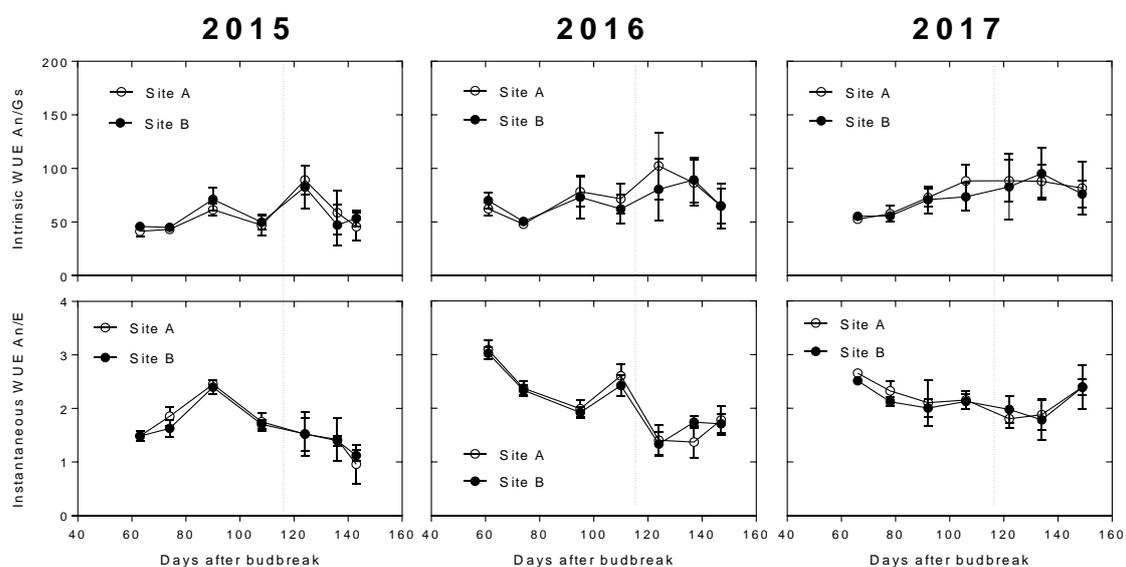


Figure 5. Intrinsic water-use-efficiency (top) and Instantaneous water-use efficiency (bottom) from flowering ( $\pm$  60 DAB) to final harvest ( $\pm$  150 DAB) for 2015 – 2017 in cv. Pinotage, Stellenbosch, South Africa (standard deviation confidence interval 95 %, --- indicates véraison).

### 3.4.6 Vegetative growth

Total leaf area (TLA) per vine displayed stable and comparable trends for both sites (albeit with a degree of variability) (Fig 6 & Table 4). Grapevines were topped and measurement only started at flowering and therefore increases in total leaf area is expected to be largely dependent on secondary leaf area (SLA) development. Reduction in primary leaf area (PLA) during ripening in 2016 and 2017 could be explained by severe leaf senescence, manifesting in a loss of basal primary leaves, due to the plants experiencing severe water deficits (Fig 3). Secondary leaf area measurements displayed a high level of variation in 2015 and 2017. In 2016 SLA development was significantly suppressed in accordance with results presented by Lebon *et al.* (2006), in reaction to increased water deficits.

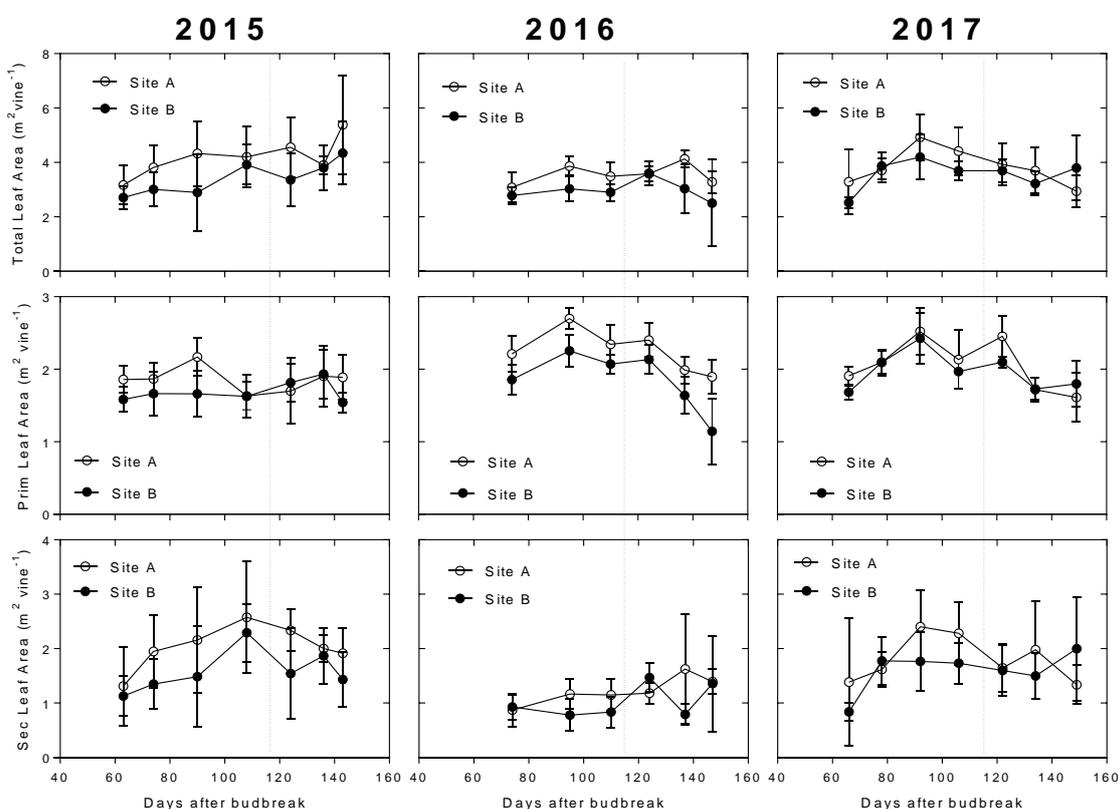


Figure 6. Total leaf area per vine (a), primary leaf area per vine (b) and secondary leaf area per vine (c) from flowering (+/- 60 DAB) to final harvest (+/- 150 DAB) for 2015 – 2017 in cv. Pinotage, Stellenbosch, South Africa (standard deviation confidence interval 95 %, --- indicates véraison).

Table 4. ANOVA results for grapevine canopy measurements for cv. Pinotage (Stellenbosch 2015-17)

	Tot LA	Prim LA	Sec LA	Prim LA:Sec LA	PAR %
Vintage	***	***	***	***	***
Site	***	***	**	*	***
DAB	**	***	<i>ns</i>	***	***
Site x Vintage	<i>ns</i>	**	<i>ns</i>	<i>ns</i>	<i>ns</i>
DAB x Vintage	<i>ns</i>	***	<i>ns</i>	***	***
Site x DAB	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	*
Site x DAB x Vintage	<i>ns</i>	<i>ns</i>	<i>ns</i>	*	<i>ns</i>

*ns* non-significant ; \*  $p \leq 0.05$  ; \*\*  $p \leq 0.01$  ; \*\*\*  $p \leq 0.001$ , Days after budbreak (DAB), Total leaf area (Tot LA), Primary leaf area (Prim LA), Secondary leaf area (Sec LA), Photosynthetic active radiation in the bunch zone (PAR %)

Growing conditions altered the primary to secondary leaf area ratio (Table 5). With lower values in the moderate 2015 season, due to a greater proportion of SLA. In 2015, SLA was promoted by early topping of the vigorous vines, while the requirement for topping was less frequent in both 2016 and 2017 seasons (data not shown). Secondary leaf area has been shown to make a significant contribution to sinks during both the ripening of grapes and storage of reserves in vine permanent structures (Hunter, 2000).

Overall vine vigour estimated by dormant pruning weights per vine confirmed seasonal impact on grapevine carbon allocation to dominant active vegetative parts. Greater water availability in the 2015 season induced significantly higher pruning weights per vine. Grapevines from the two sites also displayed different carbon allocation. The Pinotage/140 Ru combination (Site A) maintained stronger vegetative growth compared to the Pinotage/1103 P combination (Site B). This was counter to the expected reaction, since Site B was located at the lower end of the slope, which is generally considered to induce higher soil fertility and water availability. Considering the soil properties, Site B had higher gravel content in the upper 0 – 60 cm layer than Site A (Table 2), thereby possibly reducing available soil volume in those layers. However, grapevine water status measurements were maintained at similar levels for Site A & B (see **Water Status**) during the measurement window, thus showing towards possible differences in Site x Genotype interactions and its effect on vegetative expression.

Table 5. Primary and Secondary leaf area (LA) ratios and pruning weight for 2015 – 2017 in cv. Pinotage, Stellenbosch, South Africa.

Parameter	Site	2015	2016	2017	Site (S)	Site x Year (S x Y)
Leaf area ratio (Prim:Sec)*	Site A	0.67c	2.09a	1.44b	ns	p ≤ 0.05
	Site B	1.44a	1.49a	1.41a		
Pruning weight (kg vine <sup>-1</sup> )†	Site A	0.57a	0.43b	0.45b	p ≤ 0.001	p ≤ 0.01
	Site B	0.39a	0.33b	0.30b		

Mean values within rows followed by the same letter do not differ significantly (p ≤ 0.05)

\* Ratio of Primary to Secondary leaf area per vine at véraison

† Dormant pruning weight per vine

Increased light incidence in the bunch zone (Fig. 6) as ripening progressed, was supported by evidence of early cessation of secondary growth and subsequent senescence of basal primary leaves in the latter ripening stages (Fig. 5). That effectively created different microclimatic conditions for earlier (less exposed) harvested grapes compared to later ones (more exposed), thereby increasing cumulative fruit light interception of later harvested grape berries. More exposed later harvested grapes would also be expected to readily attain higher berry temperatures, as cv. Pinotage ripens early and late harvest dates coincide with the warmest month (February) of the year. Berry temperature has profound effects on berry composition (Bergqvist *et al.*, 2001; Spayd *et al.*, 2002). On a seasonal level, the high degree of water deficits seen in 2016 manifested through higher light incidence in the bunch zone, underlining the impact of seasonal water deficits and its effect on vegetative expression and resulting fruit microclimate.

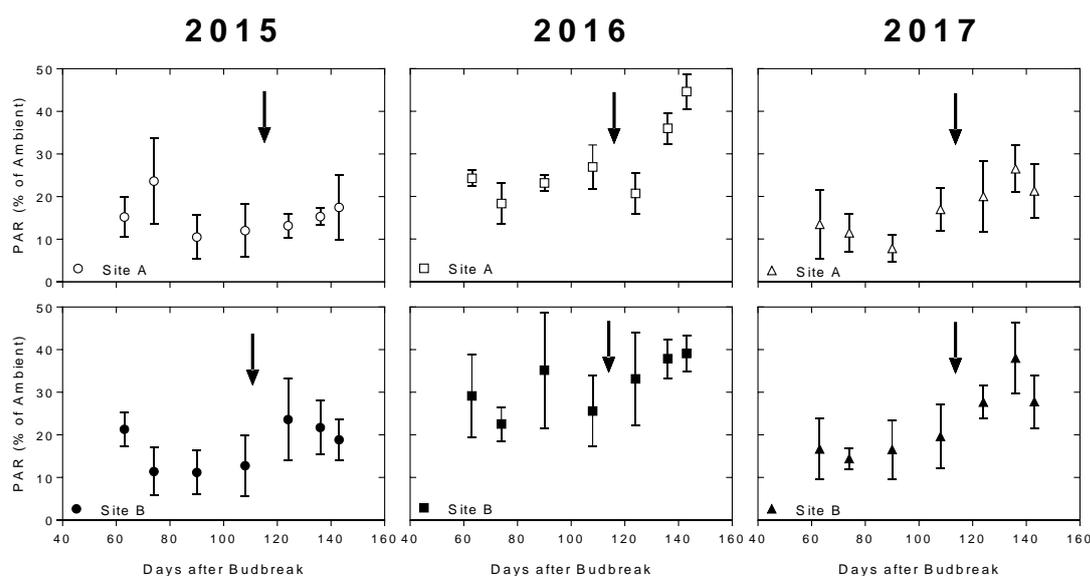


Figure 7. Light interception in bunch zone expressed as % of ambient photosynthetic active radiation (PAR) at Site A (top) and Site B (bottom) from flowering (+/- 60 DAB) to final harvest (+/- 150 DAB) for 2015 – 2017 in cv. Pinotage, Stellenbosch, South Africa (standard deviation confidence interval 95 %, ↓ indicates véraison).

### 3.4.7 Yield Components

Overall, maximum berry fresh weight was already attained at the start of the measurement window/first ripeness level (Table 6 & 7) and showed stable trends as ripening continued to 27°B; before decreasing as ripening reached the final stage (29°B). Stability of the berry fresh mass parameter in the initial ripening stages seemed likely considering the short intervals that separated early ripeness levels, which in some cases were harvested only three days apart. Berry volume remained stable as active phloem loading to the berry increased the sugar concentration and compensated for or replenished transpiration losses, thereby maintaining berry turgor. Harvest intervals during the latter ripening stages became further apart as active phloem loading decreased most probably through diminished capacity of the canopy (leaf senescence) and increased water constraints (Hunter *et al.*, 2014). This led to decreases in berry fresh weight and volume towards the final ripeness level (29°B) as transpiration continued and berry water losses were not replenished, leading to berry shrivel and concomitant solute concentration (Carlomagno *et al.*, 2018). Overall average berry weights were higher in the season with the lowest water deficit, 2015, compared to 2016 and 2017, although water deficits were only evident in the post-véraison period and unlikely to have induced early season berry size reduction effects as reported by Ojeda *et al.* (2002).

Bunch weight followed similar trends to berry mass, remaining stable through most of the ripening, with an important decrease in the final ripening stage. The contribution of the rachis to the total bunch weight has been shown to be relatively stable across ripeness levels (Hunter *et al.*, 2014) and changes were therefore largely induced by changes in berry weight. Comparing the two sites, the bunch weight recorded for Site B was significantly higher than that recorded for Site A, across vintages and ripeness levels. In view of similar berry weights, higher bunch weights may be due to a higher number of berries per bunch and, in view of the higher vigour observed for Site A, more balanced vegetative:reproductive growth balances for Site B.

Table 6. Effect of ripeness level on yield components for cv. Pinotage, Stellenbosch (2015-2017)

		Harvest		Berry weight		Berry Volume		Bunch Weight	
		st	Harvest	(gram)		(ml)		(gram)	
		(DAB)	(DAB)						
Ripeness									
Vintage	Level	Site A	Site B	Site A	Site B	Site A	Site B	Site A	Site B
2015	21	134	134	1.57a	1.56ab	1.32a	1.34a	118.4a	187.4a
	23	137	137	1.50a	1.60a	1.26a	1.37a	129.2a	181.8a
	25	140	140	1.48a	1.52abc	1.23a	1.32a	136.3a	190.3a
	27	146	146	1.45a	1.46bc	1.20a	1.26b	126.1a	166.4a
	29	151	151	1.23b	1.42c	1.04b	1.23b	112.6a	183.7a
2016	21	132	135	1.47ab	1.57a	1.17a	1.24a	151.7a	185.0b
	23	135	140	1.55a	1.44ab	1.20a	1.19a	141.0a	182.5b
	25	140	147	1.40abc	1.43ab	1.13a	1.34a	139.1a	187.2ab
	27	147	151	1.36bc	1.47ab	1.25a	1.28a	132.7a	232.1a
	29	154	154	1.28c	1.36b	1.12a	1.22a	132.5a	179.8b
2017	21	131	133	1.22a	1.46a	1.28a	1.35a	130.2a	168.0a
	23	133	136	1.31a	1.38a	1.22a	1.28a	118.9ab	191.3a
	25	136	140	1.30a	1.50a	1.20a	1.40a	121.1ab	189.3a
	27	140	150	1.35a	1.42a	1.24a	1.27a	103.2b	182.7a
	29	155	155	1.37a	1.50a	1.26a	1.36a	140.2a	177.5a

Mean values within columns for each vintage followed by the same letter do not differ significantly ( $p \leq 0.05$ )

Table 7. ANOVA results for grape berry parameters at harvest for cv. Pinotage (Stellenbosch 2015-17)

	Berry weight	Berry Volume	Bunch weight
Vintage	**	***	*
Site	***	***	***
Ripeness Level	**	*	<i>ns</i>
Site x Vintage	<i>ns</i>	<i>ns</i>	<i>ns</i>
Ripeness Level x Vintage	**	**	<i>ns</i>
Ripeness Level x Site	<i>ns</i>	*	<i>ns</i>
Ripeness Level x Site x Vintage	<i>ns</i>	<i>ns</i>	<i>ns</i>

*ns* non-significant ; \*  $p \leq 0.05$  ; \*\*  $p \leq 0.01$  ; \*\*\*  $p \leq 0.001$

Yields per vine on Site A were lower than on Site B, most probably driven by a higher number of berries per bunch, rather than higher berry weights (Table 8). Seasonal variation in terms of prevailing climatic conditions did not impact the yield per vine parameter to differ significantly over vintages.

Grapevine source/sink balance can be expressed as a ratio of leaf area to fruit weight; over cropped situations occur when inadequate LA is available to supply ripening berries (sinks) (Buttrose, 1966). In this study, TLA:Yield ratios at commercial harvest displayed values

significantly higher than those proposed in literature for VSP trained vines, i.e. 1,0 – 1,5 m<sup>2</sup>/kg (Kliewer & Antcliff, 1970; Smart, 1985), and notably higher than 0,8 – 1,2 m<sup>2</sup>/kg proposed for moderate to warm climates (Kliewer & Dokoozlian, 2005), considering the prevailing Mediterranean conditions. Higher TLA:Yield ratios, especially in Site A, were clearly driven by low yields per vine and while Site B produced higher yields per vine, TLA:Yield values were still higher than those proposed by Kliewer & Dokoozlian (2005). This most probably aided grapevines to readily ripen grapes over the spectrum of prevailing conditions, due to an adequate supply to the sinks. Consistency in yield was surely brought about by uniform bud load left at pruning and the judicious removal of all the bearing and non-bearing shoots (suckering) that were not allocated during pruning, together with low to moderate water deficits during the pré-*véraison* period, buffering water deficit related berry size reductions.

Table 8. Seasonal yield and growth balances for cv. Pinotage in Stellenbosch (2015-17)

Parameter	Site	2015	2016	2017	Site	Site x
					(S)	Year
(S x Y)						
Yield (kg vine <sup>-1</sup> )*	Site A	1.66a	1.83a	1.13b	p ≤ 0.01	ns
	Site B	2.29a	2.88a	2.11a		
Total Leaf Area:Yield (m <sup>2</sup> /kg)†	Site A	2.74ab	1.98b	3.95a	ns	ns
	Site B	1.46a	1.35a	1.97a		

Mean values within rows followed by the same letter do not differ significantly (p ≤ 0.05)

\* Yield per vine at commercial harvest (25°B)

† Total leaf area to yield ratio at commercial harvest (25°B)

### 3.5 CONCLUSIONS

Changes in growing conditions brought about by seasonal differences in rainfall and temperature had a major impact on cv. Pinotage grapevine performance under the experimental conditions. Grapevines grafted to drought tolerant rootstocks invariably adapted to warmer and drier conditions, demonstrating efficient stomatal control and sustained WUE under higher seasonal water deficits. On a whole-plant level grapevines responded with reduced/altered leaf area development, in particular suppressing secondary leaf area, while leaf senescence reduced/altered primary leaf area during late ripening stages. This influenced source/sink relationships as well as bunch microclimate during the ripening phase. Effects on reproductive components during ripening, measured in the 21 – 29°B window, demonstrated remarkable stability regarding berry weight, berry volume, bunch weight and timing of harvesting stage over a diverse range of climatic conditions and vintages. This may be attributed to the consistent moderate pre-véraison water constraints experienced by the plants, highlighting the importance of soil water availability during this critical period. Under these conditions, the genotype, specifically with regard to marked rapid and early maturation, coped with lower seasonal water availability.

This study demonstrated cv. Pinotage responses over a wide range of ripeness levels and lays an important foundation of plant behavioural information for future studies with specific reference to the possible effects of climate change and imminent higher temperatures and limited water supply.

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## Chapter 4

### **Berry composition of *Vitis vinifera* L. cv. Pinotage as related to grape ripeness level**

#### 4.1 ABSTRACT

Grape composition represents a major part of final wine qualitative potential, therefore a detailed understanding of its relation to ripeness level can improve decision making. In this field study the extent of grape compositional changes for cv. Pinotage were assessed at five (R1-R5) ripeness levels that were defined by Brix (*ca.* 21, 23, 25, 27 & 29°B) over three vintages (2015-17) and two sites, Pinotage/140 Ruggeri (A) and Pinotage/1103 Paulsen (B), under Mediterranean conditions, Western Cape, South Africa. As no significant changes in berry size were recorded from R1-R4, accumulation or decrease of berry constituents was independent of the concentration effect by dehydration (increased skin:pulp ratio) as this only manifested at R5. Late season berry size reduction (dehydration) in R5 was linked to increases in primary metabolites (sugars and acids) and minerals (both phloem and xylem bound), but to decreases in phenolic and anthocyanin content. Concentration of berry phenolic compounds and pigments increased from R1 to R4 (peak), before declining towards R5. Anthocyanin proportions changed during ripening as proportions of Malv increased at the expense of Delf, Cya, and Peo. More specifically PetAc, MalvCum and MalvCaff associated well with grapes of increasing ripeness levels and warrant further investigation as indicators of ripeness level. Generally, a decrease in abundance of potential grape aroma compounds was noted along ripening for the 20 compounds investigated [incl C6-compounds, methoxypyrazines (MP), C13-norisoprenoids (C13N), terpenes (TP), benzoids and volatile phenols (VP)]. Changes in grape aromatic profile were subtle and reaction of key components was significantly influenced by Vintage and Site. Ripening grapes were characterised by decreased C6-compounds and MP's, with different responses from norisoprenoids, terpenes and VP's. Results suggest a relatively minor shift in overall aroma profile per ripeness level (+ 2°B) during the compact harvest window (21 days). This exhaustive berry compositional field study placed ripening related changes in context to that dictated by the environment (Vintage/Climate) and Site (Soil & Genotype), indicating negative effects of extended hang time on phenolics and providing much needed insight into compositional changes during rapid sugar accumulation.

## 4.2 INTRODUCTION

Berry composition changes significantly during the course of ripening (Coombe, 1992), as a result of various biochemical processes taking place in the grape berry (Conde *et al.*, 2007), ultimately affecting wine composition and sensory attributes (Hunter, Pisciotta, *et al.*, 2004; Dennis *et al.*, 2012; Bindon *et al.*, 2014; Boss *et al.*, 2014). Due to the non-climacteric nature of grapes and the decisive impact of ripeness level on berry composition, wine quality effectively hinges on harvesting at the correct maturity level for the desired outcome.

Traditionally, ripening is monitored using classical ripening indices such as total soluble solids (°Brix), titratable acidity (TA) and pH, representing the increase in sugars and decrease in organic acids in the grape berry. These indices are simple and cost effective; however, acting alone they are insufficient to accurately predict wine composition (Jackson & Lombard, 1993), notably as many key grape derived compounds do not follow the same kinetics as sugar accumulation and acid degradation (Allen *et al.*, 1991; Adams, 2006; Vilanova *et al.*, 2012; Boss *et al.*, 2014). Secondary metabolites collectively describe key grape derived compounds that infer intrinsic qualities to wines in relatively low concentrations. The most important of these are the flavonoids (proanthocyanidins and anthocyanins) and aroma compounds, which are present in either free form or bound as glycosides (Kennedy *et al.*, 2002; Downey *et al.*, 2006; Boss *et al.*, 2014; Robinson *et al.*, 2014)

Condensed tannins (proanthocyanidins) are polymeric flavan-3-ols that are comprised of a range monomeric subunits (catechin, epicatechin, and their gallated derivatives), producing a wide range of compounds from small oligomers to large polymers. Grape tannin content is linked to wine sensory quality as they contribute significantly to wine astringency, bitterness, colour intensity and colour stability (Gawel, 1998; Vidal *et al.*, 2002; Monagas *et al.*, 2005; Ribéreau-Gayon *et al.*, 2006a). Proanthocyanidins primarily accumulate before véraison (Cadot *et al.*, 2006). Responses during ripening (after véraison) have shown to be unclear, as both increases (Ojeda *et al.*, 2002; Hunter *et al.*, 2014) and decreases (Harbertson *et al.*, 2002; Downey *et al.*, 2003) have been reported. Due to the large structural variation, measurement of proanthocyanidins proved to be highly subject to analytical methods, while they are also highly reactive to growing conditions (Ojeda *et al.*, 2002; Downey *et al.*, 2006). However, general consensus is that mean degree of polymerisation (mDP) increases during ripening (Kennedy *et al.*, 2002; Downey *et al.*, 2003; Fournand *et al.*, 2006). These increases in mDP have been implicated with a decrease in wine astringency (Gawel, 1998; Vidal *et al.*, 2002; Monagas *et al.*, 2005; Kennedy, 2008). This is thought to be brought about by a decrease in highly astringent monomeric (low mDP) content of seeds (Harbertson *et al.*, 2002). Thereby, Ristic *et al.* (2010)

could link higher grape skin tannin and anthocyanin content and lower seed tannin with increased wine sensory quality. However, seed tannin generally displayed a poor relationship to wine tannin in the same study, highlighting the importance of the skin fraction as wine qualitative denominator (Sparrow *et al.*, 2015; Bindon *et al.*, 2017). Contrary to proanthocyanidins, anthocyanins (red pigments) accumulate in grape berries with the onset of véraison and, apart from influencing wine colour, recent works have confirmed that the presence of anthocyanins increase tannin extraction during winemaking and thereby its role on wine astringency (Kilmister *et al.*, 2014; Bindon *et al.*, 2017). Differences in hydroxylation on the B-ring produces five distinct anthocyanin-3-O-glucosides, which include delphinidin- (Delf), cyanidin- (Cya), petunidin- (Pet), peonidin- (Peo), and malvidin- (Malv). Malvidin-monoglucosides are the most prevalent anthocyanin forms present in *Vitis vinifera* varieties. In addition to free monoglucosides, most varieties produce acetyl and p-coumaryl glucoside derivatives (Fong *et al.*, 1971; Roggero *et al.*, 1986). Essentially, this leads to significant varietal changes in anthocyanin acylation, which produces unique anthocyanin profiles for different varieties (de Villiers *et al.*, 2004; Guidoni & Hunter, 2012). Anthocyanins increase during ripening to reach a maximum at harvest (Ribéreau-Gayon, 1971) or decline just prior to harvest and/or during over-ripening (Roggero *et al.*, 1986). Increases of methoxylated forms (Malv, Peo and Pet) during ripening have been reported, while p-coumaryl glucoside peaks early and decreases during late ripening (Fournand *et al.*, 2006). Understanding ripeness-related changes under field conditions is challenging as anthocyanins and upstream metabolism are highly responsive to cultural conditions (Reshef *et al.*, 2017), such as UV exposure of berries (Downey *et al.*, 2006), bunch shading (Spayd *et al.*, 2002), berry temperature (Tarara *et al.*, 2008), diurnal ambient temperature (Kliewer & Torres, 1972; Mori *et al.*, 2005) and plant water status (Castellarin *et al.*, 2007).

Grape derived aroma profile is mainly dependent on the variety (Rocha *et al.*, 2010), although environmental factors certainly impact the extent of variation within the varietal profile (Jackson & Lombard, 1993). Many works have detailed the role of pungent free terpenes, denoting floral aromas in aromatic varieties such as cv. Muscat (Wilson *et al.*, 1984; Gunata *et al.*, 1988; Bureau, Razungles, *et al.*, 2000; Luan *et al.*, 2006). However, most red varieties exhibit more subtle aroma predominantly derived from various precursors present in non-odorous glycosylated form (Iland, 2001). Glycosylated precursors can either be monoglucoside conjugates or disaccharide glycosides, where glucose is linked to a sugar moiety such as  $\alpha$ -L-arabinofuranosyl,  $\alpha$ -L-rhamnopyranosyl,  $\beta$ -D-xylopyranosyl, or  $\beta$ -apiofuranosyl group, which is then bound to the aglycone (Williams *et al.*, 1995). The hydrolysis of these grape glycosides releases various volatile components, including monoterpenes (floral), norisoprenoids (floral,

berry-like), alcohols (fruity), and phenolic (spicy, smokey) compounds (Robinson *et al.*, 2014). In general, the increase of these positive glycosylated precursors in grapes is a distinctive feature of the advancement of ripening (Wilson *et al.*, 1984), although recent studies have shown less uniform (more complex) relationships with regard to ripeness level, especially regarding the latter stages of ripening (commercial harvest) (Bindon *et al.*, 2013; Boss *et al.*, 2014). Moreover, studies have shown that the rate and extent of accumulation of aroma precursors during grape ripening are compound dependent, most probably due to different metabolic origins (Yuan & Qian, 2016a). For instance, the absence of green flavours is a major qualitative trait in red varieties (Kalua & Boss, 2010; Boss *et al.*, 2014). Vegetative characteristics are generally linked to the presence of free isobutyl methoxypyrazine (IBMP), which infers a green bell pepper attribute to grapes and wines (Roujou de Boubee *et al.*, 2000; Romero *et al.*, 2006; Escudero *et al.*, 2007). The IBMP generally decreases during ripening and thus can be influenced by harvest date (Lacey *et al.*, 1991). However, increased exposure of grape berries to sunlight has shown to significantly decrease IBMP (Marais *et al.*, 1999). In addition, the C6-compounds (derived from fatty acids) have also been implicated in green and herbaceous flavours (Dubois, 1994; Escudero *et al.*, 2007). Grape C6-compounds can exist as fatty acid, acetate esters, aldehydes and alcohols, with C6-alcohols predominating the latter stages of ripening (Kalua & Boss, 2010; Vilanova *et al.*, 2012). Importantly, *de novo* generation of C6-compounds from fatty acids occurs via the lipoxygenase pathway with the disruption of berry cell walls during grape processing, releasing C6-aldehydes which are later reduced to C6-alcohols (Schwab *et al.*, 2008; Iyer *et al.*, 2010). The relationship has also been complicated by recent works elucidating the production of esters, *e.g.* hexyl acetate (fruity) and thiols, *e.g.* 3-mercaptohexan-1-ol (tropical) from C6 alcohol- and aldehyde- precursors during fermentation (Dennis *et al.*, 2012; Harsch *et al.*, 2013). Despite the underlying complexities and numerous gaps in our understanding, the collection and relative quantities of precursors and free compounds at harvest has a major impact on overall wine aromatic profile (Boss *et al.*, 2018). This, coupled with expected changes thereof due to ripeness level give producers an opportunity to manipulate wine sensory profile (Casassa *et al.*, 2013; Bindon *et al.*, 2014; Sherman *et al.*, 2017), the extent of which is yet to be fully elucidated.

*Vitis vinifera* L. cv. Pinotage is the 3<sup>rd</sup> most widely planted red variety in South Africa (SAWIS, 2017). Yet, berry composition and its relation to wine quality is poorly understood for this early ripening variety (Marais, 2003). One of the major challenges for viticulture in warm and dry Mediterranean climates (such as the Western Cape, South Africa), is that berries readily achieve full ripeness and even over-ripeness, within short timeframes. This effect will be compounded by predicted climate warming (Webb *et al.*, 2012), certainly necessitating prompt decision making during harvest. More knowledge of the environmental and compositional profiles during

ripening will aid growers to better estimate grape quality at harvest. In this study, an array of compositional parameters were studied in parallel with classical indices for a range of ripeness levels (21-29°B) in order to track changes during the commercial harvest window. This information will provide background for reasoning the extent of changes seen in resulting wine composition and sensory properties as influenced by ripeness level.

## 4.3 MATERIALS AND METHODS

### 4.3.1 Experimental Vineyard

The experiment was carried out during three consecutive seasons (2015, 2016 and 2017) in a unirrigated *Vitis vinifera* L. cv. Pinotage vineyard, grafted onto two known drought tolerant rootstocks (*Vitis Berlandieri* x *Vitis rupestris*), namely Pinotage (clone PI 50A)/140 Ruggeri (clone RU 354B) planted in 1997 and Pinotage (clone PI 48A)/1103 Paulsen (clone PS 28A) planted in 1999. The vineyard is located at Stellenbosch University Welgevallen Experimental Farm in Stellenbosch, Western Cape, South Africa. The area is under the influence of a Mediterranean climate, characterised by winter rainfall and warm and dry summers. Vines are orientated in a North–South direction and the vineyard locality (approx. 200 m altitude) is characterised by a slope (3°) with a north western aspect. The vines are spaced 2.75 m x 1 m (3636 vines/ha) and the soil classified as Oakleaf soil form originating from weathered granite (Soil Classification Working Group, 1991). Vines are trained to a uni-lateral cordon and pruned to five, two-bud spurs per plant. Foliage was managed by a vertical shoot positioned trellis system with three sets of movable foliage wires. Standard canopy management practices were applied during the growth season, including early suckering (the judicious removal of shoots not allocated during pruning), vertical positioning of shoots, and topping once shoots passed 30 cm above the top foliage wire. An annual cover crop of *Triticale spp.* was sown in autumn and controlled with herbicide before budburst, to ensure a mulch layer within the work row.

### 4.3.2 Experimental layout & Sampling

Vineyards grafted onto the two rootstocks were located directly adjacent to one another. Considering the variables [clone (A-PI 50 vs B-PI 48), age (A-1997 vs B-1999) and slope (A-Top vs B-Bottom)] of the experimental vineyard, rootstock was not considered a treatment as such, but rather the combination of rootstock and site, therefore resulting in two measurement sites: Site A (Pinotage/140 Ruggeri) and Site B (Pinotage/1103 Paulsen). Experimental plots were assigned by a randomised block design, selecting vine parcels from an area within the vineyard which displayed homogeneous vigour. Experimental plots consisted of 30 vines each and were replicated five times per ripeness level for each site.

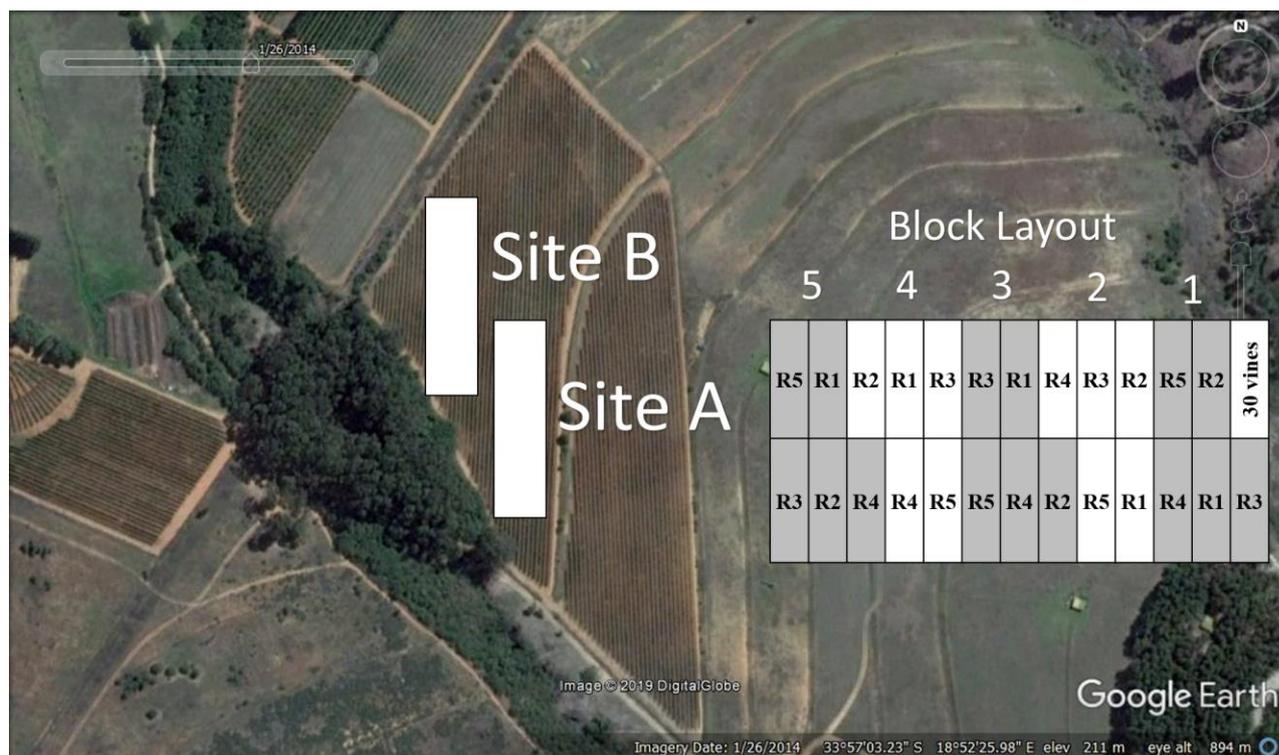


Figure 1. Experimental layout at Stellenbosch University Welgevallen experimental farm, indicating both measurement sites (A & B) and block design with five repetitions (blocks 1 -5) and five ripeness levels (R1-R5).

#### 4.3.3 Measurements and analyses

Grapes were harvested at five sequential ripeness levels coinciding with *ca.* 21°Brix, 23°Brix, 25°Brix, 27°Brix and 29°Brix, respectively. At every ripeness level, 12 bunches per replicate were randomly selected for analysis. After bunch mass was determined, all the berries were separated from the rachis (keeping the pedicel intact). From a sub-sample of 200 randomly selected berries, berry volume and weight were recorded before being crushed and must total soluble solids (°Brix), titratable acidity (g/L tartaric acid), and pH determined. In 2017, the density range of an additional 200 berries of each replicate was determined by submersing the berries in NaCl solutions, each with a pre-determined density corresponding to an increase in probable alcohol of 1% v/v ranging from 10-18% v/v as previously described by Fournand *et al.* (2006). The remaining pooled sample was used for various compositional analyses of fresh berries as described below, while approximately 500 g berry sample was immediately frozen at -80°C for analysis at a later stage.

#### 4.3.3.1 Phenolic Analyses

Two 50 g subsamples of fresh berries were randomly taken from the pooled berry sample from each replicate. The whole berries were homogenised for 60 seconds on the *HI* setting (Waring Laboratory blender, USA) in an equal volume (of the 50 g berries) of extraction solution/model wine (*i.e.* 12% ethanol, 5 g/L tartaric acid, 2 g/L  $K_2S_2O_5$ , 1N NaOH) which was corrected to pH 1 and pH 3.2, respectively. Samples were extracted in the dark for 4 hours at room temperature, before being separated by centrifugation (10 min, 5000 rpm, 15°C). Supernatants were spectrophotometrically (Cary 60, Agilent Tech, USA) analysed for total anthocyanins at  $A_{520}$ , both potential and extractable; total tannins; and total phenolic index ( $A_{280}$ ), as described by Ribéreau-Gayon *et al.* (2006). Analysis of flavan-3-ol monomers and oligomers was conducted according to the DMAC method (Vivas *et al.*, 1994) and expressed in equivalents mg of catechin per gram of berry fresh mass.

#### 4.3.3.2 Anthocyanin Profile

A subsample of 30 fresh berries were randomly taken from the pooled berry sample of each replicate. Berry weight was recorded and the skin of each berry was carefully separated from the pulp and seeds with a scalpel. Skins were blotted dry with paper towel to remove juice residues. Skins were then immersed in 120 mL of extraction solution/model wine (*i.e.* 12% ethanol, 5 g/L tartaric acid, 2 g/L  $K_2S_2O_5$  and pH adjusted to 3.2 with addition of 1N NaOH) and incubated in the dark for 72 hours at 30°C. After incubation samples were homogenized by means of a macerator (Ultra-Turrax T25—IKA, Germany) for 60 seconds at 8,000 rpm and supernatants (skin extracts) were retrieved after centrifugation at 3,500 rpm (15 min) and stored at 4°C until HPLC analyses (as described in Guidoni & Hunter, 2012).

Centrifuged skin extracts were purified by passing 1 mL of skin extract through a C18 Sep-Pack cartridge (Waters, Milford, USA), followed by elution with 5 mL of methanol. Elutes were concentrated to dryness by rotary evaporation at 30°C (IKA, Germany), before being re-dissolved in 1 mL of solvent B (formic acid/methanol/water, 10:50:40, v/v/v). Anthocyanin profiles were analysed by HPLC (Hewlett Packard 1100), fitted with a LiChroCART 250-4 Purospher RP-18 column (Merck, Darmstadt, Germany). 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 the retention time of each chromatographic peak with available data in literature (Guidoni & Hunter, 2012). All individual anthocyanins were quantified at 520 nm using malvidin 3-O-glucoside chloride (Extrasynthèse, Genay, France) as

external standard and expressed as mg/kg of berry taking into account the weight and the number of the analysed berries.

#### 4.3.3.3 Grape Aroma Potential

The grape aroma potential, represents a measure of selected compounds that have been previously identified in grape berries, after the direct acid hydrolysis of grape berry material and subsequent release of glycosidically bound compounds (Yuan & Qian, 2016b). Although enzymatic hydrolysis has shown to be more effective in hydrolysing total glycosides with limited degradation of free-volatiles (Hampel *et al.*, 2014), acid hydrolysis has shown better predictive capabilities regarding final wine volatile/sensory properties (Francis *et al.*, 1992; Loscos *et al.*, 2009).

Approximately 30 g of frozen grape berries (-80°C) were randomly selected from the pooled berry samples described previously. The berries were frozen in liquid nitrogen and blended to a fine powder using a batch mill (model A10, IKA Works GmbH & Co); 2 g of powdered berry was mixed with 8 mL of citric acid/saturate NaCl buffer (0.2 M, pH 2.5) in a 20 mL autosampler vial. The vial was tightly sealed with a PTFE-lined magnetic crimp cap, and kept in a water bath (99°C) for 1 h. After incubation, the vial was cooled in cold water for 10 min and kept at 10°C before SPME-GC-MS analysis (as described in Yuan & Qian, 2016).

Prior to analysis, 50 µL of internal standard (100 µg/L, Anisole d8) was added and vials vortexed for 30 seconds before being placed on the auto-sampler (Thermo Scientific TriPlus RSH). Vials were incubated in the auto-sampler for 10 minutes at 50°C, after which a pink 65 µm Polydimethylsiloxane/Divinylbenzene (PDMS/DVB) stableflex SPME fiber (Supelco, Belafonte, PA, USA) was exposed to the headspace for 15 minutes at the same temperature. After exposure, the fiber was injected and left for ten minutes in order to allow desorption of volatiles. The fiber was re-conditioned between samples for 10 minutes at 260°C to avoid cross contamination. The injector was operated in splitless mode. Analysis of volatiles was performed using a Thermo Scientific trace 1300 gas chromatograph (Anatech Instruments), coupled to a Thermo Scientific TSQ 8000 Triple Quadruple Mass Spectrometer (Anatech Instruments). The MS-detector was set for acquisition in single reaction monitoring (SRM) mode. Chromatographic separation of the volatiles was performed on a polar Zebron ZB-FFAP (30 m, 0.25 mm ID, 0.25 µm film thickness) capillary column. The chromatographic program was set at 35°C for 6 min, raised to 60°C at 4°C/min for 5 min, then raised to 150°C at 8°C/min for 5 min and finally raised to 240°C at 20°C/min, and held for 2 min. The injector and transfer line temperatures were both maintained at 250°C. Helium at 1 mL/min flow rate was used carrier gas.

The ionization source 171 temperature was set at 250°C and emission current of 50  $\mu\text{A}$  was used with argon collision.

A standard calibration curve for each compound was prepared by adding a series of known concentrations of pure chemical standard to a 10 mL citric acid/saturated NaCl buffer (0.2 M, pH 2.5), mixing in a 20 mL autosampler vial, and adding 50  $\mu\text{L}$  of internal standard (100  $\mu\text{g/L}$ , Anisole d8). The analysis procedure was used as described above. Results were calculated using the Chemstation software package (Agilent Technologies, Santa Clara, CA, USA).

#### 4.3.3.4 Berry mineral content

The balance of powdered berry subsample ( $\approx 28$  g, described above) was weighed and freeze dried at  $-50^\circ\text{C}$  before mineral content determination. Once dried, grape berry samples were weighed again before being transported to a commercial laboratory (Bemlab, Strand, South Africa) for analyses. Here, samples were milled and a 0.5 g subsample was used to directly determine total N content by total combustion in a TruMAC N-analyser (Leco Inc.), while the balance was placed in an oven at  $480^\circ\text{C}$  until reduced to ashes. Grape berry ash (1 g) was then added to 10 mL of HCl (32%):H<sub>2</sub>O (50:50, v/v) solution for extraction, for 2 hours (Campbell & Plank, 1998; Miller, 1998), before filtration (60 mesh). The cation (P, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup>) content of the extract was measured with a 710-ES Inductively Coupled Plasma-Optical Emission Spectrometer (Varian Inc.) and related to dry mass.

#### 4.3.4 Statistical analyses

Analysis of variance (ANOVA) was performed using statistical software, SAS version 9.2 (SAS Institute Inc., USA), and normality of data was evaluated using the Shapiro-Wilk test. To compare means, the Students' t-test for Least Significant Differences was calculated at the 5 % significance level. Results were also presented using multivariate analyses such as Principal Component Analysis (PCA), Discriminant Analysis (DA) and Partial Least Squares regression (PLS) performed with XLSTAT version 2017 (Addinsoft SARL).

### 4.4 RESULTS AND DISCUSSION

#### 4.4.1 Classical Ripening Indices

The first three ripeness levels were generally harvested in short succession (3 to 4 days apart) with harvest intervals becoming longer (7 days) in the later ripeness levels (Table 1). As ripeness levels were allocated by pre-defined TSS ( $^\circ\text{B}$ ) levels, it was important to harvest grapes as close as possible to the targeted levels of 21, 23, 25, 27 and  $29^\circ\text{B}$  respectively. In general, the overall response led to grapes being marginally over the target levels in the initial ripeness levels and

marginally under the target ripeness levels in the later ripeness levels. Interestingly, resulting wine alcohol measurements (see Chapter 5 Table 1), confirmed that ripeness levels were in fact not off the targeted °B levels. Nonetheless, the available spectrum provided a suitable range to study the ripeness level distinction. As expected, must titratable acidity (TA) displayed a decreasing trend as grape ripening continued, most probably due to decreases in malic acid, as berry size remained stable and only decreasing significantly in the last ripeness level (R5). Per berry tartaric acid (the major grape organic acid) is thought to remain stable during ripening, thereby the concentration thereof is primarily influenced by increases or decreases in berry size (Coombe & McCarthy, 2000). In parallel, the pH increased significantly as the ripening continued and stresses the importance of ripeness level and the resulting harvest decision on this qualitative parameter, especially considering the short time frame in which changes took place. Brix:TA values were comparable to those reported by Du Plessis & Van Rooyen (1982) and Hunter *et al.*, (2004) for Pinotage and Shiraz cultivated in Stellenbosch, with values ranging from 3.8 to 4 being identified as optimal for the highest potential wine quality, associated in this case with R3 & R4. Differences in Brix:pH values were less pronounced compared to Brix:TA ratios. Consequently, R2, R3 and R4 had comparable values, with only the two extremes (R1 and R5), displaying significantly different Brix:pH ratios. This possibly indicates less sensitivity of this parameter within the context of this study (Hunter *et al.*, 2004).

In accordance with results from Shiraz in Stellenbosch the highest berry fresh mass was recorded at R1 and the lowest at R5 (Hunter *et al.*, 2014). Importantly, since berry size appeared to remain stable for the spectrum of ripeness levels R1 to R4 and only decreased significantly at R5, harvest decisions between R1 and R4 would be free of potential decreases in yield, while R5 would significantly decrease overall yield potential. As expected, berry volume also displayed a similar trend as seen in berry fresh mass (Ollat *et al.*, 2002; Deloire *et al.*, 2004). However, as ripening continued, the fraction of berry H<sub>2</sub>O decreased and the fraction of dry matter increased correspondingly, indicating a possible concentration effect on berry constituents despite stable berry fresh mass. Final berry fresh mass is dependent on the flux of sugar, water and solutes into the grape berry and losses through transpiration. Measurements of dry mass per berry displayed increases in dry mass per berry until the final ripening stage, indicating a continued loading of solutes and/or sugar into the berry until the final ripening stage, albeit at a retarded rate towards the latter, as seen in the longer harvest intervals.

Table 1. Mean berry ripening parameters for cv. Pinotage (A &amp; B) grown in Stellenbosch (2015-2017).

Parameter	Ripeness Levels				
	R1	R2	R3	R4	R5
Harvest (DAB)	133e	136d	140c	147b	154a
TSS (°B)	21.63e	23.62d	24.64c	26.22b	27.57a
TA (g/L)	8.54a	7.72b	6.61c	5.82d	5.99d
pH	3.16d	3.24c	3.41b	3.60a	3.60a
Brix:TA	2.55d	3.12c	3.86b	4.58a	4.69a
Brix:pH	6.84c	7.30b	7.24b	7.32b	7.65a
Berry fresh mass (g)	1.47a	1.46a	1.44a	1.42ab	1.36b
Berry Volume (ml)	1.28a	1.25a	1.27a	1.25ab	1.20b
Dry Mass (g per berry)	0.39c	0.41c	0.44b	0.44b	0.47a
% Berry H <sub>2</sub> O	74.61a	72.79b	71.23c	69.56d	67.22e
% Berry Dry Mass	25.39e	27.21d	28.77c	30.44b	32.78a

Days after Bud Break (DAB), Total Soluble Solids (TSS), Titratable acidity (TA)

<sup>1</sup>Means followed by the same letter within a row do not differ significantly ( $p \leq 0.05$ )

Table 2. ANOVA results for mean berry ripening parameters as presented in Table 1.

	DAB	TSS	TA	pH	Brix:TA	Brix:pH	Berry FM	Berry Vol	Berry DM	% H <sub>2</sub> O	% Dry
Vintage	*	***	<i>ns</i>	***	*	***	**	***	***	**	**
Site	*	***	<i>ns</i>	***	**	***	***	***	<i>ns</i>	***	***
Ripeness Level	***	***	***	***	***	***	**	*	***	***	***
RL x Vintage	**	***	***	***	***	***	**	**	<i>ns</i>	**	**
RL x Site	<i>ns</i>	<i>ns</i>	<i>ns</i>	***	<i>ns</i>	***	<i>ns</i>	*	*	*	*
RL x Site x Vintage	**	**	**	***	**	***	<i>ns</i>	<i>ns</i>	<i>ns</i>	**	**

*ns* non-significant ; \*  $p \leq 0.05$  ; \*\*  $p \leq 0.01$  ; \*\*\*  $p \leq 0.001$

Days after Bud Break (DAB), Total Soluble Solids (TSS), Titratable acidity (TA), Fresh Mass (FM), Volume (Vol), Dry Mass (DM), Ripeness level (RL)

#### 4.4.2 Berry heterogeneity

Berry density distribution provided insight into the natural heterogeneity found within ripeness levels (Fig 2) of the winemaking substrate. Normal distributions of each ripeness level displayed a large base throughout the measurement window, implying that each category contained large amounts of berries above and below the targeted ripeness levels. Berry population heterogeneity is one of the major challenges when interpreting ripening studies (Fournand *et al.*, 2006), thus many works have endeavoured to classify berries according to size (Roby *et al.*, 2004; Melo *et al.*, 2015), colour (Lafontaine *et al.*, 2013; Hendrickson *et al.*, 2016) and density (Fournand *et al.*, 2006; Boss *et al.*, 2014) before measurement and analyses (Kontoudakis *et al.*, 2011). However, on a commercial scale, the sorting of grape berries to this detail during wine production has so far been deemed impractical. Therefore in this study the naturally

heterogeneous berry populations were used in order to emulate a commercial production system. Nonetheless, it is worth to note that the extent of heterogeneity found in this study differed significantly between Sites ( $p < 0.05$ ) when ripeness levels of each Site were compared, even though average Brix levels were similar. Therefore indicating that despite a similar ripeness level in terms of Brix, substrate make-up can differ at berry density level.

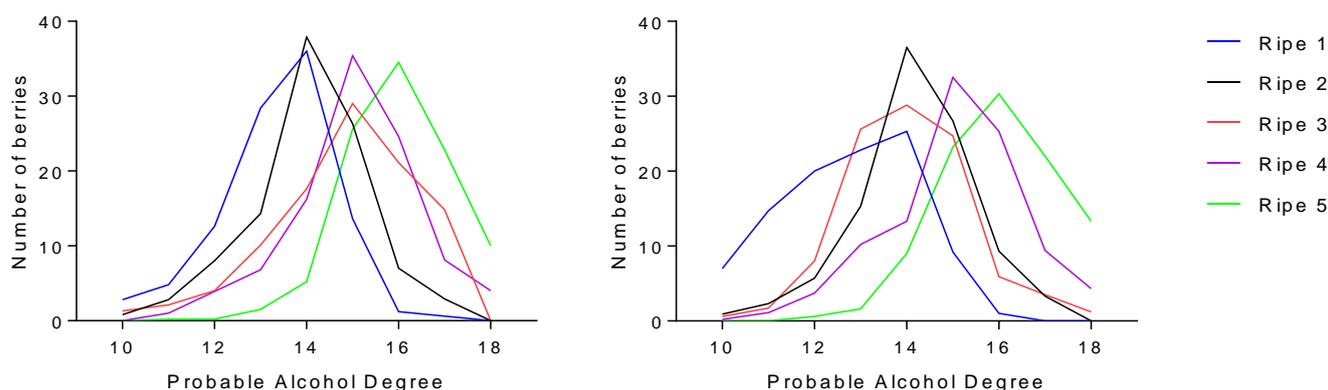


Figure 2. Mean berry density distribution per ripeness level for cv. Pinotage from Site A (left) and Site B (right) in Stellenbosch (2017).

#### 4.4.3 Berry mineral content

Overall high berry mineral concentrations were more frequent in the later ripeness levels compared to earlier ripeness levels (Table 3). Phloem bound minerals  $K^+$ ,  $P$  and  $Mg^{2+}$  generally increase as phloem loading of sugar and water to the berry starts post véraison and continues during ripening (Rogiers *et al.*, 2006). Details of mineral content per berry (Supplementary Info Fig 1) displayed that only  $K^+$  exhibited significant per berry increases during the ripening period investigated. Total stoppage/blockage of phloem flux into the berry had not occurred by the final ripening stage, as per berry  $K^+$  increased from R4 to R5, despite berry dehydration also increasing concentration at the final ripeness level (Table 2). The detrimental impact of high must  $K^+$  levels is well documented, primarily increasing wine pH by tartaric acid precipitation (Mpelasoka *et al.*, 2003; Rogiers *et al.*, 2017). Moreover, xylem bound  $Ca^{2+}$  could also increase tartaric acid precipitation, through a similar action as  $K^+$ , but is generally found in significantly lower concentrations and is therefore considered subordinate to  $K^+$ . Calcium accumulation ceases at véraison and concentration is related to berry size variation. Here, the impact of minimal berry size variation in 2017 (Chapter 3) displayed higher levels of  $Ca^{2+}$  in earlier

ripeness levels compared to 2015 and 2016. Nitrogen was generally in highest concentration at R5, although yeast assimilable (YAN) nitrogen was not measured, nitrogen is an important nutrient in fermentations and can have a major impact on yeast derived aroma in wines (Van Wyk *et al.*, 1979; Joubert, 1980; Louw *et al.*, 2010). Given the stable per berry contents of P, Mg, Ca and N from R1 to R4, increases in mineral concentration were most likely only brought about by concentration through dehydration in the final ripeness level. This trend could also be seen for  $K^+$ , despite per berry increases along the ripeness level. Nonetheless the potential advantages of increased hang time should always be weighed against the potential negative impact of increased  $K^+$  on wine pH, despite the ostensible increase seen in must titratable acidity at late ripeness. Interestingly, differences between vintages in mean mineral concentrations were non-significant for  $K^+$  while significant for all the other minerals (Table 3 & 4). In addition Site A generally produced significantly higher concentration of minerals than Site B.

Table 3. Berry mineral concentrations (g per kg of berries) for cv. Pinotage at five ripeness levels from two Sites (A and B) in Stellenbosch (2015-2017).

Vintage	Site	Ripeness Levels				
		R1	R2	R3	R4	R5
<b>K<sup>+</sup> (g/kg)</b>						
2015	Site A	2.03b	2.10b	2.26ab	2.44a	2.46a
	Site B	1.74c	1.89bc	1.92bc	2.07b	2.39a
2016	Site A	1.99c	2.04c	2.35b	2.11c	2.57a
	Site B	1.89b	1.83b	1.92b	2.42a	2.06b
2017	Site A	2.14b	2.18b	2.25b	2.15b	2.71a
	Site B	1.98a	1.92a	2.00a	2.18a	2.25a
<b>P (g/kg)</b>						
2015	Site A	0.26ab	0.25b	0.27ab	0.30a	0.28ab
	Site B	0.20c	0.25b	0.26b	0.27ab	0.31a
2016	Site A	0.25bc	0.22c	0.30a	0.28ab	0.30a
	Site B	0.24c	0.26b	0.26b	0.28ab	0.30a
2017	Site A	0.22a	0.21a	0.22a	0.22a	0.22a
	Site B	0.19b	0.22a	0.19b	0.20ab	0.22a
<b>Mg<sup>2+</sup> (g/kg)</b>						
2015	Site A	0.124ab	0.123b	0.136ab	0.137ab	0.143a
	Site B	0.093b	0.110ab	0.115a	0.130a	0.126a
2016	Site A	0.147bc	0.132c	0.165ab	0.162ab	0.177a
	Site B	0.139b	0.158b	0.146b	0.147b	0.184a
2017	Site A	0.158a	0.152a	0.148a	0.157a	0.160a
	Site B	0.145abc	0.133bc	0.126c	0.153ab	0.160a
<b>Ca<sup>2+</sup> (g/kg)</b>						
2015	Site A	0.235a	0.235a	0.242a	0.240a	0.258a
	Site B	0.180b	0.193b	0.191b	0.243a	0.200b
2016	Site A	0.253b	0.248b	0.272b	0.263b	0.376a
	Site B	0.222bc	0.257b	0.241bc	0.212c	0.315a
2017	Site A	0.311a	0.293ab	0.250b	0.283ab	0.274ab
	Site B	0.291a	0.233b	0.214b	0.230b	0.237b
<b>N (g/kg)</b>						
2015	Site A	1.62b	1.60b	1.70b	1.81ab	1.97a
	Site B	1.43b	1.49b	1.49b	1.77a	1.74a
2016	Site A	1.65c	1.77bc	1.99a	1.86ab	1.90ab
	Site B	1.52a	1.65a	1.63a	1.57a	1.68a
2017	Site A	1.59b	1.67ab	1.54b	1.69ab	1.80a
	Site B	1.32bc	1.49ab	1.23c	1.45ab	1.54a

Potassium (K<sup>+</sup>), Phosphorus (P), Magnesium (Mg), Calcium (Ca<sup>2+</sup>) and Nitrogen (N).<sup>1</sup>Means followed by the same letter within a row do not differ significantly ( $p \leq 0.05$ )

Table 4. ANOVA results for mean berry mineral concentration (g per kg of berries) for cv. Pinotage at five ripeness levels from two Sites (A and B) in Stellenbosch (2015-2017).

	K <sup>+</sup>	P	Mg <sup>2+</sup>	Ca <sup>2+</sup>	N
Vintage	<i>ns</i>	***	***	***	***
Site	**	*	**	***	***
Ripeness Level	***	***	***	**	***
RL x Vintage	<i>ns</i>	**	*	***	**
RL x Site	*	**	<i>ns</i>	<i>ns</i>	<i>ns</i>
RL x Site x Vintage	*	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>

*ns* non-significant ; \*  $p \leq 0.05$  ; \*\*  $p \leq 0.01$  ; \*\*\*  $p \leq 0.001$ , Potassium (K<sup>+</sup>), Phosphorus (P), Magnesium (Mg), Calcium (Ca<sup>2+</sup>) and Nitrogen (N).

#### 4.4.4 Berry phenolic content

Compositional results obtained from whole berry extractions using two model wine extraction mediums at pH 1.0 (total potential) and pH 3.2 (wine-like) (Ribéreau-Gayon *et al.*, 2006a) consistently displayed a general trend of increasing berry phenolic index (280nm) as ripening continued, achieving the highest values at R4 (Table 4). Importantly, R5 regularly displayed decreased phenolic index values, despite expected increases in skin:pulp ratio coupled with berry dehydration as seen in other berry constituents (sugars and minerals). This suggests that changes in extractability may have occurred because of the oxidation/transformation of phenolic compounds, changing phenolic potential. Previous works have also postulated that wine phenolic content may be detrimentally affected if harvest is delayed injudiciously to allow for over ripeness (Harbertson *et al.*, 2002; Holt *et al.*, 2010). Recent works have also highlighted the importance of the concentration gradient, showing that addition of pulp facilitated the extraction from skins to the wine matrix (Bindon *et al.*, 2017), while the increased adsorption to solids may affect phenolic content at the later ripeness levels. Total flavan-3-ols expressed as mg catechin/g of berry fresh mass displayed similar trends than those reported by Ojeda *et al.*, (2002), with higher levels at the start of ripening, most probably due to high seed flavan-3-ol content. Steady decreases in flavan-3-ols along ripening on a per gram berry fresh mass basis could indicate to a high degree of polymerisation, despite the increases expected due to berry dehydration. Total tannin (proanthocyanins) content (corrected for anthocyanin) developed in a similar fashion, peaking at the fourth ripeness level and having very low levels at the final ripeness level.

Table 5. Berry phenolic indices for cv. Pinotage at five ripeness levels from two Sites (A and B) in Stellenbosch (2015-2017).

Vintage	Site	Ripeness Levels				
		R1	R2	R3	R4	R5
<b>TPI (pH 1)</b>						
2015	Site A	73.9ab	69.2b	67.2b	80.3a	71.7ab
	Site B	51.5bc	46.2c	57.1ab	64.2a	53.9bc
2016	Site A	74.9d	59.4e	91.5b	108.3a	77.7c
	Site B	72.3d	56.2e	84.9b	100.4a	78.7c
2017	Site A	92.2b	81.8c	93.6b	102.2a	94.2b
	Site B	77.5c	87.1ab	89.8ab	93.1a	84.5b
<b>Skin Tannin (pH 3.2)</b>						
2015	Site A	36.4ab	35.7b	35.5b	39.1a	37.2ab
	Site B	33.6ab	24.9d	27.9dc	34.1a	30.1bc
2016	Site A	76.3e	59.8d	91.4b	107.9a	80.5c
	Site B	76.0d	58.7e	87.1b	102.2a	82.1c
2017	Site A	69.4b	70.0b	67.7b	75.6a	69.9b
	Site B	64.5ab	62.9b	66.1ab	66.3a	66.2ab
<b>Flavan-3-ols (pH 1)</b>						
2015	Site A	0.94ab	0.82c	0.85bc	1.00a	0.92abc
	Site B	0.89a	0.64b	0.74b	0.96a	0.91a
2016	Site A	0.83a	0.75b	0.76b	0.82a	0.76b
	Site B	0.83a	0.74c	0.77b	0.82a	0.77b
2017	Site A	0.85a	0.81b	0.82ab	0.85a	0.83ab
	Site B	0.81ab	0.85a	0.81ab	0.77b	0.77b
<b>Flavan-3-ols (pH 3.2)</b>						
2015	Site A	0.70b	0.70b	0.68b	0.79a	0.64b
	Site B	0.76a	0.50c	0.51c	0.73a	0.59b
2016	Site A	0.84a	0.75c	0.78b	0.81a	0.79b
	Site B	0.84a	0.78b	0.76b	0.83a	0.78b
2017	Site A	0.82a	0.81ab	0.79abc	0.75bc	0.74c
	Site B	0.82a	0.79a	0.78a	0.68b	0.71b
<b>Total tannins (pH 1)</b>						
2015	Site A	3.57b	4.07b	4.37b	5.74a	4.16b
	Site B	1.99d	2.81c	3.92b	4.64a	3.24bc
2016	Site A	5.26ab	5.94a	4.56b	5.10ab	2.84c
	Site B	4.56a	4.84a	3.65b	3.31ab	2.76c
2017	Site A	5.82ab	5.45b	5.05b	6.83a	5.40b
	Site B	4.53b	5.07a	4.55b	4.83ab	4.82ab

Total phenolic Index A 280 (TPI) and Total tannins measured according to Ribéreau-Gayon *et al.*, 2006, Total flavan-3-ols DMAC method (Vivas *et al.*, 1994)

<sup>1</sup>Means followed by the same letter within a row do not differ significantly ( $p \leq 0.05$ )

Colorimetric analysis of anthocyanins provided similar trends to phenolic indexes (Table 6), displaying increases in total red pigments (pH 1) and potential anthocyanins as ripening continued, reaching a peak at R4, before declining to R5. Again the highest anthocyanin potential in whole berry extractions did not coincide with concomitant decreases in post véraison

berry size at R5. Colorimetric results did not display significant differences in accumulation trend between vintages or sites, meaning that while Site A consistently produced higher values than Site B regardless of vintage, differences in absolute values between vintages were not apparent. Anthocyanin extractability in hydrochloric medium (model wine) was comparable over the range of ripeness levels, suggesting that changes in colorimetric potential was related to anthocyanin concentration and level of co-pigmentation rather than level of extractability (Canals *et al.*, 2005; Fournand *et al.*, 2006). However, frequent lower values (higher extractability) was found at the last ripeness level. This could be due to degeneration of structural parts of the berry, allowing for greater extraction despite lower concentrations.

Table 6. Berry colour indices for cv. Pinotage at 5 ripeness levels from two Sites (A and B) in Stellenbosch (2015-2017).

Vintage	Site	Ripeness Levels				
		R1	R2	R3	R4	R5
<b>Anthocyanin Pot (pH 1)</b>						
2015	Site A	1155ab	998b	965b	1206a	1006b
	Site B	628b	597b	734ab	862a	704b
2016	Site A	718c	873b	923ab	1070a	691c
	Site B	480b	630a	689a	695a	737a
2017	Site A	744c	834bc	915b	1128a	909b
	Site B	580b	752ab	869a	869a	916a
<b>Anthocyanin Pot (pH 3.2)</b>						
2015	Site A	373ab	339b	352b	411a	382ab
	Site B	241b	219b	282a	296a	289a
2016	Site A	304b	318b	302b	399a	382ab
	Site B	221b	238b	312a	326a	280a
2017	Site A	308c	392ab	328bc	453a	426a
	Site B	240c	327b	362ab	318b	398a
<b>Anthocyanin Extractability</b>						
2015	Site A	67.0a	65.9ab	63.5ab	65.9ab	61.7b
	Site B	61.6ab	63.3a	61.3ab	65.4a	58.4b
2016	Site A	63.1b	65.0ab	64.3ab	67.2ab	63.4b
	Site B	59.8ab	63.3ab	65.0a	66.3a	58.4b
2017	Site A	57.6ab	52.4b	63.5a	59.7ab	53.1b
	Site B	58.3a	56.0a	57.2a	62.9a	56.3a

Anthocyanin estimates at A520 nm measured according to Ribéreau-Gayon *et al.* (2006)

<sup>1</sup>Means followed by the same letter within a row do not differ significantly ( $p \leq 0.05$ )

Table 7. ANOVA results for mean phenolic and colorimetric indices for cv. Pinotage (2015-2017).

	TPI pH 1	Skin tannin pH 3.2	F-3-ol pH 1	F-3-ol pH 3.2	Tot Tannin	Pot A pH 1	Pot A pH 3.2	A Extract (%)
Vintage	*	***	<i>ns</i>	***	*	***	**	***
Site	*	***	<i>ns</i>	***	**	***	***	***
Ripeness Level	***	***	***	***	***	***	**	*
RL x Vintage	**	***	***	***	***	***	**	**
RL x Site	<i>ns</i>	<i>ns</i>	<i>ns</i>	***	<i>ns</i>	***	<i>ns</i>	*
RL x Site x Vintage	**	**	**	***	**	***	<i>ns</i>	<i>ns</i>

*ns* non-significant ; \*  $p \leq 0.05$  ; \*\*  $p \leq 0.01$  ; \*\*\*  $p \leq 0.001$

Total phenolic Index A280 (TPI), total tannins and anthocyanin estimates at A520 nm measured according to Ribéreau-Gayon *et al.* (2006), Total flavan-3-ols according to the DMAC method (Vivas *et al.*, 1994)

#### 4.4.5 Berry Anthocyanin profile

Five distinctive anthocyanin-3-O-glucosides namely; delphinidin- (Delf), cyanidin- (Cya), petunidin- (Pet), peonidin- (Peo), and malvidin- (Malv) could be quantified by HPLC from grape berry skins (Fig 3). Acylated and *p*-coumarylated derivatives for all the 3-O-glucosides could be detected and quantified, with the exception of Delf. The Delf *p*-coumarylated derivative was not detected in any of the berry skin samples, while the Malv caffeoyl derivative was detected in small quantities in all of the samples (n=150). In previous work on Shiraz using the same methodology, Guidoni & Hunter (2012) could not positively identify the *p*-coumarylated derivative of Peo, while it was present in all samples (n=150) of Pinotage skins analysed in the present study. This may indicate differences in varietal profiles between Shiraz and Pinotage. Varietal differences in anthocyanin profiles have been proposed as method of varietal identification/authentication (de Villiers *et al.*, 2004), for instance in cv. Pinot noir, one of the parents of Pinotage, no acylated derivatives were found in its varietal profile (Fong *et al.*, 1971).

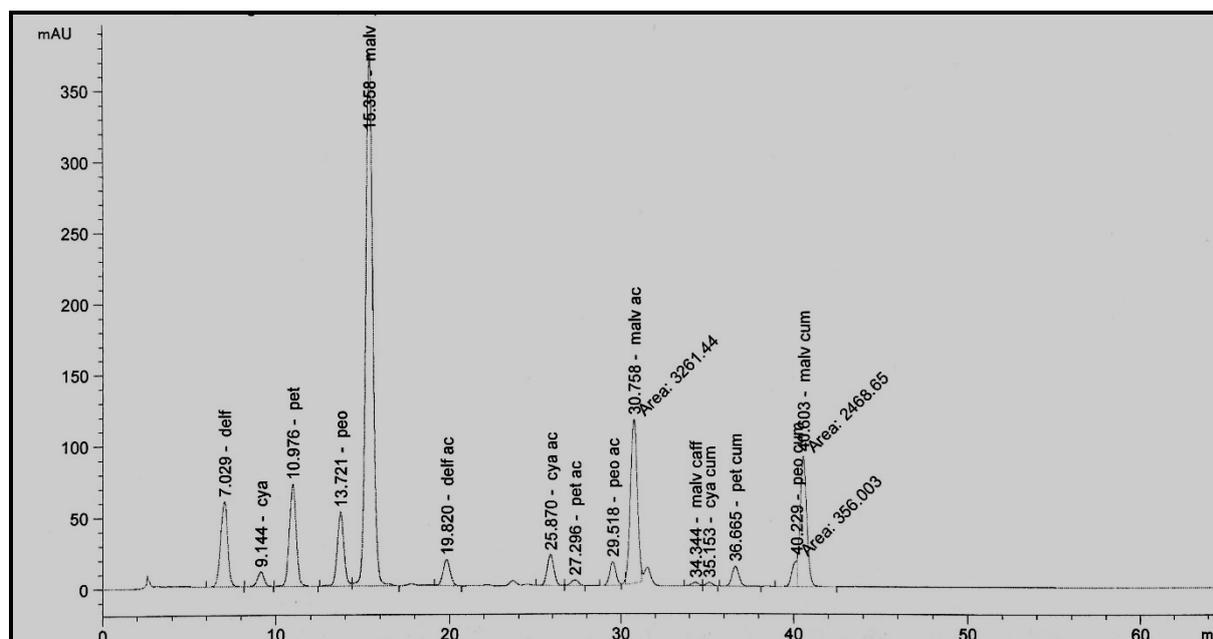


Figure 3. Example of a chromatogram (HPLC) used to identify various anthocyanin components at wavelength 520 nm after extraction from cv. Pinotage berry skins.

Principal component analysis of individual anthocyanin components could explain 95.17% of variation of the dataset (F1 80.46% and F2 14.70%) and confirmed the decisive impact of vintage on anthocyanin profiles (Fig 4), clearly separating profiles based on vintage as also displayed by individual accumulation plots (Figs 5 & 6) and ANOVA results (Table 9). From the PCA plot, site could be identified as second order separating factor on F1, with limited separation between sites in 2015 and 2016, and greater separation between sites in 2017. Differences in ripeness level were accounted for on F2 (14.70%), with riper levels in the direction of Malv derivatives and less ripe in Cya and Cya derivatives.

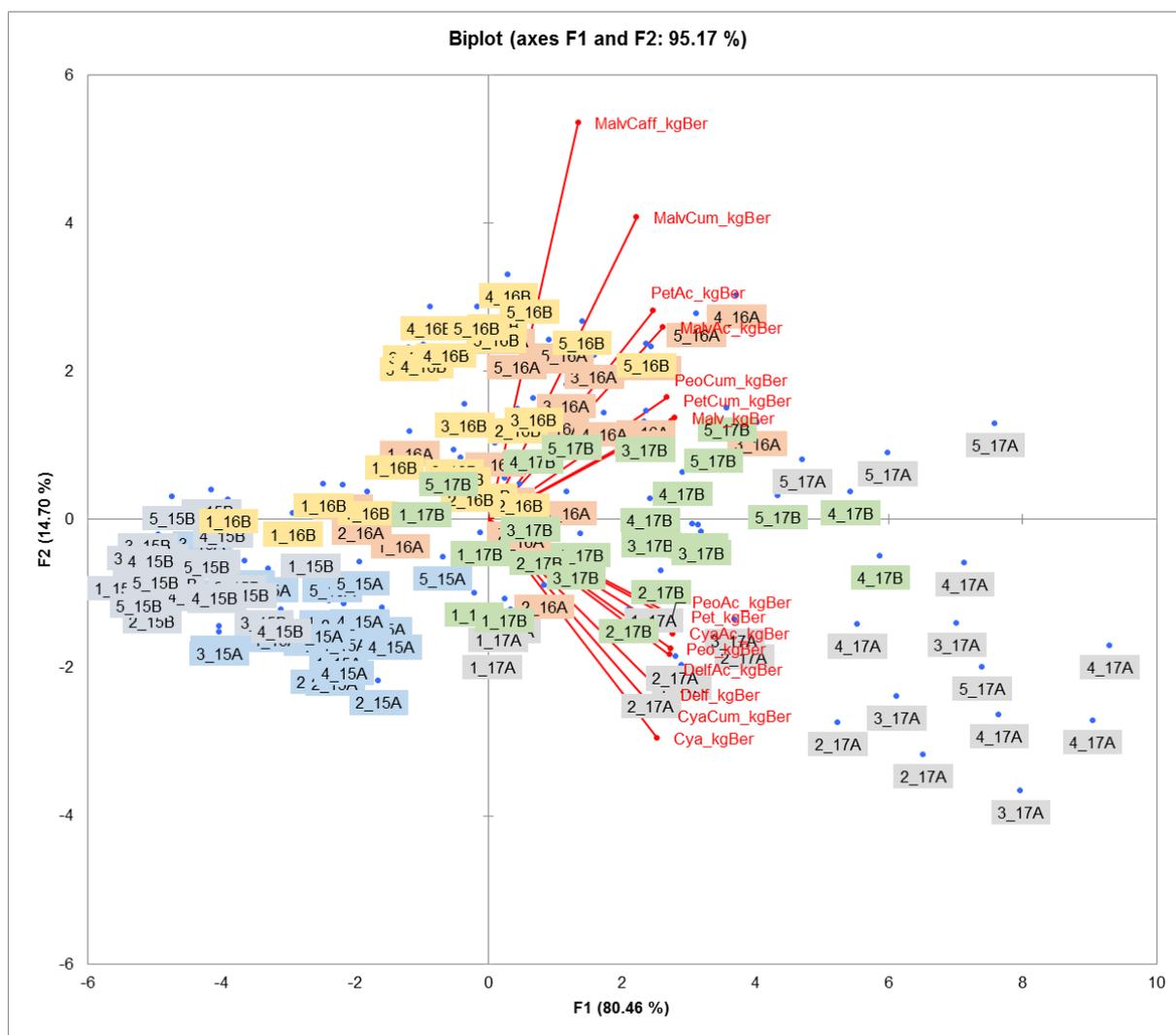


Figure 4. Principal component analysis of individual anthocyanins (mg/kg of berries) (HPLC) as influenced by ripeness level (R1-R5) of cv. Pinotage at Site A and B for Vintages (2015-17) in Stellenbosch, Western Cape, South Africa.

Trends in concentration of total anthocyanins compared well to results obtained spectrophotometrically (pH 3.2) from whole berry extracts (Tables 6 & 8). Total anthocyanins (Table 8) were found in higher concentrations than for Shiraz (Guidoni & Hunter, 2012), but were similar to those previously reported for Pinotage (Marais & October, 2005). Proportions of total free glucosides peaked at R2 at the cost of acylated and coumarylated derivatives, which were higher at both the start (R1) and end (R5) of ripening. Moreover, free glucosides were the dominant form (>65%), while acylated derivatives (>18%) were in greater proportions than coumarylated derivatives (>12%) throughout ripening. In Shiraz, coumarylated forms were in greater proportions than acylated forms (de Villiers *et al.*, 2004; Fournand *et al.*, 2006; Guidoni & Hunter, 2012). The degeneration of the anthocyanins in the latter part of ripening appeared to be predominantly at the cost of free glucosides, while MalvCaff appeared to be unaffected by the degeneration (Fig 5 & 6) and continued in an increasing trend to the final ripening stage, most

probably also at the cost of free glucosides as acylated and coumarylated derivative proportions remained stable.

Table 8. Mean proportional (%) changes in wine anthocyanins and their derivatives as affected by Ripeness Levels for cv. Pinotage (Site A & B and Vintages 2015-17).

	Tot A	T free	T Ac	T Cum	Delf	Cya	Pet	Peo	Malv
R1	924c	67.7c	19.0a	12.9a	9.1b	3.8b	10.6b	9.7a	66.9b
R2	1106b	69.1a	18.6bc	12.0b	10.6a	4.1a	11.0a	10.0a	64.3c
R3	1152b	68.7ab	18.3c	12.7a	9.5b	3.6b	10.6b	9.5a	66.8b
R4	1297a	68.2bc	18.8ab	12.7a	9.2b	3.7b	10.4b	10.0a	66.8b
R5	1249a	67.8c	19.0ab	12.8a	8.3c	3.3c	9.9c	9.9a	68.6a

<sup>1</sup>Means followed by the same letter within a row do not differ significantly ( $p \leq 0.05$ )

Total Anthocyanins mg/L (Tot A), Total free 3-O-glucosides (T free), Total Acylated glucosides (T Ac), Total Coumarylated glucosides, Delphinidin (Delf), Cyanidin (Cya), Petunidin (Pet), Peonidin (Peo) and Malvidin (Malv)

Total Caffoyel glucosides are not presented as only Malv Caff could be identified.

Table 9. ANOVA results for proportional changes in wine anthocyanins and their derivatives.

	Tot Anth	T free	T Ac	T Cum	Delf	Cya	Pet	Peo	Malv
Vintage	***	***	***	***	***	***	***	***	***
Site	***	***	***	***	***	***	***	***	***
Ripeness Level	***	**	**	**	***	***	***	<i>ns</i>	***
RL x Vintage	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	*	<i>ns</i>	*	<i>ns</i>	<i>ns</i>
RL x Site	<i>ns</i>	<i>ns</i>	*	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
RL x Site x Vintage	<i>ns</i>	**	**	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>

*ns* non-significant ; \*  $p \leq 0.05$  ; \*\*  $p \leq 0.01$  ; \*\*\*  $p \leq 0.001$

Total Anthocyanins mg/L (Tot A), Total free 3-O-glucosides (T free), Total Acylated glucosides (T Ac), Total Coumarylated glucosides, Delphinidin (Delf), Cyanidin (Cya), Petunidin (Pet), Peonidin (Peo) and Malvidin (Malv)

Total Caffoyel glucosides are not presented as only Malv Caff could be identified

Individual anthocyanin concentration (Fig 5 & 6) reflected compositional changes based on per berry (data not shown) for R1-R4 as berry fresh mass remained stable during this time. Interestingly, the dramatic per berry decreases that manifested in R5 (data not shown) were also visible as concentration, despite the opposite being expected along with significant decreases in berry size and increase in skin:pulp ratio. Due to the significant influence of vintage and site (Table 9) concentrations and the evolution thereof during ripening are presented separately (Figs 5 & 6). In addition to changes due to ripeness levels, colorimetric analyses also confirmed both significant vintage and site differences (Tables 8 & 9). However, greater articulation in anthocyanin profile was expected in the HPLC method due to greater extraction gradient (decreased ratio skin:liquid) and increased sensitivity of instrumentation. Concentrations of skin anthocyanins generally followed a trend of 2015 < 2016 < 2017. Higher concentrations presented in 2017 could be due to cooler minimum temperatures and higher day-night amplitudes during the month of ripening compared to other vintages (See Chapter 3 Table 1). This has been shown to result in increased anthocyanin concentration (Kliwer & Torres, 1972; Mori *et al.*, 2005).

Conversely, less solar irradiance in the bunch zone, coupled with mild water deficits in 2015 compared to other vintages (See Chapter 3 Figs 2 & 4), may have influenced anthocyanin biosynthesis and accumulation negatively (Castellarin *et al.*, 2007). Nonetheless, concentration of anthocyanins followed similar trends in all vintages, albeit with considerably less articulation in 2015 compared to 2016 & 2017. Site A consistently produced higher total concentration of anthocyanins than Site B over all three vintages (Table 8 & 9). This was especially notable for Peo and Cya and their derivatives. Although increased ripeness level generally increased anthocyanin concentrations, there was a clear decline in most compounds after a peak at R4 (27°B). Late season decline in anthocyanins has been reported in many studies (Fong *et al.*, 1971; Ribéreau-Gayon, 1971; Roggero *et al.*, 1986). However, decreases have been shown to manifest substantially earlier (18°B) in the absence of a concentration effect along ripening (Fournand *et al.*, 2006). For Shiraz, anthocyanins increased dramatically 3 week post véraison (Nadal *et al.*, 2004; Guidoni & Hunter, 2012; Hunter *et al.*, 2014), after which slow increases were reported (23-28°B) for Shiraz., predominantly driven by a decrease in berry size (Guidoni & Hunter, 2012). In this study anecdotal observations confirm that in many cases berries were not completely coloured at R1 (21°B) and in some cases not even at R2 (23°B). This places the results in context as R1 frequently occurred around 2 weeks after véraison (Chapter 3) compared to Shiraz in the South of France, which attained 20.8°B ( $\approx$  R1) 6 weeks after véraison, where anthocyanin content had already peaked and started declining (Fournand *et al.*, 2006).

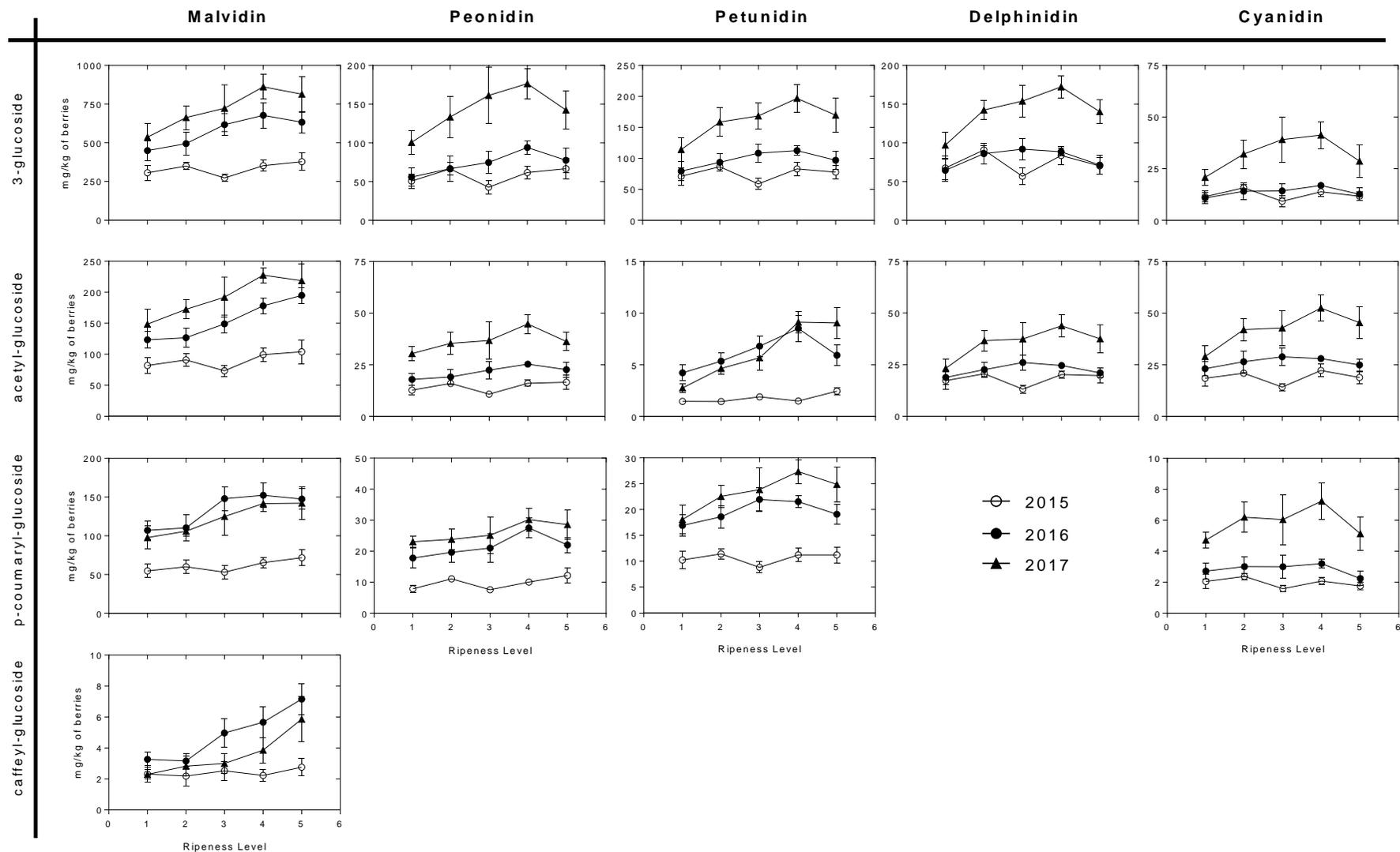


Figure 5. Concentration (mg/kg of berries) of individual anthocyanin glucosides per ripeness level for Site A, Pinotage Stellenbosch (2015-17) (standard deviation confidence interval 95 %).

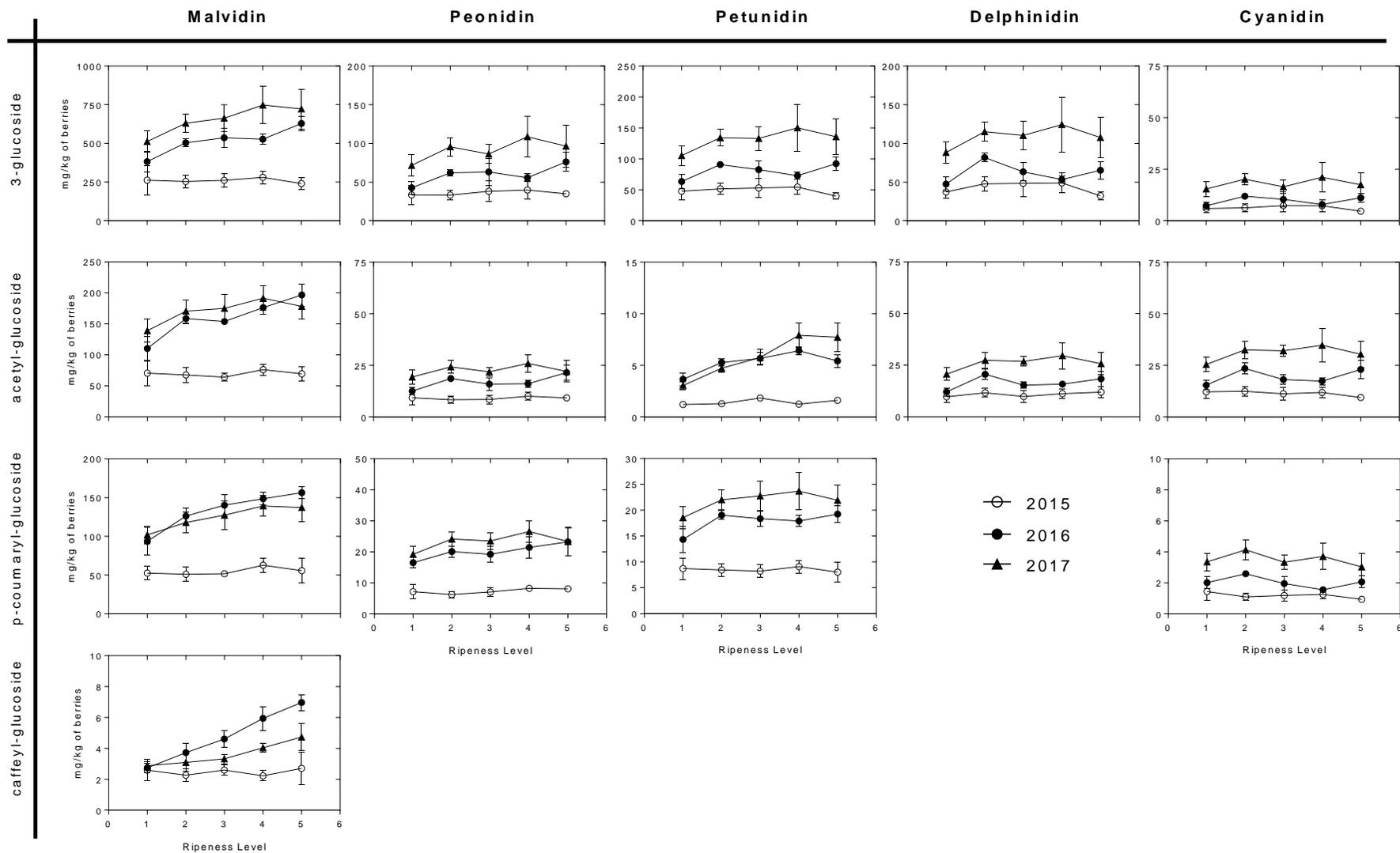


Figure 6. Concentration (mg/kg of berries) of individual anthocyanin glucosides per ripeness level for Site B, Pinotage Stellenbosch (2015-17) (standard deviation confidence interval 95 %)

In the light of the similar trends found in sites regarding accumulation during ripening, sites were combined in Discriminant Analysis (DA) in order to assess the overall ripeness level effects per vintage (Fig 7). Observations from DA models explained 88.53% in 2015 (F1 69.56% and F2 18.97%), 86.24% in 2016 (F1 62.99% and F2 23.25%) and 97.84 % in 2017 (F1 91.59% and F2 6.25%) of the dataset (Fig 6). Anthocyanin profiles of differing ripeness levels were consistently separated along F1 from R1 to R5. As expected, the abundance of anthocyanins increased as ripening continued, predominantly discriminating along F1. However, confidence ellipses frequently overlapped for adjacent ripeness levels and only R1 and R5 could consistently be discriminated from one another (no overlapping) in all of the vintages. In 2017, less overlapping was prevalent, supporting the articulate differences in accumulation during ripening, most probably brought about by climatic factors. Although the separation of ripeness levels along F1 was dominant, F2 (<24%) often discriminated R1 & R5 from the other ripeness levels, presumably driven by the reaction of MalvCaff. The consistent overlap between harvest dates reveal mostly a consistent quantitative progression, rather than major qualitative shifts in anthocyanin profiles. This reaction was in line with overlapping shifts seen in berry density distributions (Fig 1), effectively re-affirming the compact changes in anthocyanin profile in relation to significant changes in classical ripening indices, while raising questions regarding the impact of berry heterogeneity.

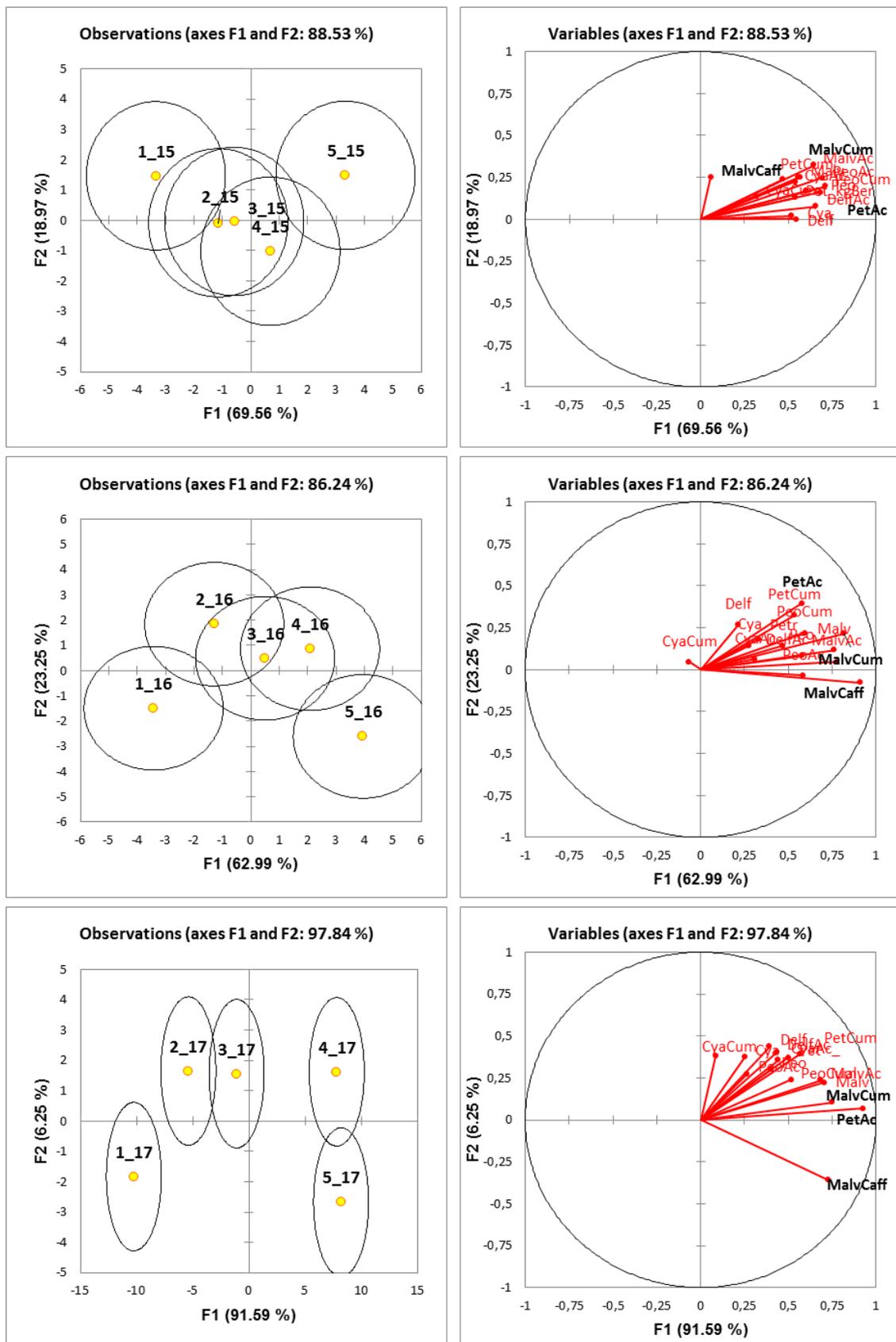


Figure 7. Discriminant analysis of grape anthocyanin content of five ripeness levels for vintages 2015 (top), 2016 (middle) and 2017 (bottom), with alpha ellipses (0.5). Key compounds (black) were confirmed (VIP > 1) with PLS modelling of ripeness level.

In the light of the consistent response displayed over vintages, an attempt was made to identify individual anthocyanin variables that performed consistently in relation to increased ripeness level using multivariate models. Partial least squares modelling was performed on DA variables in each vintage, where the various anthocyanins were included as the X loadings, while actual Brix values were given as Y values and ripeness levels plotted on the same graph as observations (Fig 7). The variables displaying the strong predictive qualities with regard to ripeness level modelling (Addendum B Table 2) were PetAc, MalvCum and MalvCaff, (presented in black in Fig 7). Interestingly, the compounds were derivatives of the dominant methoxylated groups found in the latter part of ripening (Malv and Pet), and not free glucosides (specifically Malv) as proposed previously for Pinotage (Marais *et al.*, 2001; Marais & October, 2005). For Shiraz, Guidoni & Hunter, (2012) also reported an increase for the MalvCum derivative, but a decline for both PetAc and MalvCaff in the 23-28°B window, while Fournand *et al.*, (2006) also marked a later peak for coumarylated derivatives compared to acylated and free anthocyanins. Considering the early peak of free glucosides and subsequent decline reported in many studies (Roggero *et al.*, 1986; Haselgrove *et al.*, 2000; Fournand *et al.*, 2006; Guidoni & Hunter, 2012), the three derivative compounds identified here warrant further study regarding possible additional indicators to ripeness levels.

#### 4.4.6 Grape berry Aroma Potential

Twenty aroma compounds could be identified with HSPME GC-MS analytic methods and quantified with authentic standards. Compounds included C6 aldehydes and -alcohols, methoxypyrazines, monoterpenes, norisoprenoids, aliphatic alcohols and volatile phenols (Table 10 & 11). Considering the differences in accumulation pattern and odour threshold of compounds, even of the same chemical/volatile group, concentrations were reported separately and were not added in groups. Multivariate techniques were used to display overall responses. As in the case of phenolic compounds results were presented on a concentration basis as berry size remained constant for the first four ripeness levels.

Principal component analysis confirmed the decisive impact of vintage on grape aroma potential profiles (Fig 8) separating profiles based on vintage. From the PCA plot, site differences were less pronounced when compared to anthocyanin profiles, yet the minor separation on Site was mostly based on quantitative factors rather than qualitative factors. From quantitative data 2017 appeared to display greater concentrations of monoterpenes,

such as linalool and citronellol, also following the expected increase along ripeness, compared to decreases seen in other vintages.

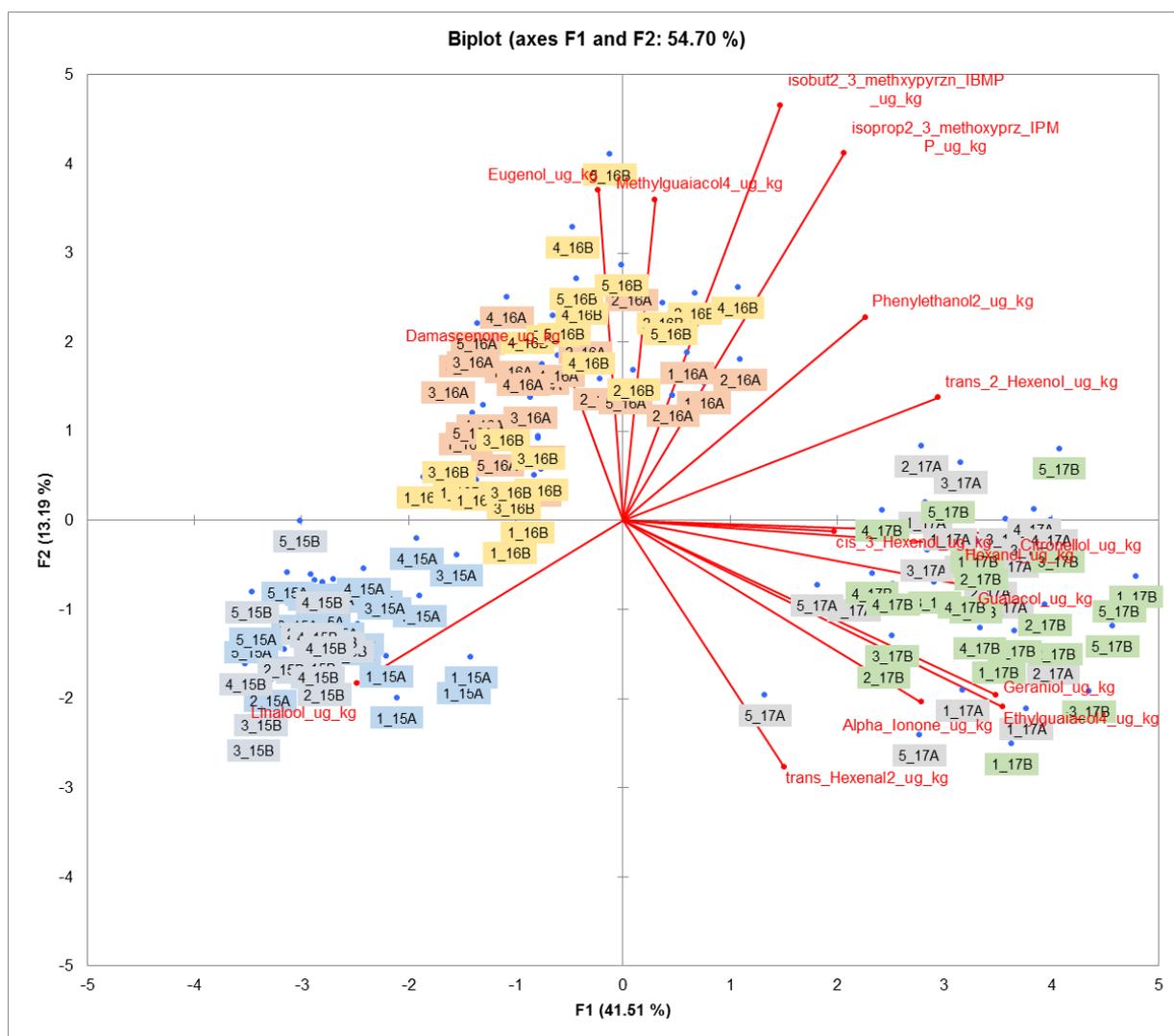


Figure 8. Principal component analysis of aroma potential groups ( $\mu$ /kg of berries) (GC-MS) as influenced by ripeness level (R1-R5) of cv. Pinotage at Site A and B for Vintages (2015-17) in Stellenbosch, Western Cape, South Africa.

TABLE 10 Grape aroma potential ( $\mu\text{g}/\text{kg}$  berries) of cv. Pinotage harvested at 5 ripeness levels for Site A, Stellenbosch (2015-2017)

OT <sup>1</sup>	2015					2016					2017					
	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5	
<b>C6-Compounds</b>																
hexanal	4.5 <sup>a</sup>	2703a	1364b	1357b	1349b	712c	2592a	1414b	640c	1087bc	681c					
( <i>E</i> )-2-hexanal	17 <sup>a</sup>	3432b	1690c	1037d	1579a	1510cd	3726a	2166b	820c	2005bc	1375bc	4529a	3860b	2401c	3961ab	2988c
1-hexanol	500 <sup>a</sup>	379a	83.7c	250b	117c	74.3c	253a	244a	200a	204a	267a	299c	506b	632a	507b	311c
( <i>Z</i> )-3-hexanol	70 <sup>a</sup>	147a	45.3b	39.2b	53.1b	22.9b	100b	149a	30.1c	55.8c	34.7c	141a	88.1b	46.8b	143a	84.6b
( <i>E</i> )-2-hexanol	1000 <sup>a</sup>	72.7ab	48.1bc	96.4a	32.5c	32.7c	229a	186ab	100c	127bc	93c	144c	185bc	261a	218ab	145c
<b>Methoxypyrazines</b>																
IPMP*	2 <sup>b</sup>	0.32a	0.32a	0.34a	0.23b	0.24b	0.58b	0.58b	0.92a	1.05a	0.60b	0.73a	0.83a	0.83a	0.81a	0.71a
SBMP*	2 <sup>b</sup>	0.64a	0.63ab	0.65a	0.51b	0.73a	0.33c	0.38bc	0.50ab	0.56a	0.34c	0.47a	0.72a	1.07a	0.38a	nd
IBMP*	2 <sup>b</sup>	0.46ab	0.47ab	0.55a	0.33b	0.44ab	0.80a	1.06a	0.95a	0.97a	0.85a	0.86a	0.82a	0.74a	0.77a	0.67a
<b>Monoterpenes</b>																
linalool	6 <sup>a</sup>	17.8b	17.3b	18.5a	16.1b	9.4c	15.5a	10.7b	7.63b	6.31b	6.51b	4.82b	4.51b	5.10ab	6.06a	5.38ab
citronellol	30 <sup>a</sup>	3.06b	2.58b	4.80a	3.20b	2.46b	7.85ab	9.75a	5.14c	4.86c	6.74bc	13.1b	13.4b	15.9a	15.3ab	14.4ab
geraniol	40 <sup>a</sup>	1.47a	1.32ab	1.65a	1.68a	0.96b	2.87ab	3.21a	2.00cd	1.74d	2.37bc	25.0a	24.1a	23.0ab	23.7ab	16.3b
nerol	30 <sup>a</sup>	2.93a	2.12a	2.32a	nd	nd	1.99b	7.54a	2.55b	4.21b	3.20b	23.5a	20.7a	22.8a	26.5a	22.8a
<b>Alcohol</b>																
2-phenyl-ethanol	140 <sup>c</sup>	182c	315c	620b	1227a	213c	428c	1197a	973ab	519bc	1279a	1187ab	1493a	1519a	2063a	350b
<b>Norisoprenoids</b>																
$\beta$ -damascenone	2 <sup>d</sup>	14.0ab	13.7ab	11.3c	14.3a	12.2cb	36.6a	20.1b	12.4c	11.9c	15.9bc	12.8a	12.1ab	11.7ab	9.47c	10.3bc
$\alpha$ -ionone	0.6 <sup>a</sup>	4.85ab	4.81b	4.85a	4.72c	4.70c	5.11a	5.05ab	4.81c	4.84c	4.95bc	6.05a	5.51a	5.15a	5.35a	5.66a
<b>Volatile phenols</b>																
guaiacol	0.84 <sup>c</sup>	0.81b	0.80b	0.96a	0.58c	0.46c	1.38a	1.35ab	0.76c	0.68c	0.96bc	1.99bc	2.33ab	2.69a	1.67c	1.83bc
4-methyl-guaiacol	21 <sup>c</sup>	1.71c	1.74c	1.73c	3.01a	2.29b	2.95a	2.50a	2.45a	2.43a	2.41a	2.19a	2.22a	2.25a	2.24a	2.18a
4-ethyl-guaiacol	4.4 <sup>c</sup>	1.23ab	1.25a	1.24a	1.24a	1.22b	1.31a	1.32a	1.26b	1.26b	1.30a	2.96a	2.93ab	2.96a	2.94ab	2.89b
eugenol	6 <sup>c</sup>	3.12a	3.04ab	3.10ab	2.97bc	2.89c	3.27ab	3.50a	3.07b	3.10b	3.19b	3.28a	3.03a	3.01a	3.07a	2.68a
4-vinyl-guaiacol	5.1 <sup>c</sup>	2.47b	4.40ab	2.00b	8.89a	3.10b	3.39a	1.96bc	2.87ab	1.67bc	0.92c	10.6a	5.94a	10.1a	10.8a	3.95a

For each Vintage, means followed by the same letter within a row do not differ significantly ( $p \leq 0.05$ ) \* concentrations of methoxypyrazines are in ng/kg of berries, nd – not detected. Missing values of hexanal in 2017 were due to lack of authentic standard for calibration.<sup>1</sup>Odour thresholds in water as published by <sup>a</sup>(Van Gemert, 2003), <sup>b</sup>(Sala *et al.*, 2004), <sup>c</sup>(Czerny *et al.*, 2008), <sup>d</sup>(Pineau *et al.*, 2007).

TABLE 11 Grape aroma potential of cv. Pinotage harvested at 5 ripeness levels for Site B, Stellenbosch (2015-2017)

OT <sup>1</sup>	2015					2016					2017					
	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5	
<b>C6-compounds</b>																
hexanal	4.5 <sup>a</sup>	1405b	1930a	1257b	1164b	383c	1562b	1130bc	2204a	536a	753cd					
(E)-2-hexanal	17 <sup>a</sup>	2062c	1947c	4180a	3337b	616d	3277b	1787c	4874a	1171c	1030c	4399a	4431a	4443a	3599b	2202c
1-hexanol	500 <sup>a</sup>	100ab	120a	137a	70.2bc	55.3c	178bc	363a	135c	183bc	265ab	435b	373b	400b	404b	812a
(Z)-3-hexanol	70 <sup>a</sup>	67.4a	30.8b	36.2ab	36.9ab	15.6b	50.6a	92.2a	93.1a	96.7a	59.4a	125b	83.5c	171a	86.8bc	55.6c
(E)-2-hexanol	1000 <sup>a</sup>	71.7a	55.0ab	10.0c	26.5bc	37.4bc	164a	149a	92.2b	159a	123ab	166b	139b	221b	153b	360a
<b>Methoxypyrazines</b>																
IPMP*	2 <sup>b</sup>	0.35a	0.32ab	0.24c	0.24c	0.27bc	0.56c	0.59bc	0.83b	1.28a	1.28a	0.77a	0.84a	0.73a	0.81a	0.77a
SBMP*	2 <sup>b</sup>	0.65a	0.66a	0.66a	0.61a	0.74a	0.30b	0.36b	0.49b	0.62a	0.72a	0.40a	0.60a	nd	0.79a	0.62a
IBMP*	2 <sup>b</sup>	0.47a	0.41a	0.40a	0.34a	0.46a	0.66b	0.75b	0.89b	1.16ab	1.24a	0.82ab	0.86a	0.71b	0.69b	0.68b
<b>Monoterpenes</b>																
linalool	6 <sup>a</sup>	19.8a	21.5a	nd	8.40a	4.53a	11.78a	8.62ab	9.34ab	5.37b	7.56b	5.49a	4.04b	6.65a	3.56b	6.51a
citronellol	30 <sup>a</sup>	2.77a	2.89a	1.17b	2.36a	2.18ab	4.94b	8.77a	4.93ab	5.73ab	6.58a	12.65c	12.67c	14.69b	13.83bc	18.30a
geraniol	40 <sup>a</sup>	1.45ab	1.68a	0.96b	1.25ab	1.23ab	2.07b	3.21a	2.34ab	2.10b	2.03b	28.73a	18.00b	26.10ab	20.58ab	20.4ab
nerol	30 <sup>a</sup>	0.94a	2.41a	nd	nd	nd	4.06a	4.04a	3.67a	6.40a	nd	24.50ab	20.59b	28.22a	19.88b	26.93a
<b>Alcohol</b>																
2-phenyl-ethanol	140 <sup>c</sup>	428a	384ab	235ab	179b	223ab	451b	1408a	366b	1036a	1217a	967a	1295a	1555a	1697a	1326a
<b>Norisoprenoids</b>																
β-damascenone	2 <sup>d</sup>	12.33a	12.43a	11.20a	12.59a	12.77a	15.3ab	20.3a	17.2a	9.45b	9.84b	11.97ab	13.50a	9.46bc	8.59c	10.72b
α-ionone	0.6 <sup>a</sup>	4.79abc	4.82ab	4.68c	4.70bc	4.90a	4.82a	4.98a	4.85a	4.82a	4.85a	6.38a	5.94ab	5.72ab	5.27b	5.1b8
<b>Volatile phenols</b>																
guaiacol	0.84 <sup>c</sup>	0.80a	0.96a	0.29b	0.46b	0.43b	1.09ab	1.35a	0.64c	0.83bc	1.08ab	2.29b	3.17a	1.41c	1.79bc	2.05bc
4-methyl-guaiacol	21 <sup>c</sup>	1.72b	1.70b	2.07ab	2.48a	1.93ab	2.48ab	2.94a	2.58ab	2.36b	2.44ab	2.19ab	2.15b	2.19ab	2.17ab	2.26a
4-ethyl-guaiacol	4.4 <sup>c</sup>	1.25a	1.24ab	1.21b	1.22b	1.22b	1.26b	1.33a	1.28b	1.26b	1.28b	2.93a	2.90a	2.93a	2.92a	2.93a
eugenol	6 <sup>c</sup>	3.01a	3.08ab	2.84b	2.87b	3.29a	3.14b	3.47a	3.11b	3.13b	3.26b	3.10a	2.72a	2.93a	2.89a	2.95a
4-vinyl-guaiacol	5.1 <sup>c</sup>	4.12a	1.15b	5.30a	1.60b	nd	3.06ab	1.83bc	4.05a	1.71bc	1.55c	7.74b	5.60b	12.92a	7.11b	7.80b

For each Vintage, means followed by the same letter within a row do not differ significantly ( $p \leq 0.05$ ), \* concentrations of methoxypyrazines are in ng/kg of berries, nd – not detected. Missing values of hexanal in 2017 were due to lack of authentic standard for calibration.<sup>1</sup>Odour thresholds in water as published by <sup>a</sup>(Van Gemert, 2003), <sup>b</sup>(Sala *et al.*, 2004), <sup>c</sup>(Czerny *et al.*, 2008), <sup>d</sup>(Pineau *et al.*, 2007).

The most abundant aroma compounds found in Pinotage grape berries were C6-aldehydes and alcohols, including hexanal, (E)-2-hexanal, 1-hexanol, (Z)-3-hexanol and (E)-2-hexanol, which contribute herbaceous aroma to grapes (Dubois, 1994). The C6-aldehydes were more prevalent in early harvest dates than later dates, while a clear relationship was not visible in all vintages. The C6-aldehydes and alcohols are released from fatty acids during grape crushing and processing. In grape juice C6-aldehydes are the dominant fraction of the C6-compounds, but during fermentation they are readily reduced to their corresponding alcohols. On the other hand, 1-hexanol is considered the dominant C6 component in wine, but its sensory threshold is relatively high (8000 µg/L) in a wine medium. In accordance with results published on Pinot noir (Yuan & Qian, 2016b), C6-alcohols generally decreased with an increase in maturity. This is however contrary to findings published on Cabernet Sauvignon and Riesling (Kalua & Boss, 2010; Boss *et al.*, 2014) in a warm climate. As C6-compounds are derived from berry skin constituents (fatty acid from cell walls), advanced stages of ripening that induce berry size reduction and softening, may increase the presence of C6 compounds by altered skin:pulp ratio. In addition, relative stage of ripening is also an important consideration as the final harvest date (20°B) in the studies on Cabernet Sauvignon and Riesling was completed before the first (21°B) started in the current study. Nonetheless, the growing understanding of C6-compounds has displayed their positive contributions to wine aroma as precursors of hexyl acetate (fruity) (Dennis *et al.*, 2012) and 3-mercaptohexan-1-ol (tropical) (Harsch *et al.*, 2013). The aroma-specific relationship of this group of compounds needs further elucidation.

In addition to C6 compounds, 3-isobutyl-2-methoxypyrazine (IBMP) is generally considered the main contributor to the green bell pepper aromas found in wine grapes (Romero *et al.*, 2006). Two additional methoxypyrazines, sec-butyl-methoxypyrazine (SBMP, green ivy leaves) and 3-isopropyl-2-methoxypyrazine (IPMP, asparagus) were detected, but all the methoxypyrazines were present at levels below sensory threshold (> 2 ng/L) (Roujou de Boubee *et al.*, 2000) and did not display clear trends according to ripeness level. In warm climates, the production of methoxypyrazines is often below threshold values, even in varieties prone to high levels, due to the diminishing effect of UV exposure and high temperatures (Lacey *et al.*, 1991; Marais *et al.*, 1999; Roujou de Boubee *et al.*, 2000; Hunter, Volschenk, *et al.*, 2004; Romero *et al.*, 2006). Except for linalool, the free terpenes, citronellol, geraniol and nerol, which are known to impart a floral character to grapes (Marais, 1983), were generally present in concentrations below their sensory thresholds. Linalool maintained a concentration above perception threshold (>6 µg/L). The

monoterpenes generally decreased during ripening in this study. While this is contrary to many reports in literature (Wilson *et al.*, 1984; Gunata *et al.*, 1988; Williams *et al.*, 1995; Coombe & McCarthy, 2000), high berry temperatures, high light incidence in the bunch zone and high levels of grape maturity have all been cited to decrease free terpene level (Marais, 1983; Bureau, Baumes, *et al.*, 2000; Bureau, Razungles, *et al.*, 2000). Linalool has been shown to be highly reactive to ripeness level and microclimatic conditions and increasing proportions of linalool oxide derivatives (which were not measured in this study) were found with increased ripening, temperature and sunlight conditions (Luan *et al.*, 2006). The benzenoid 2-phenylethanol is derived from cinnamic acid, and is present in relatively low concentration in grapes compared to yeast-derived forms present in wine. Phenylethanol has a low impact floral character, while ripeness-related changes have not been confirmed. In this study, 2-phenyl ethanol was present in concentrations above sensory threshold in water, but clear trends with regards to ripeness level were not found. Similarly, the norisoprenoids ( $\beta$ -damascenone and  $\alpha$ -ionone) as products of carotenoid breakdown generally remained stable during ripening, with higher concentrations more prevalent in early ripeness levels. Again, studies in cool climates have reported increases in  $\beta$ -damascenone and  $\alpha$ -ionone during ripening of Pinot noir (Yuan & Qian, 2016a), but also low levels in excessively warm climates (Robinson *et al.*, 2014).  $\beta$ -damascenone is thought to enhance the berry-like aromas in most red wines, and is also reported to have a synergistic effect with terpenes and esters, increasing floral flavours and masking green characters of IBMP (Pineau *et al.*, 2007). As was prevalent in Pinot noir grapes from Oregon, volatile phenols (guaiacol, 4-methyl-guaiacol, 4-ethyl-guaiacol, eugenol and 4-vinyl-guaiacol) were detected in significant amounts in Pinotage grape berries (Yuan & Qian, 2016b). Volatile phenols are associated with woody, smoky and clove aromas and are often related to oak-derived components of winemaking. On the other hand, volatile phenols such as 4-ethyl-phenol (not quantified here) can also derive from microbial spoilage of wines (Ribéreau-Gayon *et al.*, 2006b) and is generally seen in a negative light. Our results show significant concentrations of volatile phenols, albeit mostly under the sensory thresholds. Moreover, guaiacol concentration was at its lowest during the final ripeness level, while its methylated derivative 4-methyl-guaiacol was at its highest in R5 (Yuan & Qian, 2016b).

In the light of the similar trends found in sites regarding accumulation/decrease during ripening, sites were combined in Discriminant Analysis (DA) in order to assess the overall ripeness level effects per vintage. Observations from DA models explained 90.11 % in 2015 (F1 77.37 % and F2 12.74 %), 87.31 % in 2016 (F1 55.60 % and F2 31.72 %) and 88.40 % in

2017 (F1 77.42 % and F2 10.99 %) of the dataset (Fig 9). Potential aroma profiles of different ripeness levels were consistently separated along F1 from R1 to R5, with the exception of R2 in 2016. The abundance of aroma compounds was generally greater in lower ripeness levels, characterised by herbaceous compounds IBMP, IPMP, (E)-2-hexenal and guaiacol, becoming less abundant as ripening continued, and discriminating along F1. However, confidence ellipses frequently overlapped for adjacent ripeness levels and only R1 and R5 could consistently be discriminated from one another (no overlapping) in all of the vintages. In 2015, less overlapping was prevalent, clearly separating every second ripeness level, supporting the articulate differences in aroma accumulation during ripening, most probably brought about by meso and micro climatic factors (Marais *et al.*, 1999; Hunter, Volschenk, *et al.*, 2004). Although the separation of ripeness levels along F1 was dominant, F2 (<32%) often discriminated R5 from the other ripeness levels, quantitative data show generally the lowest aroma potential for this harvest date, possibly indicating towards the diminished aromatic potential related to over ripeness (Vilanova *et al.*, 2012). The consistent overlap between harvest dates reveal a consistent quantitative change, rather than major qualitative shifts in aroma profiles, during each vintage. This reaction was in line with overlapping shifts seen in berry density distributions (Fig 2) and anthocyanin profiles (Fig 7), effectively reaffirming the minor shifts in aroma potential profile in relation to significant changes in classical ripening indices.

The PLS modelling of grape potential aroma profiles (Addendum B Table 3) identified compounds (Fig 9, marked in red) with high predictability (predictive coefficients VIP > 1). These compounds were different for each vintage and originated from all the groups measured, with the exception of the benzoids (2-phenylethanol). Thereby,  $\beta$ -damascenone, IPMP and guaiacol, were identified as key components relating to earlier ripeness levels in at least two of the three vintages, although they were generally present at low concentrations. Associations between ripeness level and aromatic potential were less clear, and bring forward important questions regarding these highly sensitive parameters of vastly different metabolic origins. Detailed work on aromatic compounds in literature is rarely reported using a multi-seasonal approach. In addition, grape samples from field replicates are often pooled/sorted in order to diminish the effects of natural population heterogeneity between replicates (Boss *et al.*, 2014). Our results present insight into changes that would be reflective of a commercial vineyard setting, and gives insight into changes visible therein.

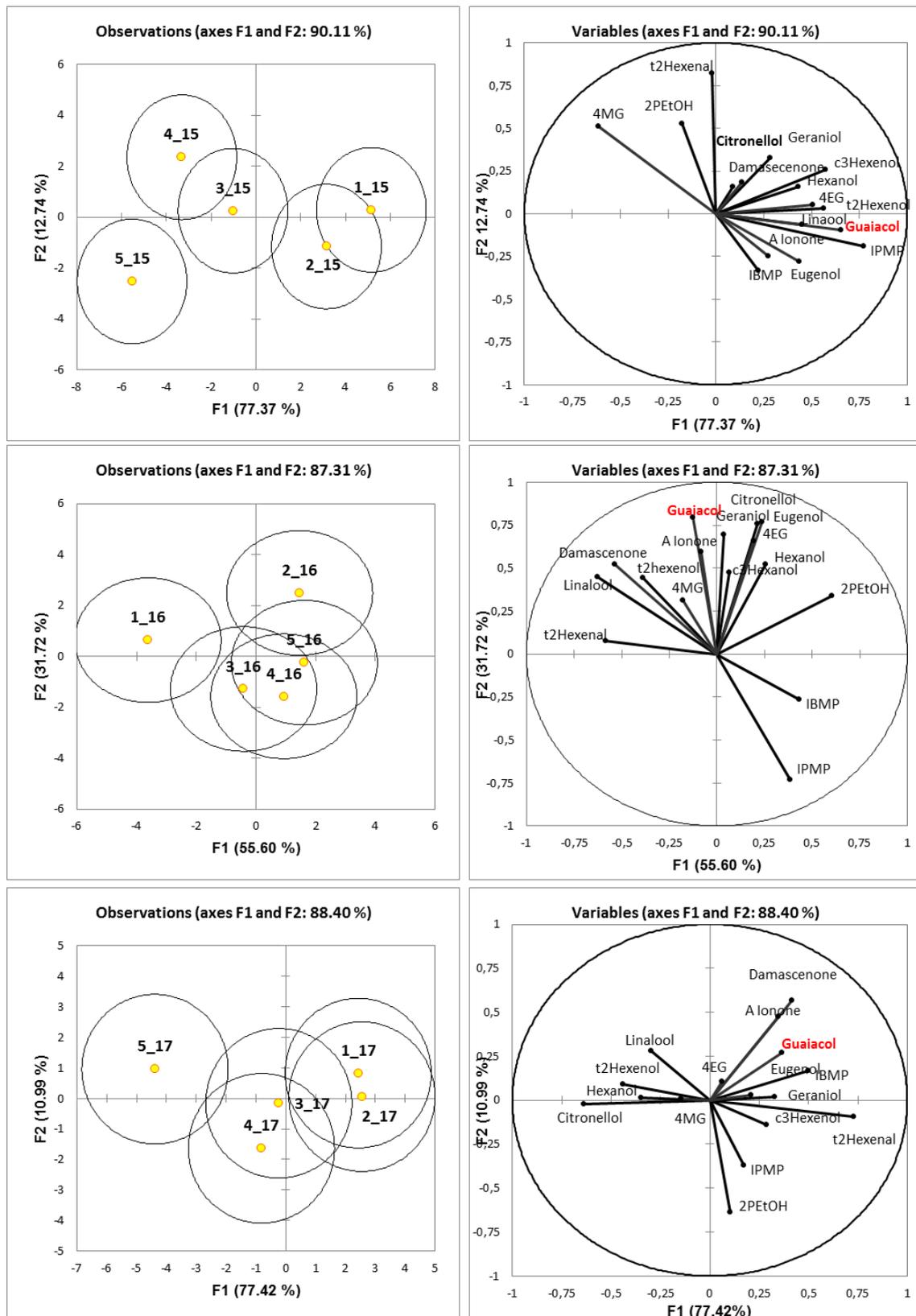


Figure 9. Discriminant analysis of grape aroma potential of five ripeness levels for vintages 2015 (top), 2016 (middle) and 2017 (bottom), with alpha ellipses (0.5). Key compounds (red) were confirmed by their contribution ( $VIP > 1$ ) with PLS modelling of ripeness level.

#### 4.4.7 Overall Compositional Response

Principal component analysis of all the measured variables managed to explain 48% of total variance in the compositional data (Fig. 10). Ripeness level could be distinguished along PC1 (29%), while differences in vintage separated along PC2 (19%). Generally, confidence ellipses overlapped for adjacent ripeness levels, yet the direction of change remained along PC1 for increasing ripeness levels. Interestingly, R3 displayed the greatest ellipse in all three vintages, undoubtedly brought about by a large degree of berry heterogeneity. Incidentally, this is generally considered the commercial harvest date ( $25^{\circ}\text{B} \approx 14.2\% \text{ Alc v/v}$ ) for Pinotage (Marais *et al.*, 1979, 1981). Ripeness levels before and after R3, tended to display more homogeneous composition. Moreover, changes in ripeness level here predominantly corresponded to increases in phenolic indicators ( $A_{280}$  and  $A_{520}$ ), classical indices, and individual anthocyanin content. Lower ripeness levels were associated with increased linalool (floral), high must titratable acidity and increased berry  $\text{H}_2\text{O}$  proportion. Apart from un-oxidised linalool content relating to less ripe grapes, potential aroma compounds were largely responsible for vintage differences opposed to driving differences in ripeness levels. From this it appears that changes in classical and phenolic indices outweighed changes in potential aroma during the measurement window. This is confirmed by the relatively minor changes that occurred in aroma potential profile along ripening. Notwithstanding, some of the aroma compounds identified (IBMP, VP and norisoprenoids) have extremely low threshold values and minor shifts can induce significant sensory impact. However, changes were not consistent and therefore challenges the concept that major changes in volatiles occur during ripening. On the contrary, in accordance with Boss *et al.* (2014), it appeared as if volatiles had already peaked earlier in ripening. This may have been a reaction mediated by climate, as seen in the high sensitivity of aromatic compounds to vintage in the current study. While aroma compounds have been proposed in numerous studies as additional ripeness indices (Williams *et al.*, 1995; Rocha *et al.*, 2010; Vilanova *et al.*, 2012), high responsiveness to growing conditions and analytical methods require many future works to understand this complex interaction.

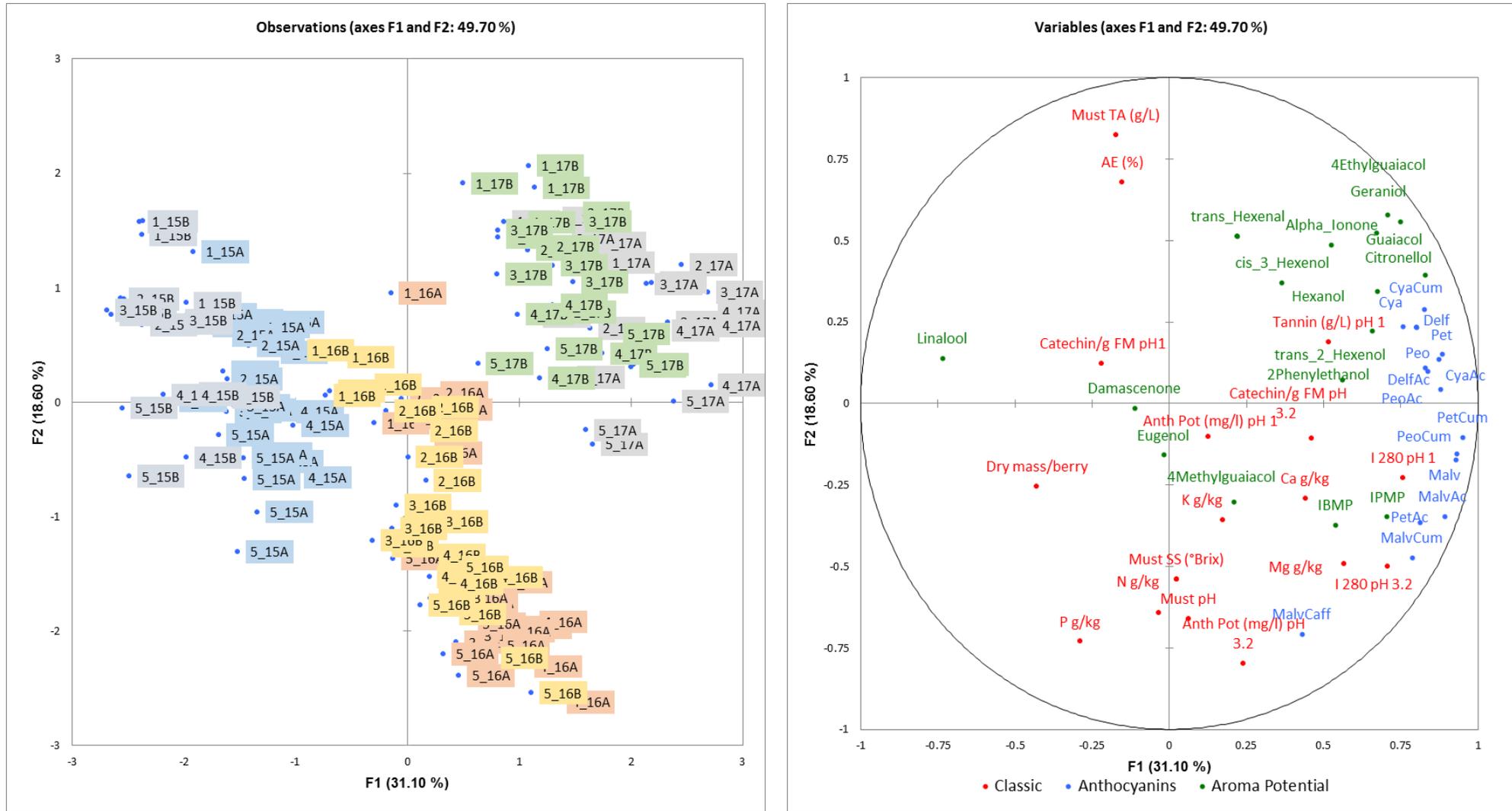


FIGURE 10. Multifactorial analysis (MFA) of all the grape compositional parameters of five ripeness levels (1-5), of cv. Pinotage at Site A and B combined for Vintages (2015-2017) in Stellenbosch, Western Cape, South Africa.

#### 4.4.8 Practical implications

On a practical level the results allude to an asynchronous development of the various berry qualitative components during the commercial harvest window (Fig 11) and necessitates the producer to take the factors into account that suits the eventual wine goal. In summary, significant increases in sugar and decreases in acid along the ripeness gradient allows for each ripeness level to be well articulated with Brix:TA ratios. Generally, grape tannin and colour content increases along ripeness level and peaks at 27°B, after which there is a decrease in phenolic and colour potential. The decrease in phenolic and colour potential associated with ‘over ripe’ grapes (29°B), should be taken in account if producers seek to increase qualitative attributes with increased hang time. Similarly while potential herbaceous (negative) aromas decreases with increasing grape maturity, potential floral-, berry and spice-linked aroma compounds are also present in abundance at early ripeness levels (21-23°B). In the field it seems that decreases in potential herbaceous characteristics are well associated with changes in Brix:TA . Thus, the producer will be able to gauge the more subtle changes (berry floral, spice) with a simple index such as Brix:TA.

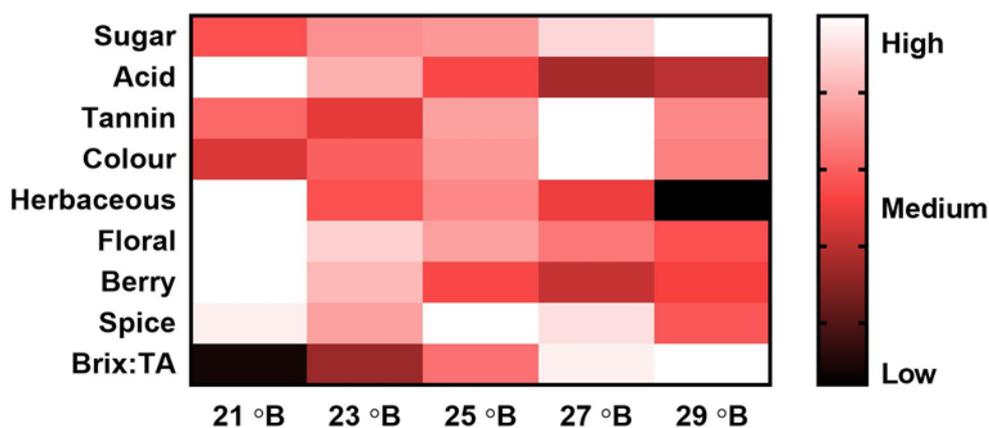


Figure 11. Simplified summary of grape compositional changes for selected variables in cv. Pinotage. Sugar (°B), Acid (titratable acidity), Tannin (A280 pH 3.2), Colour (A520 pH 3.2), Herbaceous (Tot C6-compounds), Floral (Tot Monoterpenes), Berry (Tot C13-Norisprenoids), Spice (Tot Volatile phenols) and Brix:TA (°B/titrable acidity) expressed as a relative fraction of the maximum value.

#### 4.5 CONCLUSIONS

The detailed tracking of various components under field conditions with high frequency during the commercial harvest window, gave insight to what lay in wait regarding shortened harvest windows due to a warming climate. Relatively spoken, shifts in grape primary and secondary metabolite composition appeared to be asynchronous with large degrees of overlapping between ripeness levels. Nonetheless, rapid sugar accumulation was consistently coupled with increases in phenolic content, most notably peaking at R4 (27°B  $\approx$  15.2 % Alc v/v) independent of berry size reduction, before a rapid decline in berry phenolic quality and degeneration of anthocyanins occurred in conjunction with berry size reduction. Despite decreased grape anthocyanin and phenolic content at R5 (29°B  $\approx$  16.25 % Alc v/v), extractability indexes were high for this technically overripe level. On the other hand, grape aroma potential displayed inconsistent and minor shifts over the measurement window, generally shifting to a profile that is potentially less herbaceous rather than marked by improved positive aroma compounds. Earlier ripeness levels were marked with greater abundance of compounds both potentially positive and negative (herbaceous). This raises many questions *e.g.* (1) Did high temperatures and/or the short maturation timeframe induce aromatic catabolism? (2) Did aromatic potential peak before the measurement window? (3) Did the high degree of berry heterogeneity neutralise a distinctive ripeness effect with regard to aromatic qualities, but not for phenolic and/or primary metabolism? Certainly, grape composition delineates wine composition, although many gaps exist in our understanding of the impact and interaction of winemaking variables (most notably yeast metabolism) on ripening-related sensory properties in wines. In this field study, the possible tempering role of heterogeneous berry populations on compositional results was apparent, yet undoubtedly represents the practical realities that are faced by producers. Long- and short term viticultural practices should aim to minimize berry population heterogeneity, specifically as indications of (future) shortened harvest windows will increase chances of berry population heterogeneity.

Furthermore, acknowledging that grape compositional responses are highly climate and genotype dependent, results emphasise the need for future multiple variable ripeness level indices. Finally, this study uncovered novel information regarding changes in the berry compositional profile during ripening and sheds light on and further underlines the importance of grape ripeness level as tool to manipulate the wine substrate within the boundaries dictated by environmental factors.

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4.7 ADDENDUM B

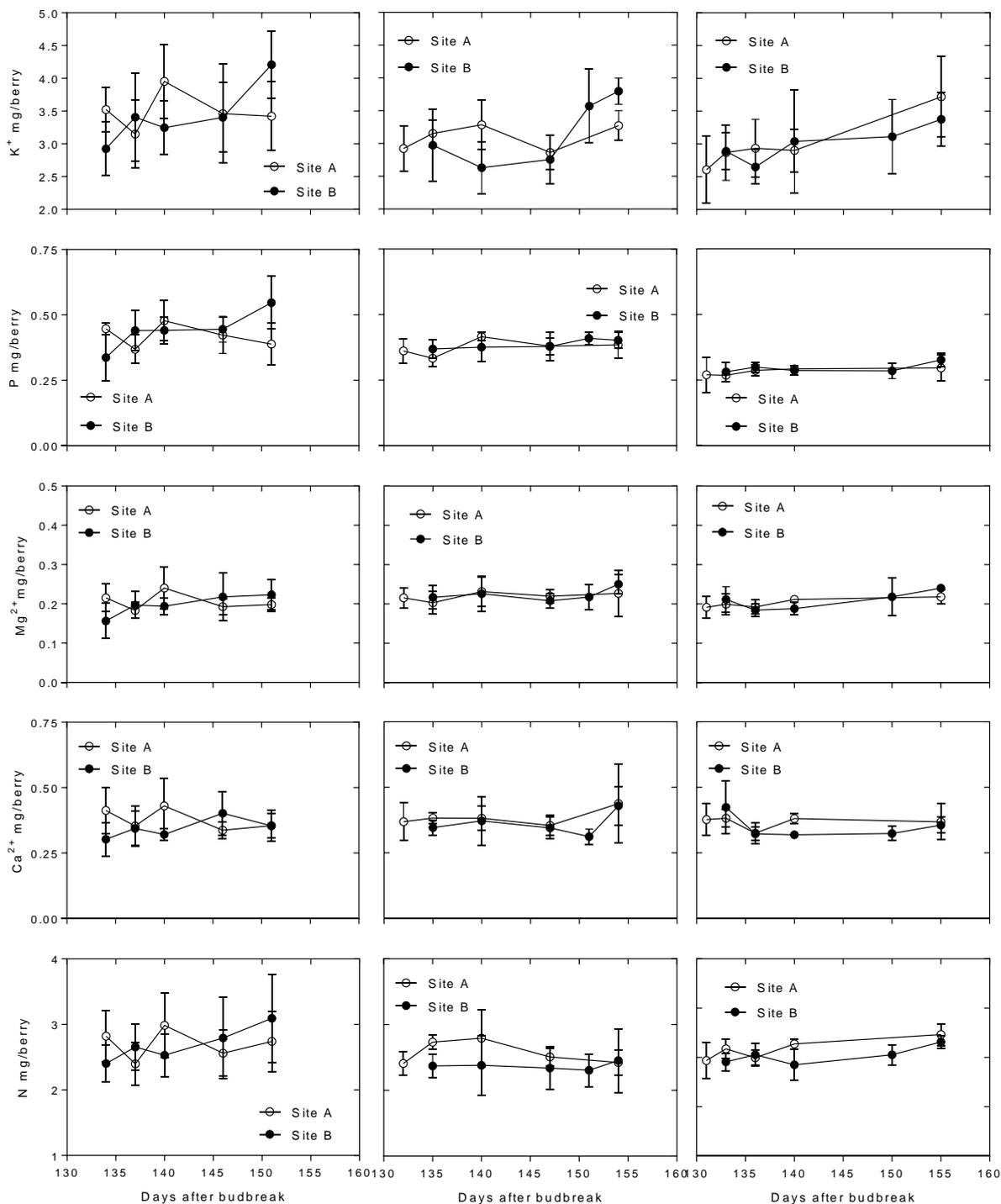


Figure 1. Mineral concentrations per berry for the 2015(left), 2016 (middle) and 2017(right) vintages at five ripeness levels (R1-R5) of cv. Pinotage at Site A and B in Stellenbosch, Western Cape, South Africa (standard deviation confidence interval 95 %).

Table 1. Variable importance in PLS projections of grape compositional (classical indices) data and ripeness level for each vintage

Variable	2015				2016				2017															
	VIP	St Dev	Lo 95 %	Up 95 %	VIP	St Dev	Lo 95 %	Up 95 %	VIP	St Dev	Lo 95 %	Up 95 %	VIP	St Dev	Lo 95 %	Up 95 %								
AE (%)	0.3	0.4	-0.5	1.0	0.9	0.3	0.2	1.6	0.1	0.3	-0.6	0.7	0.7	0.3	0.1	1.3	0.1	0.2	-0.4	0.5	0.9	1.3	-1.7	3.6
Anth Pot (mg/l) pH 1	0.4	0.3	-0.3	1.0	0.9	0.2	0.5	1.3	0.6	0.3	0.1	1.2	0.5	0.2	0.1	0.8	1.1	0.2	0.8	1.4	1.2	0.4	0.5	1.9
Anth Pot (mg/l) pH 3.2	0.6	0.2	0.1	1.1	0.7	0.2	0.4	1.1	0.9	0.2	0.6	1.3	0.7	0.1	0.4	0.9	1.1	0.2	0.8	1.4	0.9	0.2	0.6	1.2
Ca g/kg	0.6	0.2	0.1	1.0	0.5	0.2	0.2	0.9	0.5	0.3	-0.1	1.1	0.9	0.2	0.4	1.3	0.5	0.3	-0.1	1.1	0.9	1.0	-1.1	2.9
DMAC mg Cat/g FM pH 3.2	0.2	0.5	-0.7	1.1	1.3	0.2	0.8	1.7	0.2	0.5	-0.8	1.2	1.3	0.2	1.0	1.7	1.2	0.2	0.9	1.5	1.0	0.2	0.6	1.4
DMAC mg Cat/g FM pH1	0.8	0.3	0.1	1.5	1.2	0.2	0.7	1.6	0.1	0.4	-0.8	1.0	1.5	0.1	1.2	1.8	0.5	0.2	0.1	0.9	0.4	0.3	-0.1	1.0
Dry mass/berry (g)	0.6	0.3	0.0	1.1	0.6	0.2	0.1	1.1	1.0	0.2	0.7	1.3	0.7	0.1	0.5	0.9	1.5	0.1	1.3	1.7	1.3	0.1	1.0	1.6
I 280 pH 1	0.5	0.3	-0.1	1.1	0.9	0.2	0.6	1.2	1.3	0.3	0.8	1.9	1.5	0.1	1.3	1.6	0.7	0.3	0.1	1.2	0.9	0.8	-0.7	2.4
I 280 pH 3.2	0.4	0.3	-0.2	1.1	1.0	0.2	0.7	1.3	1.4	0.3	0.9	1.9	1.5	0.1	1.3	1.6	0.2	0.3	-0.3	0.8	0.2	0.4	-0.6	1.0
K g/kg	1.2	0.2	0.8	1.5	0.9	0.2	0.6	1.3	0.9	0.1	0.7	1.2	0.7	0.1	0.5	0.9	0.8	0.2	0.4	1.3	1.0	0.5	-0.1	2.1
Mg g/kg	1.0	0.2	0.6	1.3	0.8	0.2	0.4	1.1	0.8	0.2	0.3	1.2	0.7	0.2	0.3	1.1	0.4	0.3	-0.1	0.9	1.0	0.7	-0.5	2.4
Must pH	1.8	0.1	1.5	2.1	1.4	0.1	1.1	1.7	1.5	0.1	1.3	1.7	1.1	0.0	1.0	1.2	1.7	0.1	1.5	1.8	1.4	0.2	1.1	1.8
Must SS (°Brix)	1.5	0.1	1.2	1.8	1.3	0.1	1.1	1.5	1.5	0.1	1.3	1.7	1.2	0.1	1.0	1.3	1.8	0.1	1.6	1.9	1.5	0.2	1.1	1.9
Must TA (g/L)	1.7	0.1	1.4	2.0	1.4	0.1	1.2	1.6	1.5	0.1	1.4	1.7	1.1	0.1	0.9	1.2	1.6	0.1	1.4	1.8	1.4	0.1	1.1	1.7
N g/kg	1.2	0.1	0.9	1.5	0.9	0.1	0.6	1.2	0.5	0.2	0.1	1.0	0.6	0.1	0.3	0.9	0.6	0.2	0.1	1.1	1.1	0.4	0.2	2.0
P g/kg	1.1	0.2	0.7	1.5	0.9	0.2	0.5	1.2	1.1	0.1	0.8	1.4	0.8	0.1	0.6	1.0	0.2	0.3	-0.5	0.8	0.4	0.3	-0.3	1.1
Tannin (g/L) pH 1	1.1	0.2	0.6	1.5	0.9	0.1	0.6	1.2	1.0	0.2	0.6	1.4	0.8	0.2	0.5	1.1	0.2	0.3	-0.4	0.8	0.2	0.8	-1.3	1.7

Variable Importance in Projection (VIP), VIP values  $\geq 1$ , indicate high predictive value of the variable within the model  
 PLS regressions were conducted for each vintage separately, Standard deviation (St Dev)

Table 2. Variable importance in PLS projections of grape compositional (individual anthocyanin) data and ripeness level for each vintage.

Variable	2015				2016				2017				2017											
	VIP	St Dev	Lo 95%	Up 95%	VIP	St Dev	Lo 95%	Up 95%	VIP	St Dev	Lo 95%	Up 95%	VIP	St Dev	Lo 95%	Up 95%	VIP	St Dev	Lo 95%	Up 95%				
Cya_kgBer	0.9	0.6	-0.3	2.0	0.4	0.4	-0.4	1.2	0.5	0.2	0.0	1.0	0.9	0.1	0.7	1.0	0.6	0.2	0.3	0.9	0.9	0.1	0.7	1.1
CyaAc_kgBer	1.1	0.4	0.2	1.9	0.5	0.3	-0.2	1.2	0.5	0.3	-0.1	1.0	0.8	0.1	0.6	1.1	0.9	0.1	0.7	1.1	0.8	0.1	0.7	0.9
CyaCum_kgBer	0.7	0.9	-1.2	2.5	0.5	0.7	-1.0	2.0	0.2	0.3	-0.5	0.8	1.2	0.1	1.0	1.4	0.3	0.2	-0.1	0.8	1.1	0.1	0.9	1.3
Delf_kgBer	1.0	0.6	-0.2	2.3	0.5	0.4	-0.3	1.3	0.4	0.3	-0.2	1.1	1.2	0.1	1.1	1.4	0.9	0.1	0.6	1.1	0.9	0.0	0.8	1.0
DelfAc_kgBer	1.1	0.5	0.2	2.1	0.7	0.5	-0.3	1.7	0.6	0.3	0.1	1.1	0.9	0.1	0.8	1.1	0.9	0.1	0.7	1.1	0.9	0.1	0.7	1.0
Malv_kgBer	0.9	0.5	-0.2	1.9	0.7	0.5	-0.2	1.6	1.3	0.1	1.2	1.5	1.0	0.1	0.8	1.1	1.3	0.1	1.1	1.4	0.9	0.1	0.8	1.1
MalvAc_kgBer	1.1	0.9	-0.7	2.9	0.9	0.4	0.1	1.6	1.3	0.1	1.1	1.5	1.0	0.1	0.9	1.1	1.2	0.1	1.1	1.4	0.9	0.1	0.8	1.1
MalvCaff_kgBer	0.8	1.1	-1.3	3.0	1.1	0.4	0.2	1.9	1.5	0.2	1.0	2.0	1.5	0.1	1.3	1.7	1.0	0.2	0.5	1.5	1.6	0.2	1.3	1.9
MalvCum_kgBer	1.0	1.4	-1.7	3.7	1.0	0.4	0.2	1.7	1.5	0.2	1.2	1.8	1.2	0.1	1.0	1.4	1.3	0.1	1.0	1.5	1.1	0.1	1.0	1.3
Peo_kgBer	0.8	0.6	-0.3	2.0	0.8	0.6	-0.4	2.0	1.0	0.1	0.8	1.2	0.7	0.1	0.6	0.9	0.8	0.1	0.6	1.0	0.8	0.1	0.6	0.9
PeoAc_kgBer	1.0	0.6	-0.2	2.2	0.8	0.5	-0.2	1.8	0.9	0.1	0.7	1.1	0.7	0.1	0.6	0.8	0.6	0.2	0.2	0.9	0.8	0.1	0.6	1.0
PeoCum_kgBer	0.9	1.4	-1.8	3.6	1.3	0.4	0.5	2.1	1.0	0.1	0.8	1.3	0.7	0.1	0.5	1.0	0.9	0.1	0.7	1.1	0.7	0.1	0.5	0.9
Pet_kgBer	1.0	0.4	0.2	1.7	0.4	0.3	-0.2	1.1	0.8	0.2	0.5	1.2	0.9	0.0	0.8	1.0	1.0	0.1	0.9	1.2	0.9	0.0	0.8	0.9
PetAc_kgBer	1.5	2.5	-3.5	6.4	2.6	0.7	1.3	3.9	1.2	0.1	1.0	1.5	0.9	0.1	0.7	1.1	1.5	0.2	1.2	1.9	1.4	0.1	1.2	1.7
PetCum_kgBer	1.0	0.2	0.6	1.5	0.5	0.3	-0.2	1.2	1.0	0.1	0.7	1.3	0.9	0.1	0.8	1.0	1.1	0.1	1.0	1.3	0.9	0.1	0.7	1.0

Variable Importance in Projection (VIP), VIP values  $\geq 1$ , indicate high predictive value of the variable within the model  
 PLS regressions were conducted for each vintage separately, Standard deviation (St Dev)

Table 3. Variable importance in PLS projections of grape compositional (grape aroma potential) data and ripeness level for each vintage.

Variable	2015				2016				2017															
	VIP	St Dev	Lo 95 %	Up 95 %	VIP	St Dev	Lo 95 %	Up 95 %	VIP	St Dev	Lo 95 %	Up 95 %	VIP	St Dev	Lo 95 %	Up 95 %								
**Alpha_Ionone_ug_kg	1.1	0.4	0.2	1.9	0.8	0.3	0.2	1.5	1.1	0.2	0.7	1.4	0.7	0.1	0.4	1.0	1.1	0.4	0.3	1.9	0.9	0.7	-0.5	2.3
cis_3_Hexenol_ug_kg	1.0	0.4	0.3	1.7	1.3	0.3	0.6	2.0	0.8	0.3	0.2	1.4	0.6	0.2	0.1	1.1	0.7	0.5	-0.2	1.6	1.1	0.9	-0.7	2.9
**Citronellol_ug_kg	0.3	0.3	-0.3	0.8	0.3	0.3	-0.2	0.9	1.2	0.2	0.8	1.7	1.1	0.2	0.8	1.4	1.7	0.2	1.4	2.0	1.4	0.2	1.1	1.8
**Damascenone_ug_kg	0.2	0.5	-0.8	1.1	0.7	0.4	-0.1	1.5	1.1	0.3	0.6	1.6	1.1	0.1	0.9	1.4	1.1	0.4	0.2	2.0	1.3	0.5	0.3	2.2
Ethylguaiaicol4_ug_kg	0.9	0.3	0.4	1.4	0.8	0.2	0.4	1.2	1.1	0.3	0.5	1.6	1.0	0.2	0.6	1.3	0.1	0.4	-0.8	1.0	0.5	0.6	-0.6	1.7
*Eugenol_ug_kg	0.7	0.4	-0.1	1.4	0.6	0.3	0.0	1.1	1.2	0.3	0.7	1.8	1.1	0.2	0.7	1.6	0.6	0.5	-0.4	1.6	0.7	0.5	-0.4	1.8
Geraniol_ug_kg	0.3	0.4	-0.5	1.2	0.9	0.4	0.1	1.6	1.2	0.2	0.8	1.6	0.8	0.2	0.5	1.2	0.8	0.6	-0.4	1.9	1.0	0.4	0.2	1.7
***Guaiacol_ug_kg	1.3	0.2	1.0	1.6	1.0	0.1	0.8	1.3	1.4	0.1	1.2	1.7	1.0	0.1	0.7	1.2	0.8	0.5	-0.2	1.8	1.3	1.2	-1.1	3.7
*Hexanol_ug_kg	1.0	0.2	0.6	1.5	0.9	0.2	0.4	1.4	0.8	0.3	0.2	1.4	0.9	0.2	0.5	1.4	1.0	0.3	0.3	1.7	0.9	0.3	0.3	1.5
isobut2_3_methxypyrzn_IBMP_ug_kg	0.8	0.3	0.2	1.4	0.8	0.3	0.3	1.4	0.6	0.4	-0.1	1.4	0.9	0.2	0.4	1.3	1.3	0.3	0.6	2.0	1.1	0.3	0.5	1.8
**isoprop2_3_methoxyprz_IPMP_ug_kg	1.7	0.1	1.5	2.0	1.3	0.1	1.1	1.5	1.4	0.2	0.9	1.9	1.1	0.2	0.7	1.4	0.3	0.5	-0.7	1.2	0.4	1.0	-1.7	2.4
Linalool_ug_kg	1.2	0.2	0.7	1.7	0.9	0.2	0.6	1.3	1.0	0.4	0.2	1.8	1.3	0.2	1.0	1.6	0.8	0.4	-0.1	1.7	0.7	0.5	-0.3	1.7
*Methylguaiaicol4_ug_kg	1.6	0.2	1.2	2.0	1.4	0.1	1.1	1.7	0.6	0.2	0.1	1.1	0.5	0.2	0.1	0.9	0.4	0.4	-0.5	1.3	0.4	0.4	-0.4	1.1
Phenylethanol2_ug_kg	0.6	0.4	-0.3	1.5	1.0	0.5	0.0	1.9	0.4	0.5	-0.7	1.4	1.4	0.2	1.1	1.7	0.0	0.4	-0.8	0.8	0.5	1.8	-3.1	4.1
*trans_2_Hexenol_ug_kg	1.1	0.2	0.7	1.5	0.8	0.2	0.5	1.2	0.9	0.3	0.4	1.5	0.9	0.2	0.5	1.3	1.3	0.2	0.8	1.7	1.1	0.2	0.6	1.5
trans_Hexenal2_ug_kg	0.6	0.5	-0.5	1.6	1.6	0.2	1.2	2.1	0.3	0.5	-0.6	1.3	1.2	0.2	0.9	1.6	1.9	0.2	1.5	2.3	1.6	0.3	1.1	2.1

Variable Importance in Projection (VIP), VIP values  $\geq 1$ , indicate high predictive value of the variable within the model  
 PLS regressions were conducted for each vintage separately, Standard deviation (St Dev)

## Chapter 5

### **Wine composition of *Vitis vinifera* L. cv. Pinotage as related to grape berry ripeness level**

#### 5.1 ABSTRACT

This study aimed to quantify wine compositional changes over a range of commercial ripeness levels for *Vitis vinifera* L. cv. Pinotage. Wine chemical, phenolic, and volatile composition was assessed at five (R1-R5) ripeness levels that were defined by Brix (*ca.* 21, 23, 25, 27 & 29°B) over three vintages (2015-2017) and two sites (A & B) for Pinotage/140 Ruggeri and Pinotage/1103 Paulsen, under Mediterranean conditions, Western Cape, South Africa. The ripeness level range (21 days from R1-R5) corresponded to a mean increase in alcohol concentration from 11.92% to 17.05% (v/v). Wine phenolic and anthocyanin content increased significantly as ripening progressed. Changes in wine anthocyanin profile were characterised by increases in proportions of coumarylated derivatives, as opposed to acylated and non-acylated derivatives. Furthermore, proportions of Malv increased at the expense of Delf, Cya, Pet and Peo. More specifically, PetAc (grape and wine) associated well with increased ripeness levels and warrant further investigation as an indicator of ripeness level. Changes in wine volatile composition were noted for 38 volatile compounds (incl. C6-compounds, methoxypyrazines, norisoprenoids, monoterpenes, higher alcohols, fatty acids and esters). Changes in wine aromatic profile were distinctive and key components were significantly influenced by ripeness level and vintage. Wines of later ripeness levels were characterised by decreased C6-compounds (*e.g.* hexanol), methoxypyrazines (IBMP) and fatty acids (hexanoic and octanoic acid), while also characterised by increased norisoprenoids (damascenone), monoterpenes (nerol and geraniol) and higher alcohols (n-butanol). Although esters generally did not follow a clear trend during ripening, compounds linked to Pinotage, such as isoamyl acetate (increase) and ethyl hexanoate and octanoate (decrease) displayed ripeness-related trends. Results suggest a significant shift in overall compositional (chemical, phenolic and volatile) profile per ripeness level (+ 2°B) during the compact harvest window (21 days). In addition, this exhaustive wine compositional field study placed ripening related changes in context to those dictated by the environment (Vintage/Climate) and Site (Soil & Genotype). This indicated that modification of the wine substrate *via* ripeness level and subsequent alteration *via* fermentation had a major differentiating effect on final wine composition, consequently providing much needed insight into compositional changes during rapid sugar accumulation.

## 5.2 INTRODUCTION

The decision to harvest requires the consideration of complex, integrative factors as grape composition (and by extension wine composition) undergoes significant changes during ripening (Boss *et al.*, 2014; Robinson *et al.*, 2014). Thus far the understanding thereof has been complicated by the fact that grape/wine composition has proven to display high levels of plasticity according to genotype (Bigard *et al.*, 2018) and environment/terroir, *e.g.* soil (Van Leeuwen & Seguin, 2006) and climatic factors (Jackson & Lombard, 1993), such as water availability (Chaves *et al.*, 2010), temperature (Bergqvist *et al.*, 2001; Tarara *et al.*, 2008) and solar irradiance (Spayd *et al.*, 2002; Reshef *et al.*, 2017). In the context of predicted global warming many questions arise regarding the reaction of wine composition to potentially warmer and drier conditions, most of which indicate towards rapid sugar accumulation and shorter harvest windows (Petrie & Sadras, 2008; Webb *et al.*, 2012). This will place more pressure on producers regarding timing of harvest, as margin for error will decrease as harvest windows shorten.

Previous works have shown that changes in wine composition during grape ripening can infer changes in wine sensory properties (Casassa *et al.*, 2013; Bindon *et al.*, 2014; Sherman *et al.*, 2017), enabling the producer to manipulate wine style according to market needs by choosing the correct ripeness level (Hunter *et al.*, 2004). In the view of a range of optimums, each relating to a defined style, the producer will likely be able to navigate the challenges of shortened harvest windows by adapting to both climate and consumer preferences. However, this strategy necessitates a profound knowledge of the dynamics of ripening and potential effects on wine composition. Importantly, this information needs to be put into context of terroir/environment, as especially these factors often dictate grapevine reaction, beyond the intent of the viticulturist (Hunter *et al.*, 2004, 2010; Koundouras *et al.*, 2006).

In red grape varieties, qualitative and quantitative changes in wine phenolic content according to ripeness level have received a great deal of attention (Canals *et al.*, 2005; Del Llaudy *et al.*, 2008; Gil *et al.*, 2012), primarily due to the significant impact of phenolic compounds on wine sensory properties, such as astringency and bitterness (Arnold *et al.*, 1980; Gawel, 1998; Vidal *et al.*, 2004). In general, tannin contribution from grape skins increases during ripening, along with increasing extractability of colour (anthocyanins) and increased mean degree of polymerisation (mDP) of proanthocyanidins (Gil *et al.*, 2012). Wines from lower ripeness levels displayed lower proportions of skin proanthocyanidins and higher proportions of galloylated proanthocyanidins (high astringency) from seeds (Vidal *et al.*, 2004; Del Llaudy *et al.*, 2008). The decreasing seed proanthocyanidin contribution with ripening is thought to be a result of proanthocyanidins

becoming progressively bound to cell wall components during ripening as a result of an oxidation process (Kennedy *et al.*, 2000). Thus, grape skin tannin content has demonstrated a strong relationship to wine tannin and to a lesser extent to seed tannin (Ristic *et al.*, 2010). Commercial qualitative ratings have generally linked higher tannin concentrations, together with a higher proportion of skin tannin, to increased quality (Marais *et al.*, 2001; Holt *et al.*, 2010; Ristic *et al.*, 2010). Importantly, differences in the extractability of tannin along ripeness levels have been separated from that expected to be due to higher ethanol levels and greater solubility of phenolic compounds in the wine matrix. For instance, Casassa *et al.* (2013) found no significant increase in phenolic content after chaptalisation (+ 2.7% v/v alcohol). In addition, Canals *et al.* (2005) reported an increase in extractability due to increases in ethanol, but argued it subordinate to that induced by increased grape ripeness level; anthocyanin extraction was also facilitated, but at the cost of decreased co-pigmentation leading to overall wine colour loss. Increases in grape colour due to anthocyanin accumulation have been correlated with enhanced wine colour and ageing capacity (Pérez-Magariño & González-San José, 2004). Anthocyanins have the capacity to bind to tannin during vinification (Boulton, 2001) and increased tannin extraction into the wine matrix (Kilmister *et al.*, 2014; Bindon *et al.*, 2017). Changes in this class of phenolics therefore have significant implications for final wine tannin content, wine astringency, and colour stability as bisulphite-resistant pigments (Boulton, 2001; Vidal *et al.*, 2002; Kennedy, 2008).

In terms of aromatic qualities, cv. Pinotage presents an interesting case study due to its rapid ripening and sugar accumulation (Marais, 2003), as this is the predicted change for many varieties due to climate change (Webb *et al.*, 2012; Bigard *et al.*, 2018). One of the major sensory qualitative drivers in red wines is the presence or absence of vegetative/green characters (Dubois, 1994). Vegetative characteristics in red wines have been related to the presence of isobutyl methoxypyrazine (IBMP), which denotes a green pepper/asparagus attribute to wines (Allen *et al.*, 1995; Roujou de Boubee *et al.*, 2000; Ryona *et al.*, 2010). The C6 alcohols and their derivatives have also been implicated in this sensory attribute as herbaceous/green (Dubois, 1994; Escudero *et al.*, 2007; Kalua & Boss, 2010). Green aromas linked to IBMP have shown strong negative relationship to increased grape ripening, which has enabled producers to manipulate IBMP by harvest date (Lacey *et al.*, 1991; Allen *et al.*, 1995; Roujou de Boubee *et al.*, 2000). Although IBMP has been qualitatively identified in Pinotage (Weldegergis *et al.*, 2011), its contribution to sensory perception of this varietal flavour has not been investigated. Grape C6 derivatives can exist as acetate esters, aldehydes or alcohols, C6-alcohols predominating during the later stages of grape ripening (Kalua & Boss, 2009, 2010). However, there is the potential for *de novo* generation of additional C6 derivatives from fatty acid

precursors *via* the lipoxygenase pathway during grape crushing and fermentation (Iyer *et al.*, 2010). The conversion of C6 derivatives to acetates by yeast activity may alter the expected herbaceous profile, in particular since hexyl-acetate may be associated with fruity attributes and 3MHO with tropical attributes (Dennis *et al.*, 2012; Harsch *et al.*, 2013; Boss *et al.*, 2015). Impact odourants also include positive contributors, such the norisoprenoid  $\beta$ -damascenone (Escudero *et al.*, 2004; Pineau *et al.*, 2007), a grape derived product from carotenoid breakdown (Razungles *et al.*, 1993).  $\beta$ -damascenone is thought to contribute significantly to the berry/floral/tobacco aroma of red wines, and was also isolated in Pinotage (Waldner & Marais, 2002; Weldegergis *et al.*, 2011). It was postulated that it generally increases during ripening and is possibly connected to the typical plum aroma of Pinotage (Waldner & Marais, 2002; Marais & Jolly, 2004). Using omission/addition trials, Escudero *et al.* (2004) regarded  $\beta$ -damascenone as having a stand-alone sensory impact, but conceded that large changes in concentration are necessary.

While impact odourants are important in conferring wine sensory attributes, many synergistic interactions between wine volatiles can lead to enhancement or dampening effects on aroma/flavour attributes (Escudero *et al.*, 2004, 2007). For example, in terms of wine fruitiness, yeast-derived esters that are largely responsible for this attribute can either mask vegetative odours or ester aroma may be enhanced by the presence of norisoprenoids (Escudero *et al.*, 2007). In terms of assessing the effects of grape ripening on wine volatiles, it is therefore also important to recognise the significant role of yeast products in the collective aroma composition. For instance, fermentation-derived esters (*e.g.* hexyl acetate and ethyl octanoate) have been shown as important discriminators of varietal aroma in Pinotage (Marais *et al.*, 1981; Louw *et al.*, 2010), while specifically isoamyl acetate (banana flavour) has been identified (Van Wyk *et al.*, 1979; Joubert, 1980). However, the reaction of fermentation-derived aroma in relation to grape ripeness level remains complex (Boss *et al.*, 2018). Ripeness level has been shown to be related to must nitrogen status ( $\beta$ -alanine) and thereby influence the production of fermentation derived aromas (Boss *et al.*, 2015), yet much remains to be elucidated.

The current study provides a holistic overview of some key components of the important local variety Pinotage, tracking wine composition over a range of commercial grape ripeness levels. Changes in wine composition as a result of ripeness-related changes in winemaking substrate (grape) are well presented *via* the study of two sites over three vintages. Commercial grape ripeness levels (and beyond) were chosen as to provide relevant information regarding practical and alternate harvest decisions and the extent of modification of wine composition. Preceding investigations have sought to acknowledge the fundamental impact of *terroir* (Chapter 3) and investigate the multi-layered effect of ripening on grape composition (Chapter 4).

## 5.3 MATERIALS AND METHODS

### 5.3.1 Experimental Vineyard

The experiment was carried out during three consecutive seasons (2015, 2016 and 2017) in a unirrigated *Vitis vinifera* L. cv. Pinotage vineyard, grafted onto two known drought tolerant rootstocks (*Vitis Berlandieri* x *Vitis rupestris*), namely Pinotage (clone PI 50A)/140 Ruggeri (clone RU 354B) planted in 1997 and Pinotage (clone PI 48A)/1103 Paulsen (clone PS 28A) planted in 1999. The vineyard is located at Stellenbosch University Welgevallen Experimental Farm in Stellenbosch, Western Cape, South Africa. The area is under the influence of a Mediterranean climate, characterised by winter rainfall and warm and dry summers. Vines are orientated in a North–South direction and the vineyard locality (approx. 200 m altitude) is characterised by a slope (3°) with a north western aspect. The vines are spaced 2.75 m x 1 m (3636 vines/ha) and the soil classified as Oakleaf soil form originating from weathered granite (Soil Classification Working Group, 1991). Vines are trained to a uni-lateral cordon and pruned to five, two-bud spurs per plant. Foliage was managed by a vertical shoot positioned trellis system with three sets of movable foliage wires. Standard canopy management practices were applied during the growth season, including early suckering (the judicious removal of shoots not allocated during pruning), vertical positioning of shoots, and topping once shoots passed 30 cm of the top foliage wire. An annual cover crop of *Triticale spp.* was sown in autumn and controlled with herbicide before budburst, to ensure a mulch layer within the work row.

### 5.3.2 Experimental layout

Vineyards grafted onto the two rootstocks were located directly adjacent to one another. Considering the variables [clone (A-PI 50 vs B-PI 48), age (A-1997 vs B-1999) and slope (A-Top vs B-Bottom)] of the experimental vineyard, rootstock was not considered a treatment as such, but rather the combination of rootstock and site, therefore resulting in two measurement sites: Site A (Pinotage/140 Ruggeri) and Site B (Pinotage/1103 Paulsen). Experimental plots were assigned by a randomised block design, selecting vine parcels from an area within the vineyard which displayed homogeneous vigour. Experimental plots consisted of 30 vines each and were replicated five times per ripeness level for each site.

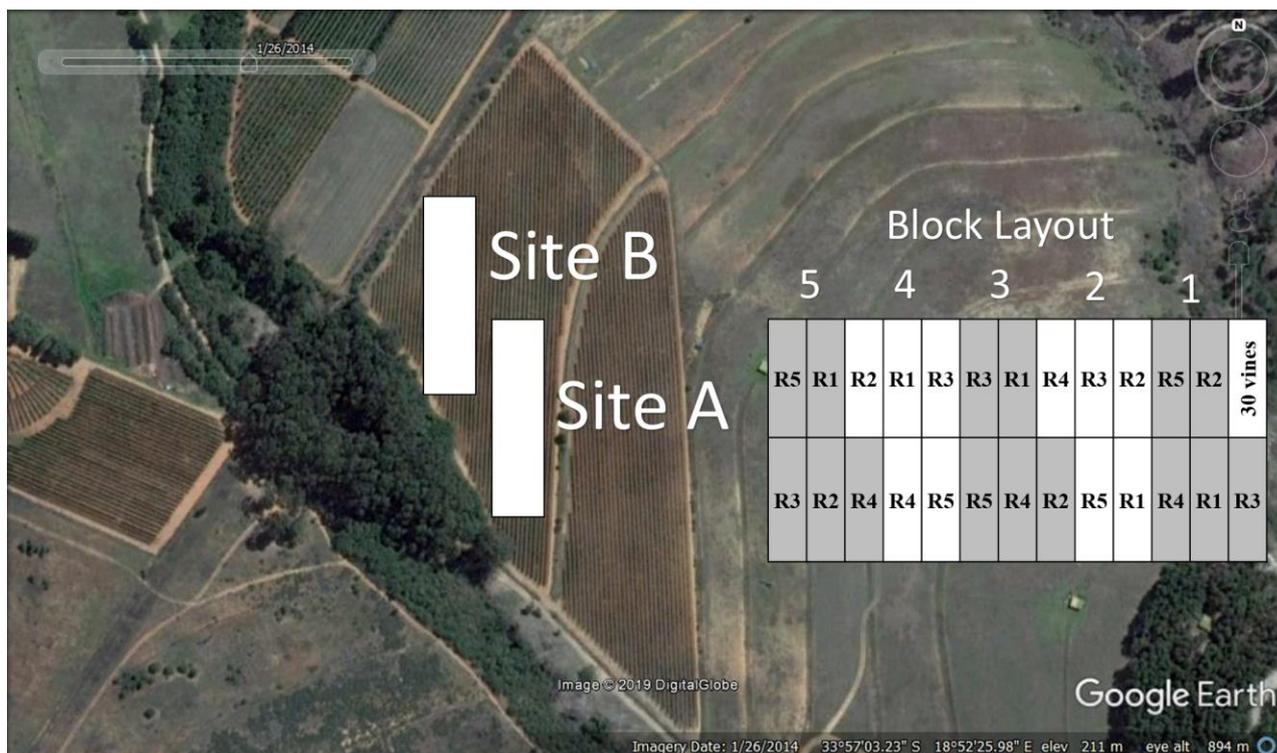


Figure 1. Experimental layout at Stellenbosch University Welgevallen experimental farm, indicating both measurement sites (A & B) and block design with five repetitions (blocks 1 -5) and five ripeness levels (R1-R5).

### 5.3.3 Experimental winemaking

Grapes were harvested at five targeted ripeness levels coinciding with *ca.* 21°Brix, 23°Brix, 25°Brix, 27°Brix and 29°Brix, respectively. Grapes were picked between 06:00 and 09:00am in 40 kg batches per field replicate, transported to the winery (6 km) and stored over-night in a cold room (10°C) before processing. Grapes were then de-stemmed and crushed, with an addition of 40 ppm SO<sub>2</sub> and immediately inoculated with a commercial yeast strain (25g/hL, VIN 13, Anchor, South Africa). Fermentation temperature was maintained at *ca.* 25°C, with a standardised addition of yeast nutrients (80 g/hL Di-ammonium phosphate) 24 hours after inoculation. Skins were punched down manually three times a day for the first five days of fermentation. At dryness, wine was strained and pomace lightly pressed (pressure 1 Bar). Wines underwent cold stabilisation (0°C for 3 weeks), fining with bentonite (50 g/hL) and sterile filtration before bottling closure (free SO<sub>2</sub> adjusted 30 – 40 ppm), during May, under screw cap. Wines did not undergo malo-lactic fermentation, due to high risk of spoilage in small scale wines, especially of increased ripeness levels with extremely high alcohol content, which is known to hinder malo-lactic fermentation.

### 5.3.4 Measurements and Analyses

#### 5.3.4.1 Routine wine analysis

Experimental wines were analysed for: % v/v alcohol (pycnometry), pH, titratable acidity (auto-titration), volatile acidity (FTIR), malic acid (Enzytec I Biopharm), residual sugar (Rebelein method), sugar-free dry extract (pycnometry) and Total and Free SO<sub>2</sub> (aspiration) by an accredited (ISO17025) commercial wine laboratory (Vinlab, Stellenbosch, South Africa) 6 months after bottling.

#### 5.3.4.2 Total Phenolic and Colour Index

Total phenolic index of wine samples were measured (100 x dilution with distilled H<sub>2</sub>O) at absorbance 280 nm (Cary 60, Agilent, USA) (Ribéreau-Gayon *et al.*, 2006a). Total anthocyanin index was measured at absorbance 540 nm, after 50 x dilution with 30:70 HCl:EtOH (v/v) (Somers, 1968).

#### 5.3.4.3 Anthocyanin Profile

Anthocyanin profiles were analysed by high performance liquid chromatography (HPLC) according to a method described by Guidoni & Hunter (2012), based on that of Di Stefano & Maggiorotto (1995). Centrifuged (10 000 rpm, 5 min) wine samples were purified by passing 0.5 ml of wine through a C18 Sep-Pack cartridge (Waters, Milford, USA), followed by elution with 5 ml methanol (99% Merck, SA). Elutes were concentrated to dryness by rotary evaporation at 30°C (IKA, Germany), before being re-dissolved in 1 ml of formic acid/methanol/water (10:50:40, v/v/v). Anthocyanin profiles were analysed by HPLC (Hewlett Packard 1100), equipped with automatic degasser (HP 1200), quaternary pump, auto sampler, UV detector and a LiChroCart 250-4 Purospher RP-18 column (Merck, Darmstadt, Germany). 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 the retention time of each chromatographic peak with available data in literature (Guidoni & Hunter, 2012). Individual anthocyanins, expressed in mg/L of wine, were all quantified at 520 nm using malvidin 3-O-glucoside chloride (Extrasynthèse, Genay, France) as external standard. Total anthocyanin concentration was calculated as the sum of concentrations of mono-glucoside, acetyl and p-coumaryl derivatives, and group concentrations were obtained by adding individual anthocyanin concentrations belonging to each group.

#### 5.3.4.4 Wine Volatiles

Liquid-liquid extraction was used for the analysis of esters and alcohols as described by Louw *et al.* (2010). Ten millilitres of experimental wine was transferred into a test tube with sealable cap, and 50  $\mu\text{L}$  of Anisole-d8 (100  $\mu\text{g}/\text{L}$ ) was added as internal standard. Volatile compounds were extracted by adding 1 mL diethyl ether (99% Sigma Aldrich, Germany) to the sample, followed by mixing (Vortex) for 30 seconds before incubation for 10 minutes in a dark room (room temperature). After incubation, the sample was centrifuged for 5 minutes at 3000 rpm. The upper diethyl ether layer was transferred into a vial containing sodium sulphate to remove any excess  $\text{H}_2\text{O}$  from the extracted liquid, after which 200  $\mu\text{l}$  of the extracted diethyl ether layer containing the volatiles was transferred into a vial (with glass insert) and tightly capped. Gas chromatographic analyses of samples were completed within 24h of extraction.

Headspace Solid Phase Micro extraction (HS-SPME) was used for the quantification of low concentration volatiles such as methoxypyrazines, monoterpenes and norisoprenoids as described by Panighel & Flamini (2014). Eight millilitres of experimental wine and 2 mL of model wine (12% Alc v/v, 5g/L tartaric acid, pH 3.50 adjusted by 1NaOH) were transferred into a 20 mL headspace vial. This was followed by the addition of 50  $\mu\text{l}$  of internal standard (100 $\mu\text{g}/\text{L}$  Anisole d8) and 2.5 mL of 20% NaCl solution and vigorous mixing (Vortex) for 30 seconds, before adsorption (extraction) and analyses. The samples were allowed to equilibrate for 5 min in the gas chromatograph autosampler incubator maintained at 50°C. After the equilibration time, a 50/30  $\mu\text{m}$  divinylbenzene/carboxen/polydimethylsiloxane coated fiber (grey) was exposed to the headspace for 15 min. The adsorbed volatiles were then desorbed into a gas chromatograph injector (10 min). Between samples the fibre was held in the conditioning chamber of the gas chromatograph for 10 min at 260 °C to ensure complete desorption/cleaning and avoid cross-contamination.

#### GC –Separation and Mass detection

Analyses of volatiles were performed using a gas chromatograph (Thermo Scientific trace 1300, Anatech Instruments), coupled to a mass spectrometer (Thermo Scientific TSQ 8000 Triple Quadruple Mass, Anatech Instruments) as detailed by (De Vries *et al.*, 2016). After exposure, the fiber was injected and left for ten minutes in order to allow desorption of volatiles. The injector was operated in splitless mode. The MS-detector was set for acquisition in single reaction monitoring (SRM) mode. Chromatographic separation of the volatiles was performed on a polar Zebron ZB-FFAP (30 m, 0.25 mm ID, 0.25  $\mu\text{m}$  film thickness) capillary column. The

chromatographic program was set at 35°C for 6 min, raised to 60°C at 4°C/min for 5 min, then raised to 150°C at 8°C/min for 5 min and finally raised to 240°C at 20°C/min, and held for 2 min. The injector and transfer line temperatures were both maintained at 250°C. Helium at 1 mL/min flow rate was used as carrier gas. The ionization source 171 temperature was set at 250°C and emission current of 50  $\mu$ A was used with argon collision.

A standard calibration curve for each detected compound was prepared by adding a series of known concentrations of pure chemical standard to 10 mL model wine solution (12% Alc v/v, 5g/L tartaric acid, pH 3.50 adjusted by 1NaOH) with 50  $\mu$ L of internal standard (100  $\mu$ g/L, Anisole d8) and 2.5 mL 20% NaCl solution. The same GC procedure was used as described above. Results were calculated using the Chemstation software package (Agilent Technologies, Santa Clara, CA, USA).

### 5.3.5 Statistical analyses

Analysis of variance (ANOVA) was performed using statistical software SAS version 9.2 (SAS Institute Inc., USA) and normality of data was evaluated using the Shapiro-Wilk test. To compare means, the Students' t-test for Least Significant Differences was calculated at the 5 % significance level. Results were also presented using multivariate analyses such as Principal Component Analysis (PCA), Discriminant Analysis (DA), Partial Least Squares (PLS), Multifactorial analysis (MFA) performed with XLSTAT version 2017 (Addinsoft SARL).

## 5.4 RESULTS AND DISCUSSION

### 5.4.1 Wine chemical composition

Intervals between ripeness levels differed significantly and increased as maturity progressed, from three days (R1 – R2), to four days (R2- R3) and seven days (R3-R4 and R4-R5). The R1-R5 range was completed within 21 days. Over the five ripeness levels ethanol concentration increased incrementally from 11.92 % (v/v) at R1 to 17.05% (v/v) at R5 (Table 1). Interestingly, all the wines (n=150) fermented to dryness (< 5 g/L RS) despite the elevated levels of ethanol at the last ripeness level, which often leads to incomplete fermentation due to yeast ethanol toxicity. Nonetheless, minor, yet significant increases in residual sugar were present in wines of increasing ripeness levels. As expected, wine pH increased and TA decreased from the first to the last ripeness level. As no acidification was made during winemaking, pH increases and TA decreases were most probably driven by a decrease in malic acid in the grape berries during ripening (Iland & Coombe, 1988) as well as increased precipitation of tartaric acid due to increases in grape potassium content (Mpelasoka *et al.*, 2003). Moreover, wine pH was significantly higher compared to that measured during must analysis prior to fermentation (data

not shown), highlighting the substantial effect of skin maceration on this parameter. During skin maceration additional potassium and calcium may be leached from grape berry solids and precipitate tartaric acid, despite the relatively short maceration time (5 days). Volatile acidity increased with increased ripeness levels, but remained under the perception threshold of 0.7 g/L (Ribéreau-Gayon *et al.*, 2006b). Sugar free dry extract (g/L) increased with increasing ripeness level, surely driven by higher skin and seed constituent extractability (Canals *et al.*, 2005). All the variables in Table 1 were significant ( $p < 0.001$ ) with regard to ripeness level in all three seasons (2015-17) and for both sites (A and B). However, mean values of variables displayed significant interactions (Table 2) due to the significant impact of both vintage and site. Despite interactions, trends along ripeness levels were consistent with the expected response, yet in many cases subordinate to those of vintage and site. Ripeness level is however the only short term variable within the context of variables in this study (ripeness level x vintage x site) that can be controlled by the viticulturist in an established vineyard and is therefore the focus of the presentation of the data.

Table 1. Mean values for wine chemical parameters of cv. Pinotage (Sites A & B and Vintages 2015-2017) harvested at five ripeness levels (R1-R5).

Parameter	Ripeness Levels				
	R1	R2	R3	R4	R5
Harvest date (DAB)	133e	136d	140c	147b	154a
Alcohol (% v/v)	11.92e	13.62d	14.21c	15.35b	17.05a
Residual sugar (g/L)	1.28c	1.39bc	1.35c	1.69ab	2.00a
pH	3.41d	3.43cb	3.50bc	3.54b	3.72a
Titratable acidity (g/L)	6.69a	6.35b	5.76c	5.40d	5.13e
Malic acid (g/L)	2.66a	2.04b	1.62c	1.35cd	1.27d
Volatile acidity (g/L)	0.29d	0.30d	0.33c	0.36b	0.43a
Sugar-free dry extract (g/L)	25.43d	26.70c	26.41c	28.71b	31.42a

<sup>1</sup>Means followed by the same letter within a row do not differ significantly ( $p \leq 0.05$ )

Table 2. ANOVA Results for wine chemical parameters presented in TABLE 1.

	DAB	Alc	RS	pH	TA	MA	VA	DE
Vintage	**	***	***	***	**	***	***	***
Site	**	***	**	**	***	<i>ns</i>	**	***
Ripeness level	***	***	***	***	***	***	***	***
RL x Vintage	**	***	***	***	***	**	***	<i>ns</i>
RL x Site	**	***	**	***	<i>ns</i>	*	**	<i>ns</i>
RL x Site x Vintage	**	***	**	***	***	*	**	**

*ns* non-significant ; \*  $p \leq 0.05$  ; \*\*  $p \leq 0.01$  ; \*\*\*  $p \leq 0.001$

Days after Bud Break (DAB), Alcohol % v/v (Alc), Residual Sugar (RS), Titratable Acidity (TA), Malic Acid (MA) Volatile Acidity (VA) and Sugar-free dry extract (DE).

#### 5.4.2 Phenolic content

Wine total phenolic index values were frequently highest in the latter two ripeness levels (R4 & R5) and lowest in the initial ripeness levels (R1-R2) (Tables 3 & 4). Although increases were prevalent during ripening, significant increases were not seen at every level. Higher phenolic contents in later ripeness levels are expected to be due to increased extraction from skins rather than seeds (Canals *et al.*, 2005; Del Llaudy *et al.*, 2008; Gil *et al.*, 2012). Increases in ethanol content along ripeness levels may have contributed to higher proanthocyanidin extractability, especially from seeds (Hernández-Jiménez *et al.*, 2012), but in general this effect is thought to be subordinate to increases in extractability from skins due to ripeness level increases (Canals *et al.*, 2005; Casassa *et al.*, 2013). Colorimetric analysis showed clear trends towards higher colour densities in the final ripeness levels. Interestingly, this is despite the fact that anthocyanin potential of whole berries in the final ripeness level was decreased (See Chapter 4, Table 5). The increased extractability due to increased ripeness level, coupled with the abundant presence of proanthocyanidins to potentially bind with anthocyanins to form more stable co-pigments, may have contributed to increased colorimetric values despite decreased grape potential (Boulton, 2001; Gil *et al.*, 2012). For both phenolic and colour indexes, vintage had a significant influence (Table 4). Here, 2016 displayed the lowest values. This was the warmest vintage, with highest maximum and minimum temperatures and highest water deficit during the month of ripening (see Chapter 3, Table 1). Excessive temperatures have been shown to be negative for phenolic accumulation (Spayd *et al.*, 2002; Tarara *et al.*, 2008) and specifically high minimum temperatures for anthocyanin accumulation (Kliewer & Torres, 1972; Mori *et al.*, 2005). As was prevalent in grape composition, Site A generally produced higher indexes than Site B throughout ripening, yet followed comparable trends during ripening.

Table 3 Mean values for wine phenolic parameters of cv. Pinotage (Sites A &amp; B) harvested at five ripeness levels (R1-R5) in Stellenbosch (2015-17).

Vintage	Site	Ripeness Levels				
		R1	R2	R3	R4	R5
<b>TPI (A280)</b>						
2015	Site A	37c	44ab	42b	45ab	48a
	Site B	31b	41a	39a	40a	44a
2016	Site A	34b	42b	42a	41a	45a
	Site B	38a	37b	36b	40ab	45a
2017	Site A	59a	59a	57a	64a	51a
	Site B	40b	42b	40b	51a	48a
<b>TAI (A540)</b>						
2015	Site A	366c	420b	566ab	644a	577ab
	Site B	307c	403b	427ab	447ab	497a
2016	Site A	241b	313ab	312ab	368a	378a
	Site B	204d	241cd	268bc	317ab	335a
2017	Site A	298c	350c	427b	521a	568a
	Site B	253c	335b	416a	440a	444a

<sup>1</sup>Means followed by the same letter within a row do not differ significantly ( $p \leq 0.05$ )

Table 4 ANOVA Results for wine phenolic parameters presented in TABLE 3.

	TPI (A <sub>280</sub> )	TAI (A <sub>540</sub> )
Vintage	***	***
Site	***	***
Ripeness Level	***	***
RL x Vintage	**	**
RL x Site	<i>ns</i>	<i>ns</i>
RL x Site x Vintage	<i>ns</i>	<i>ns</i>

*ns* non-significant ; \*  $p \leq 0.05$  ; \*\*  $p \leq 0.01$  ; \*\*\*  $p \leq 0.001$

### 5.4.3 Anthocyanin profile

Changes in the anthocyanin concentration of the finished Pinotage wines led to the identification of free glucosides of malvidin (Malv), peonidin (Peo), petunidin (Pet.) delphinidin (Delf) and cyanidin (Cya), and their respective acylated and coumarylated (except for Delf) derivatives (Fig 1). An additional caffeoyl derivative was quantified, but only for Malv. All of the aforementioned anthocyanins were also present in the Pinotage grape berries (Chapter 4).

Principal component analysis managed to explain 88.44 % of the variance in the anthocyanin data set (Fig 2). The strongest separation was seen between vintages on F1 (82.17 %), separating 2017 data from that of 2015 and 2016. The 2017 vintage displayed higher quantities of most of the individual anthocyanins. Axis F2 (6.27%), which is clearly sub-ordinate to F1, separated observations on ripeness level, with later ripeness levels primarily associating with higher MalvCum and less CyaCum. However, due to the significant effect of vintage and site, quantitative results are presented separately for both site and vintage (Figs 3 & 4).

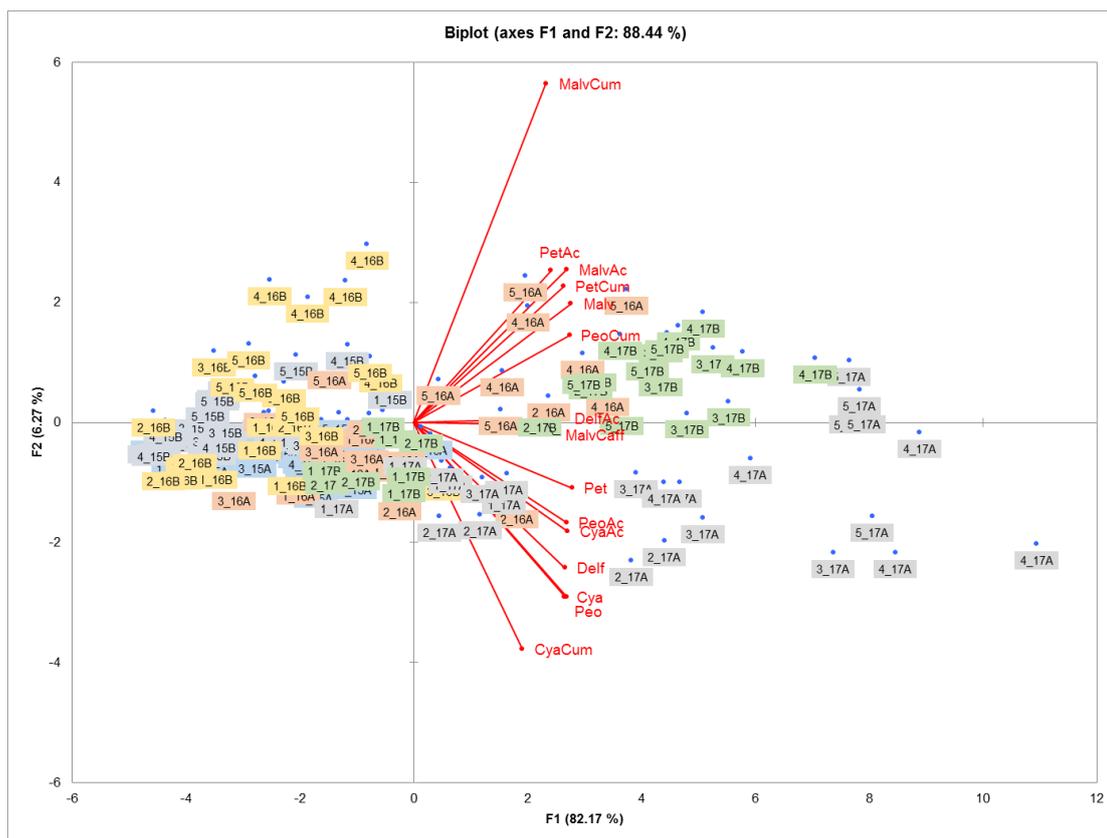


Figure 2. Principal component analysis of individual anthocyanin content of wines from 5 ripeness levels (1-5) and two sites (A & B) for cv. Pinotage in Stellenbosch (2015-17).

Total anthocyanin content in wines increased sharply from R1 to R2 (peak), slowly declined after that to R4, with a slight increase at R5 (Tables 5 & 6). In general total anthocyanin contents of wines were 30 – 40 % of the levels isolated in grapes (See Chapter 4, Table 8), Losses are generally considered to be due to adsorption to grape solids and polymerisation/precipitation to large phenolic compounds (Canals *et al.*, 2005; Guidoni & Hunter, 2012). Interestingly, the wines displaying the highest anthocyanin content did not coincide with ripeness levels of higher grape content. This could possibly be due to dissociation of anthocyanin–proanthocyanidin (copigmentation) bonds due to increased alcohol levels of wines of increased ripeness (Canals *et al.*, 2005). Proportions of free glucosides peaked early (R1) and remained relatively stable along R2 – R5. Total coumarylated anthocyanins increased throughout ripening. Coumarylated derivatives have been reported to peak after free and acylated derivatives (Fournand *et al.*, 2006). Regarding individual proportions, malvidin displayed steady increases at the cost of Delf, Cya, Pet and Peo. Despite significant mean proportional changes along ripeness levels, variables displayed significant interactions, with greater frequency in vintage than site.

Table 5. Mean proportional (%) changes in wine anthocyanins and their derivatives as affected by Ripeness Levels for cv. Pinotage (Site A &amp; B and Vintages 2015-17).

Ripeness Level	Tot A	T free	T Ac	T Cum	Delf	Cya	Pet	Peo	Malv
R1	365c	72.8a	21.5bc	5.4e	8.8a	3.5a	9.6a	9.1a	69.0d
R2	452a	70.9c	23.1a	5.7d	8.6a	3.5a	9.2b	8.5b	70.3c
R3	415b	72.2b	21.5bc	6.1c	7.5b	3.0b	8.9c	7.7c	72.9b
R4	347c	72.4ab	20.9c	6.4b	7.8b	3.0b	9.0bc	7.6c	72.6b
R5	409b	70.6c	21.8b	7.3a	6.8c	2.6c	8.3d	7.0d	75.2a

Total Anthocyanins mg/L (Tot A), Total free 3-O-glucosides (T free), Total Acylated glucosides (T Ac), Total Coumarylated glucosides, Delphinidin (Delf), Cyanidin (Cya), Petunidin (Pet), Peonidin (Peo) and Malvidin (Malv)

<sup>1</sup>Means followed by the same letter within a row do not differ significantly ( $p \leq 0.05$ )

Table 6 ANOVA results for proportional changes in wine anthocyanins presented in Table 5.

	Tot A	T free	T Ac	T Cum	Delf	Cya	Pet	Peo	Malv
Vintage	***	***	**	***	***	***	***	***	***
Site	<i>ns</i>	***	***	<i>ns</i>	***	<i>ns</i>	**	<i>ns</i>	<i>ns</i>
Ripeness Level	***	***	***	***	***	***	***	***	***
RL x Vintage	***	***	**	**	***	**	***	***	**
RL x Site	***	***	***	***	***	**	**	<i>ns</i>	**
RL x Site x Vintage	***	***	**	**	***	***	***	***	***

Total Anthocyanins mg/L (Tot A), Total free 3-O-glucosides (T free), Total Acylated glucosides (T Ac), Total Coumarylated glucosides, Delphinidin (Delf), Cyanidin (Cya), Petunidin (Pet), Peonidin (Peo) and Malvidin (Malv)

*ns* non-significant ; \*  $p \leq 0.05$  ; \*\*  $p \leq 0.01$  ; \*\*\*  $p \leq 0.001$

Individual anthocyanin concentrations provided insight regarding the drivers of differences between vintage and sites. Here, the trend of 2017 > 2016 > 2015 in terms of anthocyanin concentration was clear for most individual anthocyanins. The differences between 2015 and 2016 were less pronounced. The wines from 2016 often displayed greater amplitude during ripening, but in general concentrations of R1 and R5 were comparable to 2015. Considering the lower level of water deficit in 2015, coupled with lower solar irradiance (Chapter 3) in the bunch zone, the accumulation of anthocyanins may have been impacted negatively. Anthocyanins have been shown to represent greater concentration in due to increased sunlight exposure (Spayd *et al.*, 2002; Castellarin *et al.*, 2007a; Tarara *et al.*, 2008). On the contrary, low levels in 2016 may have been due to extremely high temperatures and excessive solar irradiance (Chapter 3), especially at the final ripeness levels due to canopy senescence. Excessively high temperatures are known to cause rapid degeneration of anthocyanins (Tarara *et al.*, 2008), which may have induced degradation as seen in 2016. Nonetheless, anthocyanin concentrations from 2017 produced strikingly higher values across most anthocyanin derivatives. Lower night time temperatures have been linked to higher anthocyanin concentrations (Kliewer & Torres, 1972; Mori *et al.*, 2005) and may be the reason for increased concentrations seen in 2017, as mean minimum temperatures during the month of ripening was 3°C less than those for 2015 and 2016 (see Chapter 3, Table 1). Comparable values regarding water deficit (Ojeda *et al.*, 2002), berry

size (Roby *et al.*, 2004) and extent of solar irradiance in the bunch zone (Santos *et al.*, 2003), ruled out possible other major drivers for anthocyanin content differences. Variation in concentrations of individual anthocyanins between sites was not as articulated in wines, as it were in extracts from grape berries, where site A displayed consistently higher concentrations than site B. However, wines presented differences in trends along ripening, especially for the articulated 2017 vintage. In 2017, concentrations increased from R1 to peak at R4 and stabilise and/or decrease towards R5. However, in site B the peak was generally attained at R3, after which stabilisation and subsequent decline towards later ripeness levels occurred. This peak was earlier than seen for grapes, implicating that anthocyanin concentrations in wines may also be related to the amount of proanthocyanidin available for co-pigmentation (Canals *et al.*, 2005).

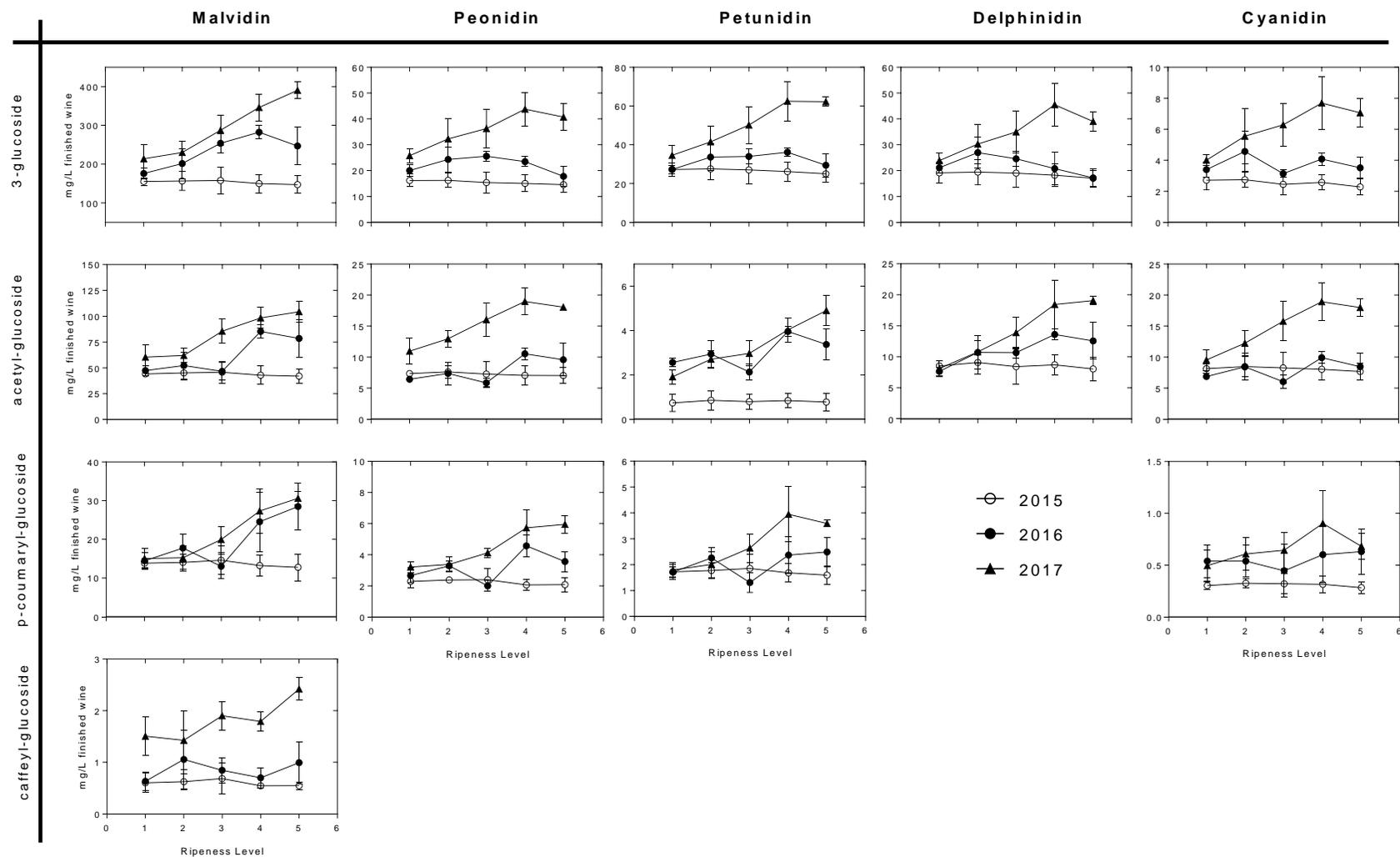


Figure 3. Concentration (mg/L) of individual anthocyanin glucosides per ripeness level for Site A, Pinotage Stellenbosch (2015-17). (standard deviation confidence intervals 95%).

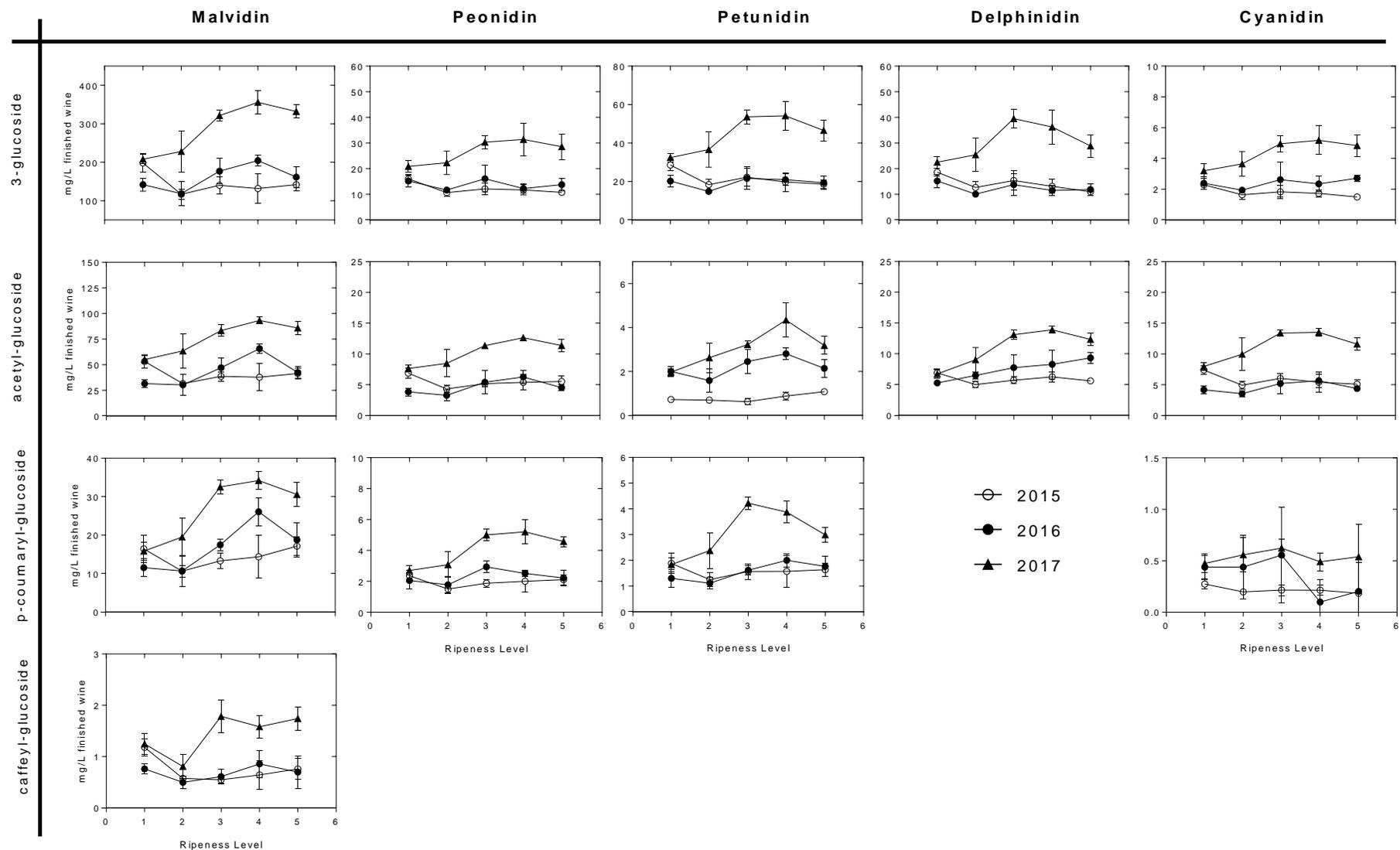


Figure 4. Concentration (mg/L) of individual anthocyanin glucosides per ripeness level for Site B, Pinotage Stellenbosch (2015-17) (standard deviation confidence intervals 95%).

In order to visualise holistic changes in the total anthocyanin profile according to ripeness level, Discriminant Analysis (DA) was performed, sites were combined per vintage, and vintages presented separately due to the significant effect thereof (Fig 2, Table 6). Observations from DA models explained 90.82% in 2015 (F1 80.00% and F2 10.82%), 93.05% in 2016 (F1 85.29% and F2 7.76%) and 92.13 % in 2017 (F1 81.04% and F2 11.09%) of the dataset (Fig 5). Here, there was a consistent shift from R1-R5 across F1 in all the vintages, with R1 & R2 often separated from R4 & R5 by F1 and R3 mostly on the border. In general the later ripeness levels displayed increased levels of most anthocyanins, with the exception of 2015 of which later ripeness levels were mostly associated with acylated and coumarylated derivatives. Despite the separation of early and later ripeness levels, most adjacent ripeness levels saw a degree of overlap, indicating that while shifts were apparent, individual ripeness levels as presented here, did not always serve as discriminating factor. This indicates that changes in anthocyanin profile did follow a ripeness level related trend, but was not well-demarcated by the chosen sugar levels. Moreover, the overlap could surely also point to grape berry heterogeneity, as is especially visible during véraison when berries seem to accumulate anthocyanins at individual rates (Ollat *et al.*, 2002). In addition, berry heterogeneity according to density was also shown to have a major impact on anthocyanin concentration and was therefore eliminated through density based sorting in studies evaluating anthocyanins during ripening (Fournand *et al.*, 2006; Kontoudakis *et al.*, 2011). However, measurement (unsorted) in this study is thought to better represent the practical situation. Lastly, F2 (< 11.09%) generally separated R1 and R5 from R2-R4, indicating that differences were not only relative to increases in quantities of anthocyanin due to increased ripeness level, but that the two extreme ripeness levels maintained unique proportions of anthocyanins compared to mid ripeness levels (R2-R4).

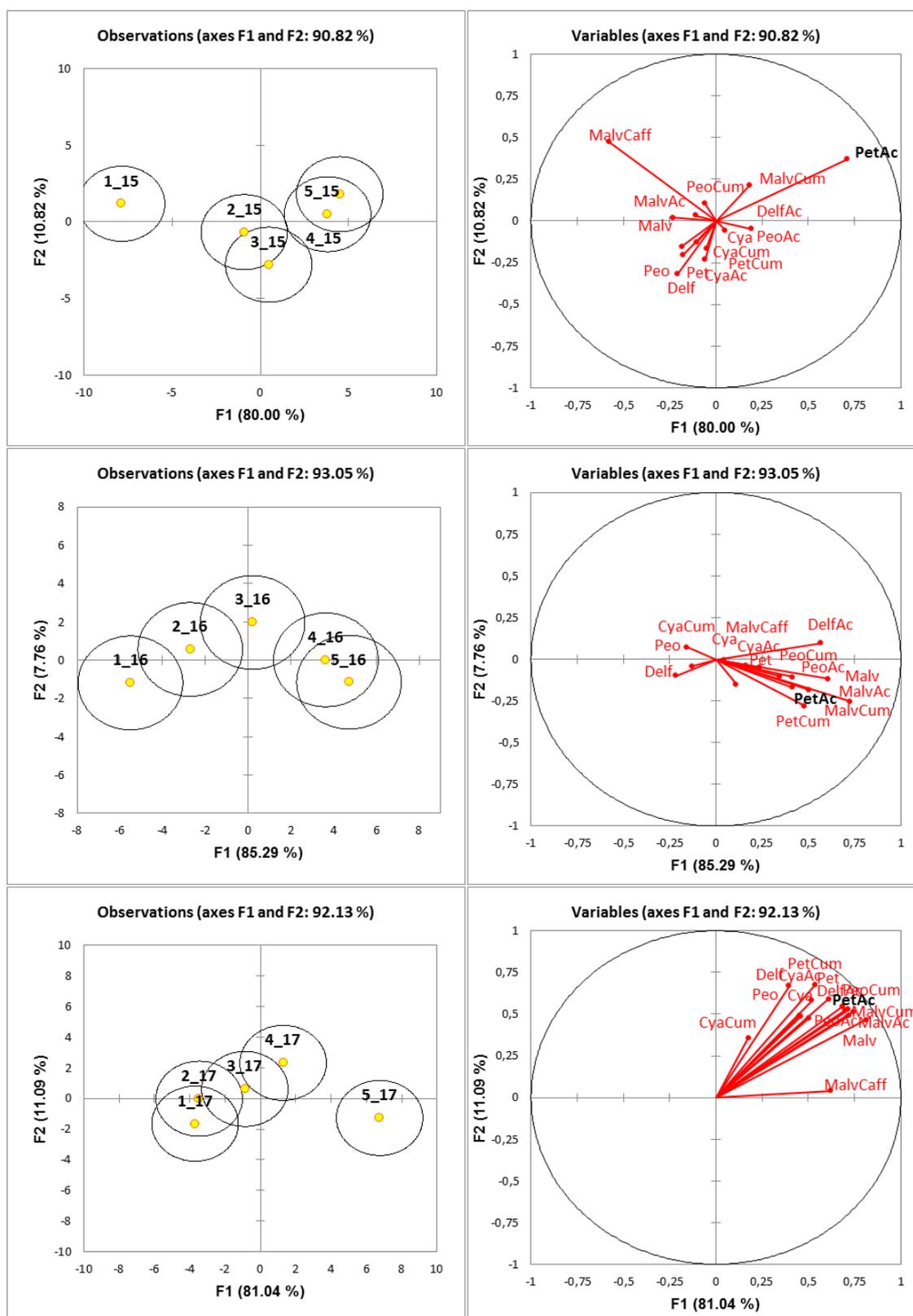


Figure 5. Discriminant analysis of wine anthocyanin content of five ripeness levels for vintages 2015 (top), 2016 (middle) and 2017 (bottom), with alpha ellipses (0.5). Key compounds (black) were confirmed (VIP > 1) with PLS modelling of ripeness level.

In an attempt to identify wine anthocyanin components that displayed consistent trends along the development of grape ripeness levels across all vintages, Partial least squares regression (PLS) was used to model anthocyanin profiles in relation to ripeness level. Here, only PetAc could be

identified across all vintages ( $VIP > 1$ ) as the variable with best predictive fit for ripeness level (Fig 5). This is encouraging as PetAc was identified along with MalvCum and MalvCaff as possible indicators to grape ripeness level. However, while MalvCum and MalvCaff both produced strong predictive fit, they were not as consistent across vintages compared to PetAc. The use of individual anthocyanins for wine qualitative/predictive assessment has been proposed for Pinotage (Marais *et al.*, 2001), but has largely focused on the most abundant form, malvidin-3-glucoside (Rossouw & Marais, 2004). The results of this study show that in addition to free glucosides, derivatives such as PetAc also provide good association with increased ripeness level, across sites and vintages. The predictive qualities of individual derivatives therefore warrant further investigation.

#### 5.4.4 Wine volatile composition

Wine volatile composition was characterised by the quantification of 38 volatile compounds (Tables 7 - 10), known to be present in Pinotage wines (Weldegergis *et al.*, 2011). The investigation included a range of compounds, including norisoprenoids, monoterpenes, methoxypyrazines, fatty acids, esters, higher alcohols and fatty acid derived C6-aldehydes and alcohols. Aroma descriptors and odour thresholds of these compounds are given in Table 11.

As in the case of anthocyanins, wine volatile composition was greatly influenced by the conditions brought about by vintage. Principal component analysis (PCA) of the volatile composition data set (Fig 6) explained 48.43 % of total variation (PC1 36.32 % and PC2 12.02 %). The different vintages were separated on the basis of wine volatile composition, with the wines from 2015 and 2016 separated from 2017 on PC1. Changes in ripeness level appeared to be displayed on the basis of separation on PC2. Importantly, separation of volatiles in terms of ripeness level was in the same direction for all vintages and therefore one could assume similar reactions in terms of ripeness level, despite the relative large influence of the vintage effect and diminished effect in terms of site. It would seem as if site effects were subordinate to those brought about by vintage and ripeness level.

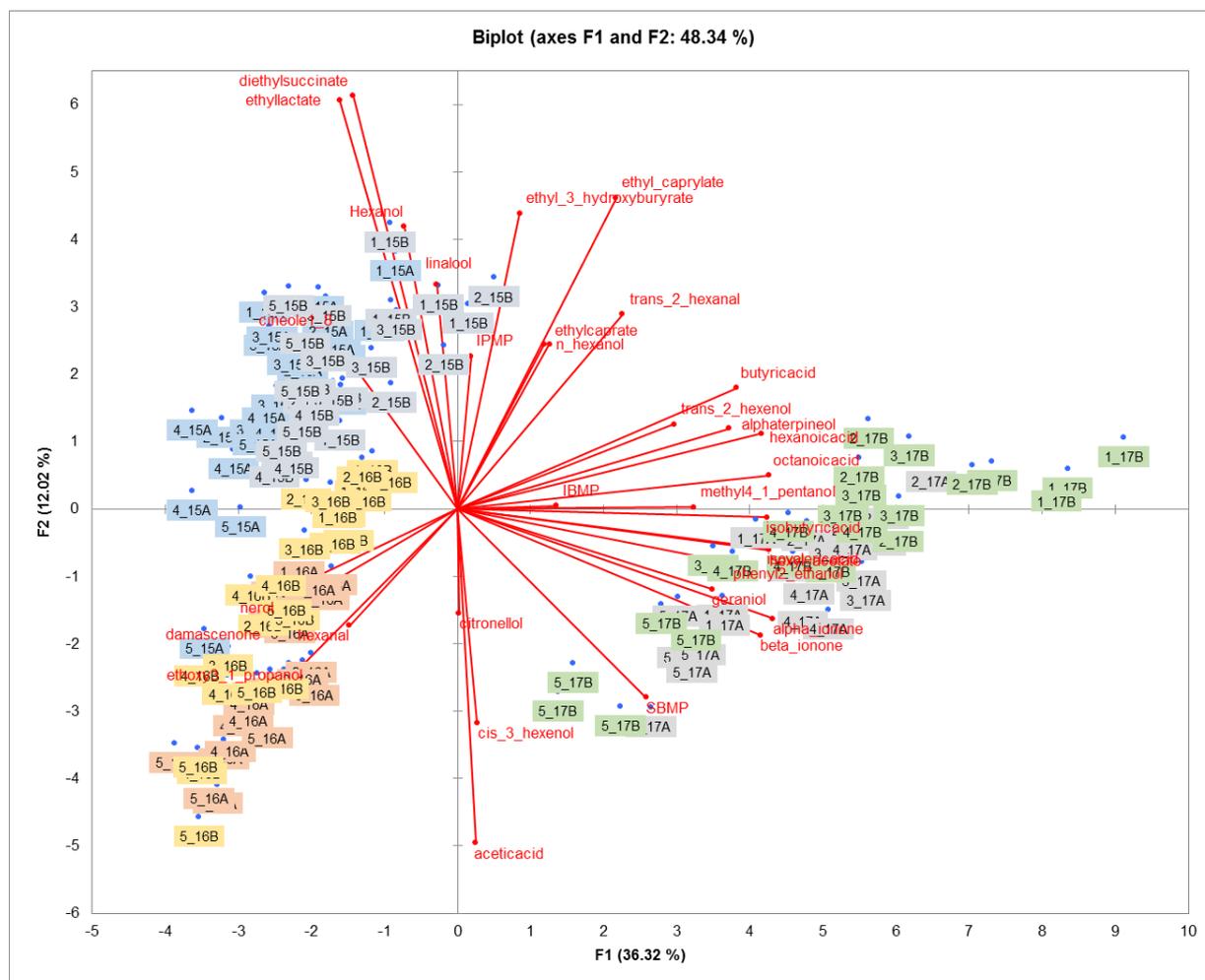


Figure 6. Principal component analysis of individual wine volatile components of wines from five ripeness levels (1-5) and two sites (A & B) for cv. Pinotage in Stellenbosch (2015-17).

The individual volatile components were initially assessed in groups (Tables 7 & 8), considering the large amount of individual compounds (Tables 9 & 10), as components from similar metabolic origins are thought to broadly share similar accumulation patterns and odour impact (Robinson *et al.*, 2014; Yuan & Qian, 2016).

Means of total norisoprenoids generally increased as ripening progressed, to achieve maximum levels at the final ripeness level. This is in accordance with previous findings regarding this group of volatiles in wines (Razungles *et al.*, 1993). The major contributor to the group was  $\beta$ -damascenone, which produced increasing trends along ripening in 2015 and 2016. However, in 2017 changes in concentrations were not as pronounced.  $\beta$ -damascenone is an important volatile in red wines, with the ability to associate in synergistic reactions with esters (Escudero *et al.*, 2007) or as impact odorant (Escudero *et al.*, 2004; Pineau *et al.*, 2007) and has been shown to increase during ripening in Cabernet Sauvignon (Bindon *et al.*, 2013). Trends regarding the other norisoprenoids  $\alpha$ -ionone and  $\beta$ -ionone were less pronounced, although they were present at adequate concentrations to impart floral-like aromas. A lesser known norisoprenoid, 6-methyl-5-

hepten-2-one (6MHO), that imparts a tomato-leaf character, was also found to increase during ripening (Robinson *et al.*, 2014).

Similarly, total monoterpenes also increased as ripening continued, showing a slight decrease at the final ripeness level. Monoterpenes denote floral aromas to red grapes and have been shown to increase due to ripening (Marais, 1983; Razungles *et al.*, 1993; Ribéreau-Gayon *et al.*, 2006a). In this study, citronellol, nerol and geraniol were the monoterpenes present in concentrations above odour thresholds. In the case of nerol and geraniol, dramatic increases were seen towards the final ripening stage. As monoterpenes are known to undergo re-arrangement in wine-like conditions (Hampel *et al.*, 2014), the reaction viewed here could be a combination of increased glycosidically bound fraction, increased release and/or preferential re-arrangement/oxidation of other monoterpenes along with an increase in ripeness level. Stable or lower concentrations of *e.g.* linalool might indicate increased oxidative metabolism of especially this monoterpene (Luan *et al.*, 2006). High temperatures, over-ripeness and excessive sunlight exposure of berries have equally imposed decreases in concentration of free linalool in wines (Bureau *et al.*, 2000a; Bureau *et al.*, 2000b).

Generally, methoxypyrazines (MP) are thought to decrease during ripening (Allen *et al.*, 1995; Roujou de Boubée *et al.*, 2000; Sala *et al.*, 2004). In this study, decreases were also noted during the measurement window. However, initial changes were not as pronounced. This is likely due to the fact that methoxypyrazine (MP) levels were already quite low at the beginning of the monitoring period. Pinotage is not known for any overt asparagus/green characters such as in the case of Cabernet Sauvignon (Allen *et al.*, 1995), the latter which may reach three times the values reported here for Pinotage under South African conditions (Lapalus, 2016). Marked influence of IBMP is only considered to start at 15 ng/L in red wines from the Bordeaux region (Roujou de Boubée *et al.*, 2000).

A major part of wine volatile components are significantly influenced by the winemaking process and are primarily yeast metabolites (Ribéreau-Gayon *et al.*, 2006b; Boss *et al.*, 2018). Fatty acids are released by yeasts throughout fermentation and decreased in wines of advanced ripeness levels, while acetic acid increased with increasing ripeness level. The dominant fatty acids in wines of early ripeness were hexanoic (green apple) and octanoic (fresh) acids, while propionic (sweaty), butyric (buttery) and isovaleric (cheese) acids were more prevalent in wines of later ripeness levels. Acetic acid is a major contributor to the distinctive flavour of wine (Bakker & Clarke, 2012) and generally contributes positive nuances to wines if below a threshold of 700 µg/L.

Yeast-derived esters peaked at R2 and R3, with the lowest level at the last ripeness levels. Ester production is highly subject to the availability of nutrients and fermentation conditions

(Ribéreau-Gayon *et al.*, 2006b), yet recent works have attempted to show the links between changes in grape composition and subsequent changes in fermentation-derived volatiles (Boss *et al.*, 2015). Results proposed by Boss *et al.* (2018) revealed the complex nature of the interactions between grape composition and resulting yeast metabolism. While the extent and drivers of effects remain largely unclear there is an undoubted impact of the state of ripening of the grape substrate on yeast-derived metabolites. In Pinotage, the ester isoamyl acetate has been related to a distinct banana aroma of young wines (Van Wyk *et al.*, 1979; Joubert, 1980). This was postulated to be due to the relatively high yeast assimilable nitrogen (YAN) content of Pinotage compared to other varieties cultivated under the same conditions (Joubert, 1980). Unfortunately, the must YAN was not determined in this study, but grape berry mineral content showed increases in nitrogen as ripening progressed. However, in a study over a similar range of ripeness levels in Cabernet Sauvignon, Bindon *et al.* (2013) noted a strong decrease in YAN along the ripeness level gradient. Nonetheless, in the current study the isoamyl acetate was detected well above perception thresholds and tended to increase towards the latter stages of ripening. Contrary to this, ethyl esters of hexanoic and octanoic fatty acids, ethyl hexanoate and ethyl octanoate were highest during the initial ripeness levels. These ethyl esters are known to impart green apple/fresh/fruity nuances to wines, and are undoubtedly linked to the relatively high quantities of hexanoic and octanoic fatty acids in wines from the early ripeness levels. Both these esters have been reported as discriminative for Pinotage wines, compared to Shiraz and Cabernet Sauvignon (Marais *et al.*, 1981; Louw *et al.*, 2010).

Higher alcohols peaked at the last ripeness level, with non-significant changes in the ripeness levels preceding R5. However, the group total was largely made up of isoamyl alcohol (3-methyl-1-butanol) with characteristic whiskey/malt like aroma, together with isobutanol imparting a solvent/chemical like aroma. Furthermore, n-butanol (fresh/grassy) and 3-ethoxy-1-propanol (ripe fruit) were the higher alcohols that displayed significant ripeness level effects. Although they were increasing to a maximum at the final ripeness level, they were present at concentrations well below the proposed threshold values (Ferreira *et al.*, 2001).

The group of C6-compounds, also called the 'leafy alcohols and aldehydes' did not display a significant trend along ripening, with lowest values present at R3. However, similar to Cabernet Sauvignon (Bindon *et al.*, 2013), decreases in hexanol were characteristic of increased ripeness levels. Hexanol can impart grassy/green flavours, along with cis-3-hexanol and trans-2-hexanol. The aldehyde trans-2-hexenal was also present in quantities above odour threshold, but displayed limited articulation during ripening. Trans-2-hexenal can readily be reduced to hexanol during fermentation (Iyer *et al.*, 2010).

Table 7. Means of volatile group totals during ripening of cv. Pinotage (2015-2017).

Vintage	Ripeness Levels				
	R1	R2	R3	R4	R5
Norisoprenoids	11.74b	13.29b	13.92b	18.36a	17.81a
Monoterpenes	91.22bc	71.66c	100.25ab	111.63a	103.27ab
Methoxypyrazines	26.98a	24.79ab	19.18bc	23.61abc	18.66c
Fatty Acids	8.41a	7.84ab	7.45b	5.88c	5.41c
Acetic Acid	270d	322c	332c	378b	486a
Esters	15.73b	18.62a	17.94a	15.70b	13.48c
Higher Alcohols	202b	201b	217a	218a	225a
C6-aldehyde and alcohols	8.92a	8.45a	6.64b	7.69ab	8.91a

<sup>1</sup>Means followed by the same letter within a row do not differ significantly ( $p \leq 0.05$ )

Table 8. ANOVA results for mean wine volatile groups presented in TABLE 7.

	Iso-prenoids	Mono-terpenes	Methoxypyrazines	Fatty acids	Acetic acid	Esters	Higher alcohols	C6
Vintage	***	**	***	***	***	***	***	***
Site	***	<i>ns</i>	***	***	***	**	***	**
Ripeness Level	***	**	**	***	***	***	**	<i>ns</i>
RL x Vintage	*	***	**	***	***	***	**	*
RL x Site	*	**	**	***	**	**	**	<i>ns</i>
RL x Site x Vintage	<i>ns</i>	**	**	***	**	**	**	**

*ns* non-significant ; \*  $p \leq 0.05$  ; \*\*  $p \leq 0.01$  ; \*\*\*  $p \leq 0.001$

Table 9. Concentration of volatile compounds (mg/L) in cv. Pinotage wines from 5 ripeness levels in Site A, Stellenbosch (2015-2017).

	OT	2015					2016					2017				
		R1	R2	R3	R4	R5	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5
<i>Norisoprenoids</i> †																
β-damascenone	2-7 <sup>1</sup>	7.12c	8.35bc	6.92c	12.03a	11.01ab	6.27c	7.48bc	12.00ab	13.27a	9.09abc	2.91b	4.44a	4.81a	4.58a	4.46a
α-ionone	0.60 <sup>2</sup>	0.20ab	0.20a	0.18b	0.19ab	0.20ab	0.20b	0.20ab	0.23a	0.23ab	0.21ab	2.34a	2.38a	2.37a	2.38a	2.31a
β-ionone	0.09 <sup>2</sup>	0.36a	0.29ab	0.16dc	0.09d	0.25bc	0.25b	0.28b	0.77a	0.77a	0.98a	2.27b	2.16bc	2.05c	2.50a	2.49a
6-M-H-O	50 <sup>2</sup>	0.57b	0.83ab	0.63ab	0.99ab	1.45a	0.71b	0.66b	2.91a	3.00a	2.32a	-	-	-	-	-
<i>Monoterpenes</i> †																
α - terpineol	330 <sup>2</sup>	5.35a	4.39b	3.43c	2.95c	3.72bc	2.08b	2.43ab	3.12a	3.03a	3.11a	7.10b	8.81ab	12.09a	10.75ab	8.18ab
1-8-cineole	400 <sup>2</sup>	13.0b	13.9b	13.7b	22.3a	9.6b	9.1a	3.5a	6.1a	8.6a	9.8a	3.5bc	8.7a	5.0ab	3.4bc	0.2c
Linalool	6 <sup>2</sup>	8.92a	6.37a	9.70a	7.83a	8.22a	6.01a	5.63ab	3.58b	4.12ab	1.39c	5.95ab	4.42b	7.09ab	8.16a	6.95ab
citronellol	30 <sup>2</sup>	8.70b	9.30b	8.67b	13.52a	15.67a	5.33c	7.67bc	10.95a	10.46ab	10.98a	7.27b	14.04a	13.03a	9.95ab	12.24a
Geraniol	40 <sup>2</sup>	8.54a	9.83a	9.06a	9.10a	11.65a	8.77b	14.23a	17.17a	16.79a	13.83a	111.3b	140.7ab	177.33a	137.5ab	56.06c
Nerol	30 <sup>2</sup>	8.80c	20.37bc	39.40ab	62.30ab	74.97a	13.02c	23.65c	57.75b	52.86b	89.24a	66.15a	21.14ab	32.6ab	46.36ab	17.68b
<i>Methoxy-pyrazines</i>																
IPMP	2 <sup>3</sup>	6.36a	4.40b	4.59b	3.47b	3.23b	3.87ab	4.05ab	5.21ab	5.90a	3.38b	4.44ab	4.14ab	5.66ab	10.93a	2.64ab
SBMP	2 <sup>3</sup>	2.24a	0.93a	1.03a	0.56a	0.46a	3.45ab	5.02a	1.33ab	8.18ab	6.79b	10.00a	5.92b	6.41b	8.43a	8.27b
IBMP	2 <sup>3</sup>	2.72a	4.32a	3.69a	2.30a	2.74a	0.60c	1.40bc	5.52a	2.85b	1.58bc	6.06a	5.92a	6.68a	7.20a	2.38b
<i>Fatty Acids</i>																
acetic acid	200 <sup>4</sup>	293c	350abc	309bc	370ab	398a	226c	283c	409ab	396b	460a	343c	362bc	380bc	405b	572a
propionic acid	20 <sup>5</sup>	1.53d	1.56d	2.64c	4.09b	4.94a	1.28b	1.42b	2.03a	1.81ab	2.13a	0.68c	0.83bc	0.77c	1.05a	0.93ab
isobutyric acid	2.3 <sup>5</sup>	0.71a	0.58a	0.57a	0.67a	0.77a	0.82a	0.77a	0.49c	0.45c	0.60b	1.61c	2.92a	2.53ab	2.23bc	2.22bc
butyric acid	0.17 <sup>5</sup>	0.95b	0.82b	0.95b	1.25a	1.30a	0.54c	0.57bc	0.67a	0.62ab	0.61ab	1.57b	1.89a	1.75ab	1.65ab	1.26c
isovaleric acid	0.03 <sup>5</sup>	0.06c	0.03c	0.08bc	0.16b	0.32a	0.09a	0.05ab	0.01b	0.01b	0.05ab	1.00b	1.40a	1.29a	1.25a	1.33a
hexanoic acid	0.42 <sup>5</sup>	1.80a	1.43abc	1.52a	1.16bc	1.03c	1.14b	1.12b	1.28a	0.95c	0.83c	3.33b	3.87a	3.17b	2.98b	1.65c
octanoic acid	0.50 <sup>5</sup>	1.70a	1.50ab	1.55ab	1.17bc	1.12c	1.29ab	1.37a	1.47a	1.14b	0.92c	4.22a	4.27a	4.12a	3.72a	2.20b

Means followed by the same letter within a row, within a vintage do not differ significantly ( $p \leq 0.05$ ) † concentration in µg/L, \* concentration in ng/L 3-isobutyl-2-methoxypyrazine (IBMP), sec-butyl-methoxypyrazine (SBMP) and 3-isopropyl-2-methoxypyrazine (IPMP), OT = Odour Threshold, 1 (Pineau *et al.*, 2007), 2 (Van Gemert, 2003), 3 (Roujou de Boubee *et al.*, 2000), 4 (Guth, 1997), 5 (Ferreira *et al.*, 2001), 6 (Bakker & Clarke, 2012), 7 (Peinado *et al.*, 2004). OT thresholds in water/ethanol.

Table 9. Concentration of volatile compounds (mg/L) in cv. Pinotage wines from 5 ripeness levels in Site A, Stellenbosch (2015-2017). (Continued)

	OT	2015					2016					2017				
		R1	R2	R3	R4	R5	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5
<i>Esters</i>																
isoamyl acetate	0.03 <sup>4</sup>	1.45c	2.81b	4.12a	4.38a	4.71a	1.39b	1.75b	1.24b	2.52a	2.55a	4.41c	6.23b	6.20b	8.71a	2.53d
ethyl hexanoate	0.014 <sup>5</sup>	0.60a	0.57ab	0.57ab	0.49b	0.56ab	0.41b	0.41b	0.46a	0.36b	0.37b	0.55b	0.70a	0.67a	0.60ab	0.38c
hexyl acetate	1.5 <sup>6</sup>	0.03b	0.02b	0.01b	0.01b	0.04a	0.05a	0.05a	0.02b	<i>nd</i>	<i>nd</i>	0.20b	0.32a	0.24b	0.30a	0.13c
ethyl lactate	154.6 <sup>5</sup>	21.54a	25.32a	23.13a	16.72b	15.18b	5.41a	4.29a	1.40b	1.26b	0.37b	6.94a	5.83ab	4.53ab	3.98b	1.09c
ethyl-octanoate	0.005 <sup>5</sup>	0.41a	0.44a	0.42a	0.34a	0.37a	0.29b	0.31ab	0.35a	0.27bc	0.22c	0.43a	0.45a	0.39a	0.42a	0.26b
ethyl-butyrate	0.02 <sup>4</sup>	0.22a	0.29a	0.29a	0.31a	0.29a	0.01a	0.01a	0.01a	0.01a	0.01a	0.12c	0.19bc	0.23b	0.35a	0.18bc
ethyl-decanoate	0.2 <sup>5</sup>	0.09b	0.10b	0.10b	0.11b	0.15a	0.10b	0.11ab	0.14a	0.10b	0.11b	0.13a	0.13a	0.12a	0.13c	0.08b
diethyl succinate	200 <sup>6</sup>	3.60ab	3.48ab	4.23a	4.48a	2.81b	0.12a	0.19a	0.16a	0.21a	0.31a	0.44c	0.57ab	0.64a	0.63a	0.48ab
<i>Higher Alcohols</i>																
isobutanol	30 <sup>6</sup>	20.85ab	20.06ab	20.37ab	19.04b	22.71a	13.86b	16.44ab	15.94ab	15.45ab	18.90a	20.57b	23.95ab	21.60b	20.77b	26.30a
n-butanol	150 <sup>4</sup>	1.67c	2.12b	2.65a	2.83a	2.95a	1.49c	1.62bc	2.07ab	2.52a	2.53a	2.03a	2.22a	2.14a	2.32a	2.32a
isoamyl alcohol	40 <sup>4</sup>	178b	194ab	208a	196a	199a	134b	123bc	112c	131b	168a	138b	176a	158ab	157ab	162a
4-methyl-1-pentanol	5 <sup>2</sup>	0.08a	0.09a	0.03a	0.06a	0.07a	0.02b	0.05ab	0.08a	0.06a	0.06a	0.36c	3.38a	2.30b	0.76c	0.27c
3-ethoxy-1-propanol	50 <sup>7</sup>	2.84c	3.40b	3.58b	3.77b	4.78a	1.99b	1.95b	3.31a	3.57a	4.04a	2.14c	2.73bc	2.99ab	3.43a	2.78b
2-phenyl-ethanol	14 <sup>6</sup>	5.58a	6.04a	6.44a	6.75a	5.87a	3.90c	5.03b	5.11b	6.52a	6.66a	12.07c	17.76ab	15.22bc	16.96ab	20.52a
<i>C6-compounds</i>																
n-hexanol	8 <sup>4</sup>	3.04a	2.22b	1.73c	1.17d	2.23b	2.90a	2.53a	2.99a	1.25b	1.70b	2.21bc	3.57a	2.49b	2.04cd	1.67d
(Z)-3-hexanol	0.4 <sup>4</sup>	1.18a	1.28a	1.30a	1.83a	1.45a	1.15c	0.86c	1.95bc	4.14a	3.56ab	4.63ab	1.32d	1.88cd	7.18a	4.07bc
(E)-2-hexanol	0.4 <sup>4</sup>	3.65a	2.28b	1.51bc	0.53cd	0.98d	0.66b	0.98ab	1.29a	0.80b	0.83b	4.11ab	3.07bc	2.19c	2.94bc	5.10a
(E)-2-hexanal	0.017 <sup>4</sup>	0.47a	0.41ab	0.37b	0.36b	0.38ab	0.21a	0.22a	0.24a	0.24a	0.18a	0.51a	0.52a	0.63a	0.51a	0.67a

Means followed by the same letter within a row, within a vintage do not differ significantly ( $p \leq 0.05$ ) † concentration in  $\mu\text{g/L}$ , \* concentration in  $\text{ng/L}$  3-isobutyl-2-methoxypyrazine (IBMP), sec-butyl-methoxypyrazine (SBMP) and 3-isopropyl-2-methoxypyrazine (IPMP), OT = Odour Threshold, 1 (Pineau *et al.*, 2007), 2 (Van Gemert, 2003), 3 (Roujou de Boubee *et al.*, 2000), 4 (Guth, 1997), 5 (Ferreira *et al.*, 2001), 6 (Bakker & Clarke, 2012), 7 (Peinado *et al.*, 2004). OT thresholds in water/ethanol.

Table 10. Concentration of volatile compounds (mg/L) in cv. Pinotage wines from 5 ripeness levels in Site B, Stellenbosch (2015-2017).

	OT	2015					2016					2017				
		R1	R2	R3	R4	R5	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5
<i>Norisoprenoids</i> †																
β-damascenone	2-7 <sup>1</sup>	8.70b	9.30b	6.92b	10.91ab	14.27a	16.45c	17.97c	19.85bc	24.84ab	26.23a	4.51a	4.76a	4.58a	4.54a	4.44a
α-ionone	0.60 <sup>2</sup>	0.23a	0.22ab	0.20b	0.21ab	0.24a	0.27c	0.31b	0.26c	0.27bc	0.36a	2.39a	2.39a	2.35a	2.29b	2.29b
β-ionone	0.09 <sup>2</sup>	0.80a	1.08a	0.37b	0.19b	0.42b	0.72b	1.02a	0.43c	0.45c	0.40c	2.65a	2.45ab	2.25bc	2.21c	2.22c
6-M-H-O	50 <sup>2</sup>	1.12b	0.96b	0.97b	1.42bb	2.26a	2.13d	4.02bc	2.85cd	4.57b	7.85a	-	-	-	-	-
<i>Monoterpenes</i> †																
α-terpineol	330 <sup>2</sup>	9.35a	8.45ab	6.22abc	5.19bc	4.12c	4.17a	3.97ab	3.41b	4.06a	3.62ab	10.91ab	11.42a	9.22abc	8.62bc	7.70c
1-8-cineole	400 <sup>2</sup>	12.60ab	7.80ab	5.95b	5.45b	17.11a	7.74b	8.19b	8.71b	16.89a	6.00b	7.78a	3.83a	4.72a	6.69a	6.21a
linalool	6 <sup>2</sup>	5.59b	5.78b	7.63ab	9.91a	10.31a	15.37a	11.19b	9.75b	5.88c	5.39c	6.40b	10.13a	4.64b	6.14b	6.36b
citronellol	30 <sup>2</sup>	8.48b	6.87b	8.65b	15.95a	15.28a	11.24b	13.61b	11.93b	17.69a	13.08b	14.27a	8.53c	12.87ab	11.10bc	14.11a
geraniol	40 <sup>2</sup>	7.92b	10.08ab	14.22a	10.77ab	11.96ab	19.65ab	25.43a	22.53ab	18.99b	21.39ab	16.49a	67.38b	46.13	52.71b	28.08b
nerol	30 <sup>2</sup>	11.7c	15.5bc	32.2abc	62.4a	50.2ab	19.7a	30.7c	39.0a	32.3a	56.0a	34.84a	32.47a	29.13a	33.85a	41.62a
<i>Methoxypyrazines</i>																
IPMP	2 <sup>3</sup>	5.35a	6.70a	10.70a	6.27a	5.66a	3.34b	9.89a	1.28ab	2.06ab	6.43a	3.76a	9.02a	4.70a	7.11a	8.22a
SBMP	2 <sup>3</sup>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IBMP	2 <sup>3</sup>	1.09a	2.63a	1.04a	1.88a	1.04a	3.03a	1.94ab	1.18b	1.42b	1.87ab	1.63a	1.68ab	1.51b	1.54ab	1.52b
<i>Fatty Acids</i>																
acetic acid	200 <sup>4</sup>	216d	270c	253cd	310b	357a	228c	329b	319bc	389b	625a	312c	340b	321c	400b	544a
propionic acid	20 <sup>5</sup>	1.99c	2.22c	2.59b	3.10a	3.05a	1.46c	1.64bc	1.75b	2.14b	2.91a	0.76ab	0.70b	0.76ab	0.76ab	0.86a
isobutyric acid	2.3 <sup>5</sup>	1.41a	1.22b	1.07bc	0.96c	0.89c	1.09a	0.63b	0.79ab	0.74b	0.68b	2.80a	2.41ab	2.52ab	2.64a	2.12b
butyric acid	0.17 <sup>5</sup>	0.84b	1.10a	1.16a	1.14a	1.09a	0.67ab	0.70ab	0.64b	0.61b	0.75a	1.96a	1.98a	1.76ab	1.48bc	1.09c
isovaleric acid	0.03 <sup>5</sup>	0.21ab	0.13b	0.45a	0.24ab	0.45a	0.26a	0.07b	0.18ab	0.07b	0.04b	1.60ab	1.48ab	1.51ab	1.65a	1.41b
hexanoic acid	0.42 <sup>5</sup>	1.96a	1.42b	1.30bc	1.01c	1.09c	1.69a	1.52ab	1.28bc	0.95d	1.10dc	5.73a	4.01b	3.27bc	2.75c	1.68d
octanoic acid	0.50 <sup>5</sup>	1.72a	1.54ab	1.38bc	1.12c	1.08c	1.88a	1.77ab	1.46bc	0.97d	1.10dc	5.85a	5.13a	5.50a	3.93b	2.26c

Means followed by the same letter within a row, within a vintage do not differ significantly ( $p \leq 0.05$ ) † concentration in µg/L, \* concentration in ng/L 3-isobutyl-2-methoxypyrazine (IBMP), sec-butyl-methoxypyrazine (SBMP) and 3-isopropyl-2-methoxypyrazine (IPMP), OT = Odour Threshold, 1 (Pineau *et al.*, 2007), 2 (Van Gemert, 2003), 3 (Roujou de Boubee *et al.*, 2000), 4 (Guth, 1997), 5 (Ferreira *et al.*, 2001), 6 (Bakker & Clarke, 2012), 7 (Peinado *et al.*, 2004). OT thresholds in water/ethanol.

Table 10. Concentration of volatile compounds (mg/L) in cv. Pinotage wines from 5 ripeness levels in Site B, Stellenbosch (2015-2017) (Continued)

	OT	2015					2016					2017				
		R1	R2	R3	R4	R5	R1	R2	R3	R4	R5	R1	R2	R3	R4	R5
<i>Esters</i>																
isoamyl acetate	0.03 <sup>4</sup>	1.07d	2.14c	2.38bc	3.01ab	3.33a	2.02c	2.27bc	3.36a	2.77ab	3.22a	7.30ab	6.81b	8.83a	5.99b	3.32c
ethyl hexanoate	0.014 <sup>5</sup>	0.62a	0.58a	0.55ab	0.48b	0.54ab	0.47ab	0.52a	0.48a	0.39b	0.45ab	0.75a	0.67ab	0.70a	0.53bc	0.42c
hexyl acetate	1.5 <sup>6</sup>	0.01b	0.02a	0.01b	0.01b	0.03a	0.09a	0.07a	0.03b	0.02b	0.03b	0.51a	0.28b	0.31b	0.18c	0.14c
ethyl lactate	154.6 <sup>5</sup>	17.37c	23.08ab	26.30a	22.84b	18.87bc	4.16b	6.40b	4.29b	2.64b	11.25a	4.25ab	5.53a	5.28a	2.21c	2.98bc
ethyl-octanoate	0.005 <sup>5</sup>	0.40ab	0.46a	0.42ab	0.38b	0.37b	0.37bc	0.49a	0.43ab	0.26d	0.33dc	0.55a	0.56a	0.55a	0.39b	0.28c
ethyl-butyrate	0.02 <sup>4</sup>	0.28b	0.29b	0.46a	0.58a	0.52a	0.01b	0.06ab	0.11ab	0.09ab	0.17a	0.21b	0.33ab	0.25ab	0.40a	0.26ab
ethyl-decanoate	0.2 <sup>5</sup>	0.11b	0.15a	0.14a	0.13a	0.15a	0.11ab	0.12a	0.13a	0.09b	0.13a	0.17a	0.15a	0.16a	0.11b	0.08c
diethyl succinate	200 <sup>6</sup>	3.66a	2.84a	3.55a	2.57a	2.71a	0.21b	0.29b	0.31b	0.26b	0.57a	0.51bc	0.64ab	0.66a	0.54abc	0.51c
<i>Higher Alcohols</i>																
isobutanol	30 <sup>6</sup>	28.85a	23.95b	25.11ab	24.87b	23.71b	19.75a	19.91a	21.08a	22.87a	22.27a	29.46ab	26.19b	29.09ab	34.12a	28.97ab
n-butanol	150 <sup>4</sup>	1.22c	1.46a	1.91b	2.25a	2.56a	1.31d	1.75c	1.82c	2.32b	3.14a	1.37b	1.69ab	1.79ab	1.96a	2.06a
isoamyl alcohol	40 <sup>4</sup>	228a	197a	214a	194a	215a	142b	148ab	180a	172ab	173ab	193ab	167b	193ab	210a	177b
4-methyl-1-pentanol	5 <sup>2</sup>	0.16a	0.09b	0.07b	0.07b	0.11ab	0.06a	0.08a	0.07a	0.06a	0.06a	3.64a	0.93b	0.93b	0.57b	0.53b
3-ethoxy-1-propanol	50 <sup>7</sup>	1.56c	1.61c	2.30b	2.52b	3.54a	1.35b	2.23b	2.30b	4.08a	4.51a	1.39b	1.62b	1.39b	1.35b	2.20a
2 phenyl-ethanol	14 <sup>6</sup>	12.78a	8.00b	8.36b	8.20b	8.07b	7.51c	5.66c	8.13abc	8.39ab	10.35a	23.48b	17.01c	26.42b	34.99a	22.79b
<i>C6-compounds</i>																
n-hexanol	8 <sup>4</sup>	3.88a	2.25c	1.88d	1.11e	2.60b	3.21a	2.91a	1.74b	1.98b	1.91b	3.97a	2.52b	1.62d	2.02c	1.40d
(Z)-3-hexanol	0.4 <sup>4</sup>	1.53b	1.95ab	2.48ab	3.18a	1.90ab	3.44b	4.74b	5.29b	4.94b	15.04a	4.16b	8.05a	1.41b	3.98b	1.99b
(E)-2-hexanol	0.4 <sup>4</sup>	2.34a	2.49a	1.32b	0.48c	1.45b	1.79a	1.60a	0.95b	1.60a	0.66b	3.15a	3.51a	3.20a	2.43a	2.48a
(E)-2-hexanal	0.017 <sup>4</sup>	0.56a	0.47ab	0.47ab	0.44b	0.49ab	0.29b	0.43a	0.36ab	0.37ab	0.39ab	0.59a	0.55a	0.52a	0.46a	0.31a

Means followed by the same letter within a row, within a vintage do not differ significantly ( $p \leq 0.05$ ) † concentration in  $\mu\text{g/L}$ , \* concentration in  $\text{ng/L}$  3-isobutyl-2-methoxypyrazine (IBMP), sec-butyl-methoxypyrazine (SBMP) and 3-isopropyl-2-methoxypyrazine (IPMP), OT = Odour Threshold, 1 (Pineau *et al.*, 2007), 2 (Van Gemert, 2003), 3 (Roujou de Boubee *et al.*, 2000), 4 (Guth, 1997), 5 (Ferreira *et al.*, 2001), 6 (Bakker & Clarke, 2012), 7 (Peinado *et al.*, 2004). OT thresholds in water/ethanol.

Table 11. Aroma descriptors and odour thresholds of volatile compounds.

Compound	OT	Descriptor	Compound	OT	Descriptor
<u>Norisoprenoids†</u>			<u>Esters</u>		
β-damascenone	2-7 <sup>1</sup>	floral/berry/tobacco	isoamyl acetate	0.03 <sup>4</sup>	banana
α-ionone	0.60 <sup>2</sup>	floral	ethyl-hexanoate	0.014 <sup>5</sup>	green apple
β-ionone	0.09 <sup>2</sup>	floral	hexyl-acetate	1.5 <sup>6</sup>	pear, floral
6-M-H-O	50 <sup>2</sup>	tomato leaf	Ethyl-lactate	154.6 <sup>5</sup>	lactic, fruity
<u>Monoterpenes†</u>			ethyl-octanoate	0.005 <sup>5</sup>	sweet, fruity
α-terpineol	330 <sup>2</sup>	lily of the valley	ethyl-butyrate	0.02 <sup>4</sup>	fruity apple
1-8-cineole	400 <sup>2</sup>	eucalyptus	ethyl-decanoate	0.2 <sup>5</sup>	grape
linalool	6 <sup>2</sup>	rose, floral	diethyl succinate	200 <sup>6</sup>	fruity melon
citronellol	30 <sup>2</sup>	citronella	<u>Higher Alcohols</u>		
geraniol	40 <sup>2</sup>	rose	isobutanol	30 <sup>6</sup>	fusel alcohol
nerol	30 <sup>2</sup>	rose	n-butanol	150 <sup>4</sup>	fusel medicinal
<u>Methoxy-pyrazines*</u>			isoamyl alcohol	40 <sup>4</sup>	alcoholic harsh
IPMP	2 <sup>3</sup>	green pea	4-methyl-1-pentanol	5 <sup>2</sup>	fruity
SBMP	2 <sup>3</sup>	green pea	3-ethoxy-1-propanol	50 <sup>7</sup>	ripe fruit
IBMP	2 <sup>3</sup>	bell pepper	2 phenyl-ethanol	14 <sup>6</sup>	rose
<u>Fatty Acids</u>			<u>C6-compounds</u>		
acetic acid	200 <sup>4</sup>	vinegar	n-hexanol	8 <sup>4</sup>	grass, green
propionic acid	20 <sup>5</sup>	rancid, pungent	(Z)-3-hexanol	0.4 <sup>4</sup>	fresh, green, grass
isobutyric acid	2.3 <sup>5</sup>	acidic	(E)-2-hexanol	0.4 <sup>4</sup>	green, citrus
butyric acid	0.17 <sup>5</sup>	rancid, cheese, sweat	(E)-2-hexanal	0.017 <sup>4</sup>	green, grass
isovaleric acid	0.03 <sup>5</sup>	blue cheese			
hexanoic acid	0.42 <sup>5</sup>	sweat, cheese			
octanoic acid	0.50 <sup>5</sup>	rancid, harsh			

\* concentration in ng/L 3-isobutyl-2-methoxypyrazine (IBMP), sec-butyl-methoxypyrazine (SBMP) and 3-isopropyl-2-methoxypyrazine (IPMP), OT = Odour Threshold, 1 (Pineau *et al.*, 2007), 2 (Van Gemert, 2003), 3 (Roujou de Boubée *et al.*, 2000), 4 (Guth, 1997), 5 (Ferreira *et al.*, 2001), 6 (Bakker & Clarke, 2012), 7 (Peinado *et al.*, 2004). OT thresholds in water/ethanol.

Overall the large variety of compounds gives insight into the complex interaction of both grape and fermentation derived components that are involved in wine volatile composition. In order to visualise changes in volatile profile during ripening, DA was conducted per vintage and sites combined. Observations from DA models explained 86.86% in 2015 (F1 66.33% and F2 20.53%), 92.36% in 2016 (F1 81.26% and F2 11.11%) and 85.70% in 2017 (F1 67.69% and F2 18.01%) of the dataset (Fig 7). Changes in wine volatile composition along ripeness levels were consistent among vintages, separating early (R1 & R2) from later (R4 & R5) ripeness levels on F1, with R3 as the intermediary ripeness level. Generally speaking, the early ripeness levels had increased levels of fatty acids (hexanoic acid, octanoic acid) and their esters (ethyl hexanoate, ethyl octanoate), hexyl acetate and green associated alcohols, specifically hexanol. On the other hand, riper levels were discriminated from earlier ripeness levels due to increasing levels of  $\beta$ -damascenone, nerol, geraniol, n-butanol, 3-ethoxy-1-propanol and acetic acid. Isoamyl acetate was also generally related to later ripeness levels. Interestingly, there seemed to be far less overlap between ripeness levels regarding wine volatile composition, compared to grape aroma potential (See Chapter 4). After the fermentation process wines could be discriminated for the major part due to a distinct collection of volatile components. Furthermore, it is encouraging to note that odour impacting compounds, such as  $\beta$ -damascenone and ethyl esters of fatty acids previously identified as key components in Pinotage (Marais *et al.*, 1981; Waldner & Marais, 2002; Louw *et al.*, 2010), displayed a significant change along ripeness level. This emphasises the importance of ripeness level with regard to the expression of varietal aroma. Nonetheless, literature (Escudero *et al.*, 2004, 2007; Pineau *et al.*, 2007) has also underlined the importance of ‘background’ components, many below odour thresholds and most of which were not quantified here. In concert with the impact components, the ‘background’ components provide the platform from and within which the impact components may act.

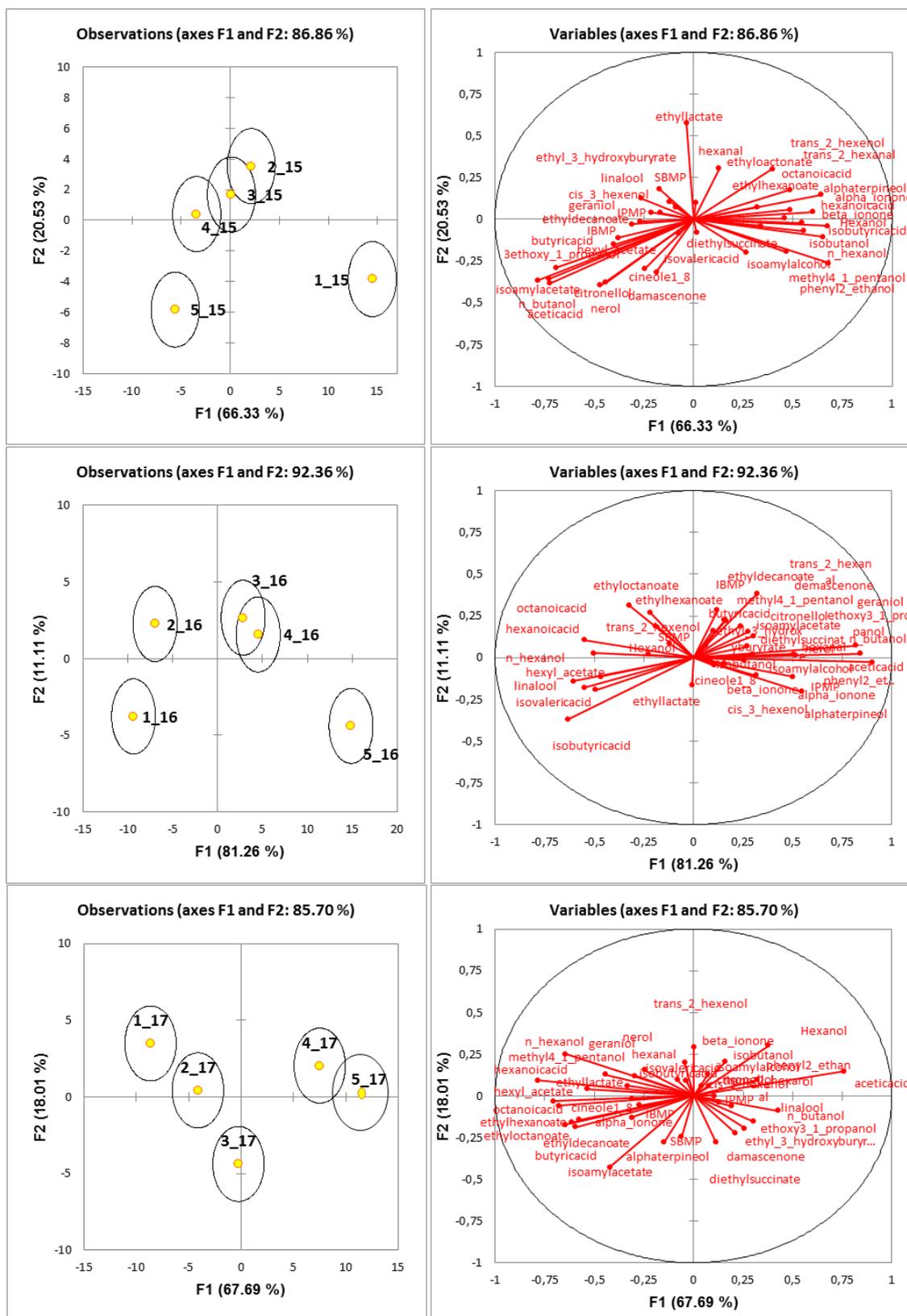


Figure 7. Discriminant analysis of wine volatile content of five ripeness levels for vintages 2015 (top), 2016 (middle) and 2017 (bottom), with alpha ellipses (0.5).

#### 5.4.5 Overall response

In terms of overall wine compositional response (including all variables measured) observations on multifactorial analysis (MFA) separated vintages 2015 and 2016 from 2017 on F1 (Fig 7). Interestingly, 2017 was consistently separated from 2015 and 2016 regarding most of the compositional parameters. This is most probably due to specific climatic factors of the particular vintage. Secondary metabolites, including anthocyanins, norisoprenoids and monoterpenes, are known to react positively to cooler minimum temperatures during the month of ripening (Kliewer & Torres, 1972; Tonietto & Carbonneau, 2004; Mori *et al.*, 2005; Etchebarne *et al.*, 2015). It is conceivable that the  $\approx 3^{\circ}\text{C}$  cooler minimum temperatures during ripening in 2017, compared to 2015 and 2016, may have had such an effect in this case (see Chapter 3, Table 1), generally producing wines with higher anthocyanin content and overall aroma (Table 7). Nonetheless, ripeness level also maintained separation on F2 on the observations plot. The correlation analysis provided insight into associations between the measured variables. As expected, wine Alc content was placed furthest from wine titratable acidity (TA). These compounds effectively represent the ripeness level axis. Thereby *e.g.* higher alcohols (butanol and 3-ethoxy-1-propanol) were closely associated with higher extract, wine alcohol content, pH, VA and acetic acid. Anthocyanins displayed good correlation with each other, increasing with increased alcohol content (ripeness level), but also showed a high level of correlation with wine phenolic index (TPI280) and were strongly influenced by vintage (2017). Importantly, C6 aldehydes and alcohols and as well as fatty acids (hexanoic and octanoic) and their ethyl esters were mostly grouped together, displaying their inter-related origins. These compounds are generally associated with earlier ripeness levels and this response can also be seen from the variable plot, although the influence of the 2017 vintage is also prominent. As a general observation it would seem as if the classical chemical variables were of more explanatory value to higher ripeness levels, than lower ones. The opposite was true for wine volatiles (included in this study), displaying greater abundance in lower ripeness levels than in higher levels. The large amount of variables in the centre of the plot (effectively R3) also indicate that many of the variables do not follow a simple linear increasing or decreasing pattern during ripening. For instance, impact odorants for Pinotage, such as  $\beta$ -damascenone, generally increased during ripening and peaked at R4, before declining towards the later ripeness level. Similarly, fermentation acetate ester, iso-amyl acetate increased along ripening, only to plateau and/or decrease towards the final ripeness level (Tables 9 & 10). Thus, many positive associated components were present at high levels during the intermediary ripeness levels (R2-R4).

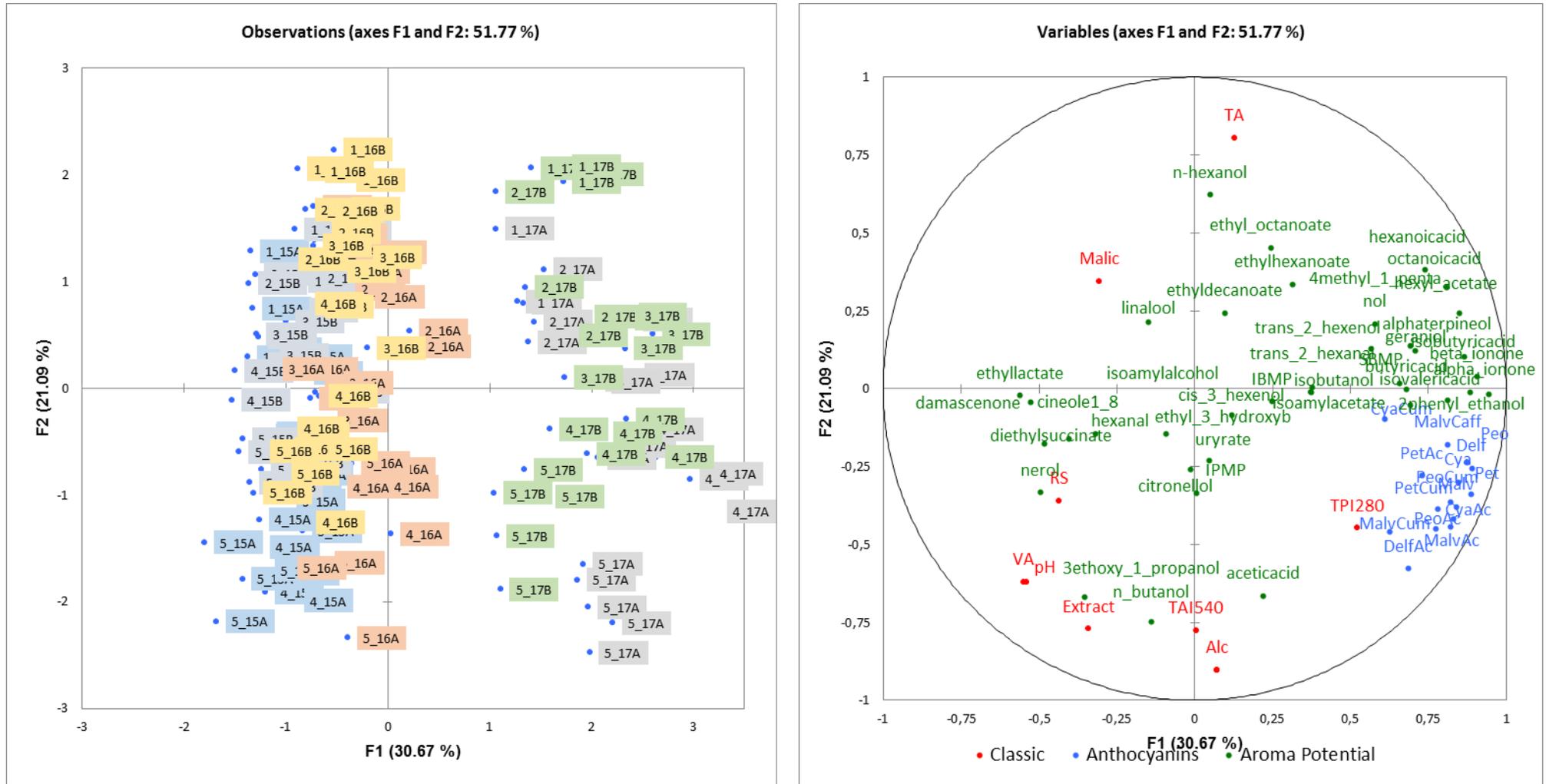


Figure 8. Multifactorial analysis (MFA) of compositional parameters combined of five ripeness levels (1-5), of cv. Pinotage at Site A and B for vintages (2015-2017) in Stellenbosch, Western Cape, South Africa.

#### 5.4.6 Practical implications

From a practical perspective producers may expect changes in terms of wine compositional profile, which will affect wine sensory attributes (Fig 9). Delaying harvesting will increase alcohol level and decrease acidity. In parallel producers may expect an increase in both wine tannin and colour in wines up until late ripening. With regards to fruity characters, wines from 23- 25°B may be characterised with greater fruitiness than wines from earlier or later harvested grapes. Berry and floral-related flavours will be more prominent in later ripeness levels (peak at 27°B), while green and rancid flavours will decrease from the first to the last ripeness level. Fusel and vinegar attributes will increase along the ripening gradient, these aromas in low quantities may add complexity to wines, but in high quantities may have a negative impact. Overall the different attributes presented by the various ripeness levels allow producers to manipulate wine chemical flavour profile, by simple choice of ripeness level.

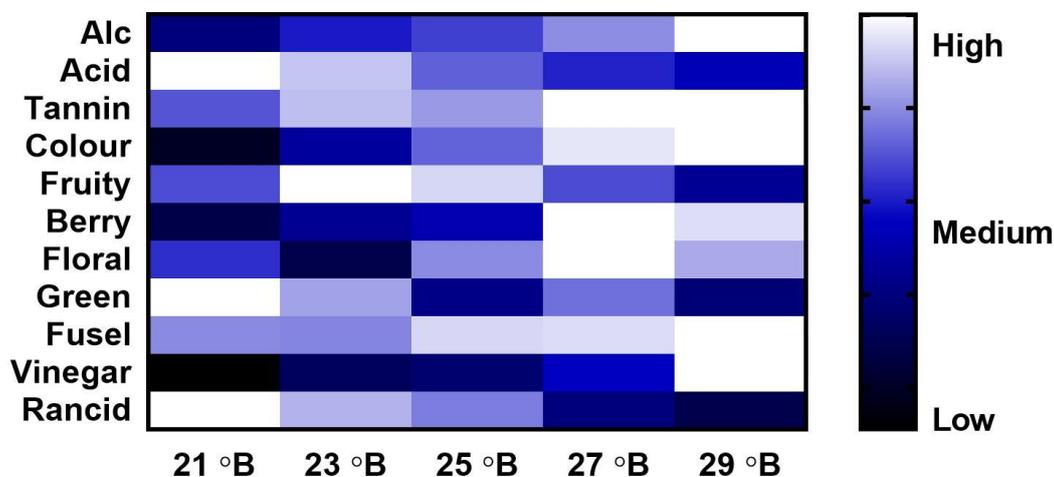


Figure 9. Simplified summary of wine compositional changes for selected variables in cv. Pinotage. Alcohol (Alc % v/v), Acid (titratable acidity), Tannin (IPT 280), Colour (TAI 540), Fruity (Tot Esters), Berry (Tot Norispreoids), Floral (Tot Monoterpenes), Green (Tot Methoxypryazines), Fusel (Tot Higher alcohols), Vinegar and rancid (Tot Fatty acids) expressed as a relative fraction of the maximum value.

## 5.5 CONCLUSIONS

The results of this study demonstrated the significant impact of grape ripeness level on wine composition and affirms that harvest decisions require careful consideration. The selection of ripeness level will not only dictate wine alc % level, but has far-reaching effects regarding wine phenolic, anthocyanin and volatile composition. In general wines of increasing ripeness levels display increased phenolic content and colour densities, while total anthocyanin content already peaks during early ripeness levels. The reaction of wine volatiles to ripeness level is complex, as they differ regarding metabolic origin (yeast-derived *versus* grape-derived). Generally speaking, wines of later ripeness levels were characterised by decreased green-associated compounds (C6-compounds, methoxypyrazines and fatty acids), while also characterised by increased positive associated compounds (norisoprenoids, monoterpenes and higher alcohols). However, the sensory scope separating these points may allow for the elaboration of wines with many unique characteristics. In addition, decreases in many volatile components were demonstrated in the final ripeness level, suggesting that over ripe grapes may have a detrimental effect on volatile composition. Results from this study suggest a significant shift in overall compositional (chemical, phenolic and volatile) profile per ripeness level, emphasising the dictating effect induced by climatic factors, as demonstrated in this study by large compositional effects imposed by different vintages. Many of these changes have an impact on wine sensory properties and could potentially be related to specific wine styles. The next challenge is therefore to be able to relate the compositional response to sensory data, due to the multiple interactions that induce sensory response.

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## 5.7 ADDENDUM C

Table 1. Variable importance in PLS projections of wine compositional (individual anthocyanin) data and ripeness level for each vintage

Variable	2015				2016				2017															
	VIP	St Dev	Lo 95%	Up 95%	VIP	St Dev	Lo 95%	Up 95%	VIP	St Dev	Lo 95%	Up 95%	VIP	St Dev	Lo 95%	Up 95%								
Cya	0.7	0.7	-0.8	2.1	0.8	0.3	0.2	1.4	0.1	0.3	-0.5	0.8	0.9	0.2	0.6	1.2	0.8	0.1	0.7	1.0	0.8	0.1	0.5	1.1
CyaAc	0.6	0.7	-0.8	2.0	0.8	0.2	0.3	1.3	0.7	0.2	0.2	1.2	0.9	0.1	0.8	1.0	1.0	0.0	0.9	1.1	0.9	0.1	0.7	1.2
CyaCum	0.5	0.8	-1.0	2.0	0.8	0.3	0.3	1.4	0.5	0.4	-0.4	1.3	0.9	0.3	0.4	1.5	0.4	0.2	0.1	0.7	0.8	0.5	-0.2	1.8
Delf	1.1	0.7	-0.3	2.6	0.9	0.5	-0.1	1.8	0.2	0.4	-0.6	1.1	1.5	0.2	1.0	1.9	0.9	0.1	0.8	1.0	1.1	0.2	0.7	1.6
DelfAc	0.2	0.8	-1.4	1.9	1.1	0.5	0.0	2.1	1.1	0.2	0.6	1.5	0.8	0.2	0.3	1.2	1.1	0.0	1.1	1.2	0.9	0.1	0.8	1.1
Malv	1.0	0.7	-0.4	2.3	0.8	0.4	-0.1	1.7	1.4	0.1	1.2	1.5	1.0	0.1	0.8	1.2	1.2	0.0	1.1	1.3	1.2	0.2	0.9	1.5
MalvAc	0.6	0.8	-1.0	2.2	0.8	0.3	0.1	1.4	1.6	0.1	1.3	1.8	1.1	0.1	0.9	1.3	1.2	0.1	1.1	1.3	1.1	0.1	0.8	1.3
MalvCaff	1.6	1.3	-1.1	4.3	1.1	0.9	-0.7	2.9	0.3	0.3	-0.3	1.0	0.3	0.2	-0.1	0.7	0.8	0.1	0.5	1.0	1.5	0.6	0.2	2.7
MalvCum	0.5	1.2	-1.9	3.0	1.0	0.3	0.3	1.7	1.8	0.2	1.4	2.2	1.4	0.1	1.2	1.6	1.1	0.1	1.0	1.3	1.1	0.2	0.7	1.4
Peo	0.9	0.6	-0.2	2.1	0.8	0.3	0.2	1.5	0.1	0.4	-0.7	0.9	1.3	0.1	1.0	1.5	0.8	0.1	0.7	1.0	0.8	0.1	0.5	1.1
PeoAc	0.4	0.7	-1.1	1.8	0.9	0.3	0.2	1.5	1.1	0.1	0.8	1.3	0.8	0.1	0.7	1.0	0.9	0.1	0.8	1.0	0.8	0.1	0.6	1.0
PeoCum	0.5	0.9	-1.4	2.3	0.8	0.3	0.2	1.4	1.0	0.2	0.6	1.4	0.8	0.1	0.7	0.9	1.2	0.0	1.1	1.2	1.0	0.1	0.8	1.2
Pet	1.0	0.6	-0.2	2.1	0.9	0.3	0.3	1.4	0.5	0.3	-0.1	1.1	1.0	0.1	0.8	1.2	1.1	0.0	1.0	1.1	0.9	0.1	0.8	1.0
PetAc	2.5	1.1	0.3	4.7	2.0	0.5	1.1	3.0	1.2	0.1	1.0	1.4	0.9	0.1	0.7	1.0	1.1	0.0	1.0	1.2	0.9	0.1	0.6	1.2
PetCum	0.1	0.6	-1.1	1.4	0.9	0.4	0.1	1.7	1.3	0.1	1.1	1.5	0.9	0.1	0.7	1.1	1.1	0.1	1.0	1.2	1.0	0.1	0.7	1.2

Variable Importance in Projection (VIP), VIP values  $\geq 1$ , indicate high predictive value of the variable within the model  
 PLS regressions were conducted for each vintage separately, Standard deviation (St Dev)

## Chapter 6

### **Wine sensory profile of *Vitis vinifera* L. cv. Pinotage as modulated by grape ripeness level**

#### 6.1 ABSTRACT

The sensory evaluation of wines is a crucial step in assessing the impact of viticultural practices in a holistic manner. This study aimed to describe the changes in wine sensory profiles manifested over a range of commercial ripeness levels for *Vitis vinifera* L cv. Pinotage. Grape ripeness levels (R1-R5) were defined by °Brix (ca. 21, 23, 25, 27 & 29°B) and investigated during three vintages (2015-2017) and two sites (A & B) for Pinotage/140 Ruggeri and Pinotage/1103 Paulsen, under Mediterranean conditions, Western Cape, South Africa. The ripeness level range corresponded to a mean increase in alcohol concentration from 11.92% to 17.05% (v/v). Various sensory evaluation techniques were employed, including rapid untrained- and trained descriptive analysis and projective mapping (Napping) with both untrained and expert panels, to assess sensory effects. Wine sensory profiles were significantly influenced by harvest date, progressively moving from herbaceous, red berry aroma profile to dark berries and prune aromas. High scores of additional positive aroma descriptors, such as tobacco, vanilla, sweet spice and roses, were often associated with R4 and R5. Taste descriptors were the major drivers with regard to overall sensory response and perception (R1-R5). Decreased perception of acidity, coupled with increased perception of concentration, body, astringency, alcohol and length, in wines from progressing ripeness levels were apparent across all sensory modalities tested. Associations between wine chemical (phenolic and volatile) composition and sensory profile were assessed, and provided ample evidence that compositional data can be used to assess plausible sensory outcomes. Results suggest a significant shift in overall sensory profile as ripening proceeded during the compact harvest window (21 days). Furthermore, sensory profiles could be linked to different wine styles according to grape ripeness level, but maintaining similar preference/quality ratings. This field-based sensory study placed grape ripening related changes in context to the environment (Vintage/Climate) and Site (Soil & Genotype) and highlighted the predictive value of basic ripeness indices, such as sugar (density) and acidity and ratios thereof, as indicators of expected changes in wine sensory profile as ripening progresses.

## 6.2 INTRODUCTION

There is general consensus among producers and researchers alike that wines from earlier harvested grapes can be dominated by vegetative flavours, while later harvested grapes display greater fruit intensity (Berg & Ough, 1977; Du Plessis & Van Rooyen, 1982; Van Rooyen *et al.*, 1984). However, more recent studies on Shiraz/Syrah (Hunter *et al.*, 2004; Nadal *et al.*, 2004) and Cabernet Sauvignon (Heymann *et al.*, 2013; Bindon *et al.*, 2014) have pointed towards greater complexity between these two opposites. In fact, these studies not only indicated differences, but also suggested the potential of stylistic modulation of wine sensory profiles along a ripeness gradient (Hunter *et al.*, 2004; Hunter & Volschenk, 2018). Yet, details regarding the extent to which viticulturists can manipulate wine style with choice of ripeness level remains largely undocumented, with no information available for Pinotage. On a practical level viticulturists continue to primarily use grape sugar content (density measurements: Baumé, Brix, Balling and Probable alcohol) as basis for making harvest decisions.

This is most probably related to ease of measurement and the inescapable fact that grape berry sugar content increases during ripening (Coombe, 1976) and is invariably linked to an increase in alcohol (mainly ethanol) content of wines. Studies have revealed ethanol to have a multi-layered effect on wine sensory perception by amplifying bitterness (Fischer & Noble, 1994; Vidal *et al.*, 2004; Fontoin *et al.*, 2008), subduing sourness (Fischer & Noble, 1994), generally increasing the perception of sweetness (Scinska *et al.*, 2000; Zamora *et al.*, 2006), and reducing astringency (Vidal *et al.*, 2004; Fontoin *et al.*, 2008), unless in the presence of high tannin concentrations (Holt *et al.*, 2010; Obreque-Slifer *et al.*, 2010). Wine tannin content generally follows an increasing trend along the ripening gradient (in parallel to ethanol), due to increased extractability of grape skins rather than seeds (Nadal *et al.*, 2004; Holt *et al.*, 2010; Ristic *et al.*, 2010). Ethanol has been shown to have limited increasing effect on tannin extraction (Canals *et al.*, 2005; Del Llaudy *et al.*, 2008) as well as limited influence on wine viscosity and body, in the absence of ripeness-related increases in pH and glycerol (Gawel *et al.*, 2007; Demiglio & Pickering, 2008). Moreover, high ethanol content is also capable of suppressing fruit characters in wines by masking the perception of esters (Escudero *et al.*, 2007; Golnder *et al.*, 2009) and contributing a hot/burning sensation (Gawel *et al.*, 2007; Heymann *et al.*, 2013). Importantly, recent studies using chaptalisation to increase the ethanol content of wines made of low maturity grapes, reported a change of sensory descriptors (more sweetness, viscosity, floral notes) as opposed to unchaptalised (vegetative) Merlot wines (Casassa *et al.*, 2013). Furthermore, in a study where must was both chaptalised and alcohol reduced through *saignée* (removal of juice after limited skin and seed contact) and subsequent replacement with water, Sherman *et al.*

(2017) stated that the resulting wine ethanol content had a stronger effect on wine sensory profile than grape ripeness level *per se*.

Vegetative flavours in certain red wines are thought to arise primarily from the potent grape-derived aroma compound isobutyl methoxypyrazine (IBMP) (Allen *et al.*, 1995; Roujou de Boubée *et al.*, 2000; Bindon *et al.*, 2013), and propensity to high levels is related to ripening stage, genotype and climate (Lacey *et al.*, 1991; Romero *et al.*, 2006). High levels are generally encountered at early maturity and are exasperated by cool climatic conditions and limited grape berry sunlight exposure (Lacey *et al.*, 1991; Marais *et al.*, 1999; Ryona *et al.*, 2010). Certain genotypes, such as Bordeaux varieties (Merlot, Cabernet Sauvignon and Cabernet franc) are prone to high levels (Roujou de Boubée *et al.*, 2000), while low levels are reported in Syrah, Pinot noir and Pinotage (Waldner & Marais, 2002; Romero *et al.*, 2006). Vegetative or green flavour attributes can also be derived from other compounds, such as the C6 compounds (green leaf volatiles), including hexanal, hexanol, (*E*)-2-hexenal and (*Z*)-3-hexen-1-ol (Escudero *et al.*, 2007; Preston *et al.*, 2008; Kalua & Boss, 2009, 2010). These C6 compounds can be present in the grape berry (Vilanova *et al.*, 2012), but primarily derive from the degradation of fatty acids *via* the lipoxygenase pathway, when cell membranes are disrupted during grape processing (Iyer *et al.*, 2010). Recent studies have also implicated C6 compounds as precursors for fermentation derived esters, such as hexyl acetate (fruity) (Dennis *et al.*, 2012). As much as the impacting role of ripeness level on volatiles from yeast metabolism is recognised (Boss *et al.*, 2015) the extent thereof remains unclear (Boss *et al.*, 2018). For instance, fruit maturation (20-26°C) increased fermentation-derived volatiles in Cabernet Sauvignon (Bindon *et al.*, 2013), while Fang & Qian (2006) reported decreases with increasing maturity (21 – 33°C), with the exception of short chain fatty acid esters. The varietal aroma of Pinotage is closely related to esters (including esters derived from short chain fatty acids) as Pinotage wines were successfully discriminated from Merlot, Cabernet Sauvignon and Shiraz/Syrah wines due to higher concentrations of butyric acid, acetic acid, octanoic acid, propanol, hexanoic acid, ethyl hexanoate, ethyl octanoate, ethyl lactate and isoamyl acetate (Louw *et al.*, 2010). Particularly, isoamyl acetate (fruity, banana), hexyl acetate (fruity, pear, floral) and ethyl octanoate (fruity, sweet) have been highlighted as potential impact odorants (Marais *et al.*, 1979; Van Wyk *et al.*, 1979). Similarly, the norisoprenoids,  $\beta$ -damascenone (floral, berry) and  $\beta$ -ionone (floral), have also been implicated in the varietal aroma of Pinotage (Waldner & Marais, 2002), yet their relationship to ripeness level appears to be unclear, as Fang & Qian (2006) reported increases while other studies found no significant effect of ripeness level (Marais *et al.*, 1992; Ristic *et al.*, 2007). Nonetheless,  $\beta$ -damascenone is considered an important impact odorant in wines (Escudero *et al.*, 2004), but also interacts with other compounds, for example through the

masking of green flavours by IBMP and enhancing of fruity flavours of esters (Escudero *et al.*, 2007; Pineau *et al.*, 2007).

Despite the recent advances in chemical analysis allowing qualification/quantification of a large number of volatile compounds (Weldegergis *et al.*, 2011; Ilc *et al.*, 2016), many works have indicated that the contribution of individual components cannot assuredly be isolated due to major matrix effects on their perception (Escudero *et al.*, 2004, 2007; Pineau *et al.*, 2007; Demiglio & Pickering, 2008; Wilson *et al.*, 2018). This presents a major challenge to interpret compositional results without sensory qualification. Moreover, qualitative sensory results are highly complex as it is subject to human error, while the physiological perceptive abilities of tasters also vary greatly (Fischer *et al.*, 1994). Consequently, quantitative descriptive analysis (QDA) has emerged as a useful sensory analysis tool aimed at removing the preferences of judges and providing a rich descriptive dataset (Lawless & Heymann, 2010). The sample-based development of a unique sensory lexicon and subsequent training of judges with the use of reference standards before testing, increases the accuracy of analysis, and repeatability of judges, but is very time consuming and costly (Lawless & Heymann, 2010). In addition, QDA generally allows for a relatively small amount of samples to be assessed per session, necessitating a rigorous experimental layout and sufficient time in studies with large sample sets (Dehlholm *et al.*, 2012). Therefore more rapid alternatives have gained popularity in recent times, such as Projective Mapping (PM) and specifically the variant, Napping, as proposed for wine analysis (Pagès, 2005; Pagès *et al.*, 2010). Napping tasks the taster to visually organise wines on a 2D plane, generating data regarding similarities/dissimilarities based on relative distances between samples (Pagès, 2005). This technique allows for the rapid and simultaneous evaluation of a larger number of samples in a shorter amount of time, but generally leads to less detailed description compared to QDA (Dehlholm *et al.*, 2012).

In the context of the exploration of wine styles, sensory analysis is a key measurement tool in order to characterise the extent of differences due to ripeness level. Certainly, wine sensory profiles will create a better market-related picture of what is to be expected at which ripeness level. Furthermore, if this can be related to compositional data, both viticultural and oenological strategies can be adapted (Hunter *et al.*, 2004; Cadot *et al.*, 2012; Bindon *et al.*, 2014) in order to achieve wine stylistic goals. Changes regarding ripeness level have recently been brought into the spotlight with the assumptions of grape metabolism modification and altered wine composition due to higher temperatures and rapid sugar accumulation expected with climate change (Jones *et al.*, 2005; Petrie & Sadras, 2008; Webb *et al.*, 2012; van Leeuwen *et al.*, 2013). Therefore, this study investigated the extent of change in wine sensory profiles according to ripeness levels of an early and rapid maturing variety, *Vitis vinifera* L. cv. Pinotage, in an

unirrigated situation in a warm Mediterranean climate. Importantly, multiple vintages (three) and sites (two) were assessed, conceding that environmental conditions related to vintages can modify expected sensory profiles (Heymann *et al.*, 2013), while the combination of genotype and site/soil can also modify expected response (Koundouras *et al.*, 2006; Van Leeuwen & Seguin, 2006; Cadot *et al.*, 2010; Hunter *et al.*, 2010). In summary, with the use of multiple sensory evaluation techniques this study endeavoured to assess the effect of ripeness level, over site and vintage differences, on sensory profiles, to gain a holistic perspective on changes brought about by ripeness level. To our knowledge, this is the first of its kind conducted on variety *Vitis vinifera* L. cv. Pinotage.

## 6.3 MATERIALS AND METHODS

### 6.3.1 Experimental Vineyard

The experiment was carried out during three consecutive seasons (2015, 2016 and 2017) in a unirrigated *Vitis vinifera* L. cv. Pinotage vineyard, grafted onto two known drought tolerant rootstocks (*Vitis Berlandieri* x *Vitis rupestris*), namely Pinotage (clone PI 50A)/140 Ruggeri (clone RU 354B) planted in 1997 and Pinotage (clone PI 48A)/1103 Paulsen (clone PS 28A) planted in 1999. The vineyard is located at Stellenbosch University Welgevallen Experimental Farm in Stellenbosch, Western Cape, South Africa. The area is under the influence of a Mediterranean climate, characterised by winter rainfall and warm and dry summers. Vines are orientated in a North–South direction and the vineyard locality (approx. 200 m altitude) is characterised by a slope (3°) with a north western aspect. The vines are spaced 2.75 m x 1 m (3636 vines/ha) and the soil classified as Oakleaf soil form originating from weathered granite (Soil Classification Working Group, 1991). Vines are trained to a uni-lateral cordon and pruned to five, two-bud spurs per plant. Foliage was managed by a vertical shoot positioned trellis system with three sets of movable foliage wires. Standard canopy management practices were applied during the growth season, including early suckering (the judicious removal of shoots not allocated during pruning), vertical positioning of shoots, and topping once shoots passed 30 cm of the top foliage wire. An annual cover crop of *Triticale spp.* was sown in autumn and controlled with herbicide before budburst, to ensure a mulch layer within the work row.

### 6.3.2 Experimental layout

Vineyards grafted onto the two rootstocks were located directly adjacent to one another. Considering the variables [clone (A-PI 50 vs B-PI 48), age (A-1997 vs B-1999) and slope (A-Top vs B-Bottom)] of the experimental vineyard, rootstock was not considered a treatment as such, but rather the combination of rootstock and site, therefore resulting in two measurement

sites: Site A (Pinotage/140 Ruggeri) and Site B (Pinotage/1103 Paulsen). Experimental plots were assigned by a randomised block design, selecting vine parcels from an area within the vineyard which displayed homogeneous vigour. Experimental plots consisted of 30 vines each and were replicated five times per ripeness level for each site.

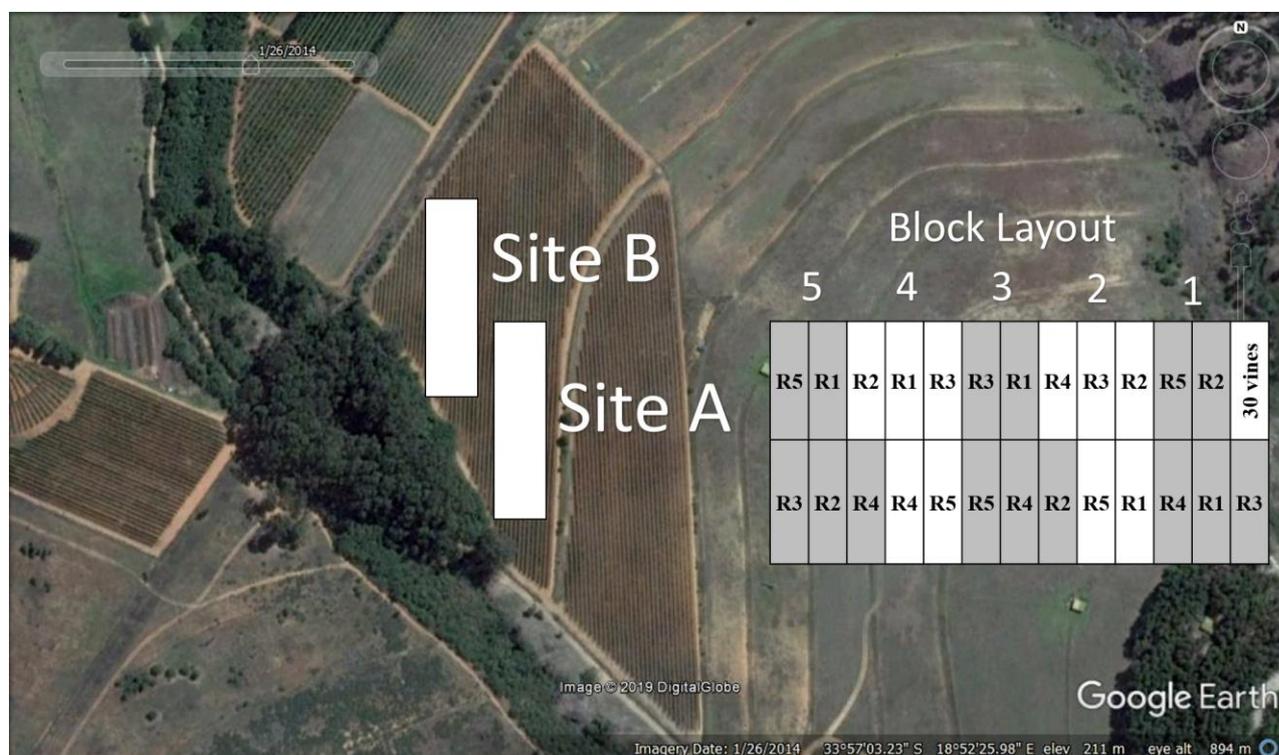


Figure 1. Experimental layout at Stellenbosch University Welgevallen experimental farm, indicating both measurement sites (A & B) and block design with five repetitions (blocks 1 -5) and five ripeness levels (R1-R5).

### 6.3.3 Experimental winemaking

Grapes were harvested at five targeted ripeness levels coinciding with *ca.* 21°Brix, 23°Brix, 25°Brix, 27°Brix and 29°Brix, respectively. Grapes were picked between 06:00 and 09:00 am in 40 kg batches per field replicate, transported to the winery (6 km) and stored over-night in a cold room (10°C) before processing. Grapes were then de-stemmed and crushed, with an addition of 40 ppm SO<sub>2</sub> and immediately inoculated with a commercial yeast strain (25g/hL, VIN 13, Anchor, South Africa). Fermentation temperature was maintained at *ca.* 25°C, with a standardised addition of yeast nutrients (80 g/hL Di-ammonium phosphate) 24 hours after inoculation. Skins were punched down manually three times a day for the first five days of fermentation. At dryness, wine was strained and pomace lightly pressed (pressure 1 Bar). Wines underwent cold stabilisation (0°C for 3 weeks), fining with bentonite (50 g/hL) and sterile filtration before bottling closure (free SO<sub>2</sub> adjusted 30 – 40 ppm), during May, under screw cap. Wines did not undergo malo-lactic fermentation.

### 6.3.4 Measurements and Analyses

Sensory and wine chemical analyses (Chapter 5 Table 1 & 2) of experimental wines, were conducted during the months of August and September, following the vintage. Wines were stored horizontally in a dark, temperature regulated cellar (16-20°C) after bottling (three months) before sensory analyses.

#### 6.3.4.1 Rapid Descriptive Analysis - Untrained Panel

A sensory panel of ten judges was used for the descriptive analyses of wines of both sites (Site A & B) and vintages (2015, 2016 & 2017). Panellists were recruited on the basis of previous experience of wine sensory analysis and were mainly employees of the ARC Infruitec-Nietvoorbij Research Institute. The judges were not remunerated, but regularly participated in other sensory evaluations with similar format at the Institute. The judges were informed of the scope of the study prior to the evaluation session. Thirteen descriptors were provided based on previous sensory evaluation of Pinotage (Van Schalkwyk & Schmidt, 2009) including visual, aroma, taste and overall descriptors (See Results and Discussion Table 1). Evaluations took place in off-white individual sensory booths in a quiet, well-ventilated, odourless 20°C air-conditioned room. For the testing sessions, wines were poured into clear glasses (25 mL aliquots) and presented in a randomised order to the judges, one sample at a time. Judges were prompted to rate the intensity of each descriptor along an unstructured line scale from “none” to “intense” using a pen and paper provided. Water and neutral crackers were available to counter sensory fatigue. Judges performed the sensory evaluation of 25 wines (five ripeness levels x five field replicates per site) on one day with each site (A & B) evaluated on separate days.

#### 6.3.4.2 Descriptive analysis – Trained Panel

The sensory panel for the quantitative descriptive analyses (QDA) of experimental wines consisted of eleven judges (10 females and 1 male) in 2015 and ten judges in 2016 (10 females). Panellists were recruited on the basis of previous experience of wine sensory analysis as well as the availability to take part in regular sessions for the duration of the experiment. The judges were remunerated and regularly participated in other sensory evaluations at the Department of Viticulture and Oenology at Stellenbosch University. The judges were not informed of the nature or goal of the study. Six one-hour training sessions preceded actual testing. During consensus training, descriptors generated by the panellists were defined using authentic aroma reference standards or model wine solutions for taste descriptors. References were used to familiarise all the panellists with the descriptors and to standardise their intensity scores for the various

descriptors. Initially, a large number of descriptors were identified. However, as the consensus training process progressed, the final lexicon was narrowed to 14 aroma descriptors and 6 taste descriptors (See Results and Discussion). For testing sessions, wines were poured in black glasses (25 mL aliquots) and covered with plastic Petri dish lids, one hour before serving. Each glass was labelled with a unique, random three-digit code. All evaluations took place in off-white individual sensory booths in a quiet, well-ventilated, odourless air-conditioned room (20°C). Five samples (one of each ripeness level) were presented to the judges in randomised order according to Williams Latin square design (Dahl *et al.*, 2008). Judges were prompted to rate the intensity of each descriptor along an unstructured line scale from “none” to “intense” using Compusense® five software (Release 5.6). Aroma was evaluated first, followed by taste descriptors. Water and neutral crackers were available to counter sensory fatigue. Judges performed DA on three sensory repetitions per sample set (five wines, one of each ripeness level) per day with a 15-minute break between sessions. Each of the five field replicates per site were evaluated individually on separate days.

#### *6.3.4.3 Napping Experienced Panel*

The sensory panel for the analyses of experimental wines consisted of 25 judges (7 males and 18 females). Panellists were recruited on the basis of previous experience of wine sensory analysis as well as the availability to take part in regular sessions for the duration of the experiment. The judges were remunerated and regularly participated in other sensory evaluations at the Department of Viticulture and Oenology at Stellenbosch University. Judges were not informed of the goal of the study, nor did they receive any training beforehand.

The sensory evaluation was conducted in a well-ventilated, odour free and temperature-controlled (20°C) room. Wines were poured in black glasses (25 mL aliquots) and covered with plastic Petri dish lids, one hour before serving. Glasses were coded with random three digit codes. Judges received 15 wines (5 ripeness levels x 3 field replicates) per session of each Site (A & B) and each judge received a different order of wines according to a Williams Latin square design. Evaluation of the wines were conducted in triplicate (on separate days). Aroma and taste evaluations were also tested separately with a 15 min break in between. Judges had to freely describe the aroma of each of the 15 wines using three to five descriptors and jot them down on the post-it note provided. After all the wines were described, judges were asked to freely arrange the wines on a blank A2 page, fixing the post-it notes with descriptors to the page and marking the final position with an X. They were informed that wines placed close together would be more similar than those placed far from each other.

#### 6.3.4.4 Napping Expert Panel

The expert Panel consisted of 25 wine industry professionals (12 male and 13 female), the majority were members of the interest group called the Pinotage Association. The participants attended regular tasting events as part of their occupation. No training or information regarding the study was provided before the sensory analysis, and the judges were not remunerated for their participation. The sensory evaluation was conducted as described in 6.3.4.3 *Napping Experienced Panel*, however, in this case the evaluation was conducted during a single session for each site (A & B) with a 15 min break in between.

Table 1. Various sensory analyses conducted during the study.

	Untrained DA	Trained DA	Panel NAP	Expert NAP
2015	✓	✓		
2016	✓	✓		
2017	✓		✓	✓

#### 6.3.5 Statistical analysis

In untrained rapid descriptive analysis, data was subject to analysis of variance (ANOVA), using the restricted maximum likelihood (REML) function to discard outliers. The significance threshold was set at  $p = 0.05$  and the Fishers's LSD post-hoc test was used to show significant differences of least squared (LS) means.

In trained descriptive analysis, the multiple sensory repetitions (3x) allowed for the evaluation of the performance of the panel using PanelCheck (V1.4.2), according to the workflow suggested by Tomic *et al.* (2007). The discriminability and consensus of the panel were evaluated by means of analysis of variance (ANOVA) and Tucker-1 plots. The significance threshold was set at  $p = 0.05$  and the Fisher's LSD post-hoc test was used to show significant differences of LS means. Principal component analysis (PCA) was also performed to illustrate correlations between attributes and samples.

Multiple Factor Analysis (MFA) was used for the combination of wine compositional data and sensory data from descriptive analysis using the trained panel. This analysis allows for the combination of various data tables, by using the Eigen values of PCA and Multiple Correspondence Analysis (MCA) of the compositional and sensory data tables to carry out a weighted PCA of the combined table. In this study, the individual factor map was used to display relative grouping of ripeness levels (treatments), while the correlation circle (PCA) display correlation (association) between variables on the same plane.

Napping data was captured by representing the position of individual descriptors of the wines as X and Y coordinates according to methodology proposed by Pagès, (2005). These coordinates were related to categorical data provided by descriptors being present or absent. Correspondence analysis (CA) was used to display the relative correspondence between columns (ripeness levels) and rows (descriptors) for both expert and sensory panel Napping analysis.

## 6.4 RESULTS AND DISCUSSION

### 6.4.1 Rapid descriptive analysis

Scores of rapid descriptive analysis of wines from different grape ripeness levels were combined for both sites and vintages (Table 2), as ANOVA results displayed negligible significance for these variables in addition to interactions limited to palate descriptors, acidity, body, persistence/length and quality (Table 3). As expected from grape and wine compositional analyses (See Chapters 4 & 5), the wine colour intensity rating increased from early ripeness levels to peak at R4. Aroma intensity, fruitiness, and vegetative characters showed similar increases with an advancement in ripening, stabilising at later ripeness levels. Surprisingly, vegetative characters were not perceived intensely in early ripeness levels, but increased as ripening progressed. This seems contrary to the expected course of development in aroma profile. After investigating the wines which showed high vegetative intensity, the commentary section of the tasting sheets revealed descriptors such as cooked vegetables and cabbage, rather than grassy, leafy, asparagus notes. It seems as if fermentation-derived off flavours, in particular di-methyl sulphide (Escudero *et al.*, 2007), could have played a role in vegetative perception rather than greenness related to ripeness level from e.g. IBMP (Allen *et al.*, 1995). Sweet spice and jammy flavours increased in parallel to increased perceived alcohol, body and persistence. Significant differences were lowest for the descriptors of floral notes and astringency. The latter is a difficult descriptor to define (Harrison, 2018), as it is related to a tactile sensation (Kennedy, 2008), linked to the interaction of phenolic compounds with salivary proteins and in combination with various other wine components (Gawel, 1998) that may also subdue the astringency perception. Astringency is thus also subject to large variation in perception amongst tasters, due to differential physiological abilities (Fischer *et al.*, 1994; Gawel, 1998). Therefore, this result may point to a greater need for training/calibration, rather than insignificant responses. Finally, regarding overall quality, ripeness levels R2-R5 displayed comparable levels. This surely reflects varied preferences/perception of the various untrained judges. Quite importantly, it also highlights that good preferences/quality scores were obtained over a range of ripeness levels (R2-R5), despite significant differences in perceived intensities of visual, aroma and taste attributes. This finding encouraged the further investigation of sensory profiles in an attempt to

define the perceived differences with more detailed descriptors and to affirm trends seen in rapid descriptive analysis. In particular, further investigations were also aimed at assessing the effects of vintage and site, which were minor in rapid descriptive analysis, but proved to be highly significant for grape and wine composition.

Table 2. Mean scores (%) for rapid descriptive analysis of Pinotage wines of five ripeness levels, sites (A & B) and vintages (2015-2017) combined.

	Ripeness Level				
	R1	R2	R3	R4	R5
Colour	66.36d	79.41c	81.33bc	86.83a	85.35ab
Aroma Intensity	55.77c	60.02b	63.65a	64.88a	64.98a
Fruitiness	37.49c	42.75b	46.68ab	49.34a	46.68ab
Vegetative	24.63b	29.96ab	33.06a	33.75a	33.86a
Floral	20.30a	22.27a	22.12a	21.13a	22.11a
Sweet Spice	18.88c	24.72b	27.63b	28.59ab	32.25a
Jammy	15.47c	18.42bc	22.52ab	25.85a	27.64a
Alcohol	42.78d	47.77c	50.19bc	52.22ab	55.03a
Astringency	50.20a	54.63a	53.67a	53.72a	53.69a
Acidity	55.45a	52.47ab	51.84b	50.32b	50.50b
Body	40.56d	52.57c	55.77bc	58.23ab	60.25a
Persistence/Length	42.83d	54.31c	57.04bc	59.66ab	60.58a
Quality	41.84c	53.53b	56.78ab	57.70a	57.21ab

<sup>1</sup>Means followed by the same letter within a row do not differ significantly ( $p \leq 0.05$ )

Table 3. ANOVA results of mean scores in descriptive analysis of Pinotage wines as presented in Table 2.

	Vintage	Site	RL	V x S	RL x V	RL x S	RL x S x V
Colour	<i>ns</i>	<i>ns</i>	***	<i>ns</i>	<i>ns</i>	**	<i>ns</i>
Aroma Intensity	<i>ns</i>	<i>ns</i>	***	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
Fruitiness	<i>ns</i>	<i>ns</i>	**	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
Vegetative	<i>ns</i>	<i>ns</i>	**	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
Floral	<i>ns</i>	<i>ns</i>	***	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
Sweet Spice	<i>ns</i>	<i>ns</i>	***	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
Jammy	<i>ns</i>	*	***	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
Alcohol	<i>ns</i>	<i>ns</i>	***	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
Astringency	<i>ns</i>	<i>ns</i>	***	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
Acidity	<i>ns</i>	<i>ns</i>	***	<i>ns</i>	***	<i>ns</i>	<i>ns</i>
Body	<i>ns</i>	<i>ns</i>	***	*	***	<i>ns</i>	***
Persistence/Length	<i>ns</i>	<i>ns</i>	***	<i>ns</i>	***	*	<i>ns</i>
Quality	<i>ns</i>	<i>ns</i>	***	<i>ns</i>	***	**	*

*ns* non-significant ; \*  $p \leq 0.05$  ; \*\*  $p \leq 0.01$  ; \*\*\*  $p \leq 0.001$

#### 6.4.2 Descriptive analysis – Trained Panel

In general, wines were aromatic and allowed for the identification of numerous detailed descriptors (14 aroma and 6 taste) during consensus training (Table 4). The majority of the descriptors identified were previously noted in the official Pinotage aroma wheel (See Chapter 2.2, Figure 4) (Marais & Jolly, 2004) and served as general confirmation that wines were of high quality and typical of variety. However, there were some slight deviations compared to that published by Marais & Jolly (2004). The Pinotage aroma wheel only included the floral descriptor noting violets, whereas the panel identified floral notes as more similar to roses and elderflowers, although violets were mentioned during initial training sessions (Table 4). Furthermore, two negative attributes (acetone and cooked vegetables) were also included in the QDA lexicon and were not present in the aroma wheel. The acetone descriptor is also referred to as fusel/solvent like and is thought to be due to the increased presence of higher alcohols (isobutanol, butanol, isoamyl alcohol) resulting from yeast metabolism (De-la-Fuente-Blanco *et al.*, 2016). The concentration of higher alcohols have been related to both yeast strain and the concentration of free amino acids (valine and leucine) in the fermenting must (Giudici *et al.*, 1990). Similarly, the cooked veg descriptor is often related to sulphur-related off-flavours in wines. Both H<sub>2</sub>S (Ribéreau-Gayon *et al.*, 2006) and di-methyl sulphide (Bindon *et al.*, 2014) are linked to yeast metabolism stresses, such as high fermentation temperature, high ethanol content and low must nitrogen composition (Ribéreau-Gayon *et al.*, 2006). Furthermore, descriptors that have been identified before as impact odours were also present within the lexicon and included banana (isoamyl acetate), prunes ( $\beta$ -damascenone) and vanilla (ethyl octanoate) (Van Wyk *et al.*, 1979; Waldner & Marais, 2002; Marais & Jolly, 2004; Louw *et al.*, 2010).

Table 4. Lexicon developed during consensus training, including reference standards and additional descriptors for Pinotage wines.

Selected Descriptors	Reference standard	Additional descriptors as recorded during training <sup>1</sup>
<b>Aroma</b>		
Acetone	Nail polish remover	Solvent, chemical
Banana	Fresh cut banana	
Cooked veg	Brine from canned green beans	sulphur, reductive, cabbage
Dark berries	Crushed black and blue berries (1:1)	Black berries, mulberry
Elderflower	<i>Vérdenne Elderflower</i> (2mL) in water (10mL)	Violets, fresh flowers
Eucalyptus	Crushed leaves (fresh)	Menthol, spearmint
Herbaceous	Kikuyu grass (fresh)	Cut grass
Prunes	Dried prune (cut into pieces)	Raisin, dried fruit, confectioned
Red berries	Crushed raspberry and strawberry (1:1)	Raspberry, cranberry
Rose	<i>Vérdenne Rose</i> (2mL) in water (10mL)	Potpourri
Savoury	Soya sauce	Soya, animal, marmite
Sweet spice	Cinnamon, nutmeg and cloves (2:1:1)	Cinnamon, nutmeg, clove
Tobacco	Tobacco from cigarette	Oak, wood, pencil shavings
Vanilla	Vanilla essence	Caramel, candy, sweet
<b>Palate</b>		
Acidity	Tartaric acid in water (1 – 5 g/L)	Sourness
Alcohol	Ethanol (99%) in water (10 – 15 % v/v)	Heat, burning sensation
Astringency		Tannic
Body	Methyl cellulose in water (0.1 – 0.5 g/L)	Mouth feel
Concentration	Wine samples with added H <sub>2</sub> O (5 – 15 %)	Palate weight
Length	Time (seconds) of flavour persistence	After taste, persistence

<sup>1</sup>Redundant/synonymous descriptors identified during consensus training

Similar to rapid descriptive analysis, ripeness level was consistently significant in terms of intensity scores for most sensory descriptors identified during training (Tables 5 & 6). Only the aroma descriptors banana and eucalyptus were non-significant with regard to ripeness level out of the 20 that were assessed. The banana aroma is caused by the acetate ester isoamyl acetate and is an important odorant in Pinotage (Van Wyk et al., 1979). From the scores one can deduce that the banana aroma was prominent in the wines and may have been so prominent that differences according to ripeness level were difficult to assess. The effects of vintage and site were more prominent than observed during rapid descriptive analysis, yet remained for the most part insignificant. Of those that were significant, some were negatively associated descriptors, such as acetone, cooked veg and herbaceous, while both vanilla and body showed some significance regarding vintage. The savoury descriptor was the only significant attribute regarding site. Overall, increasing ripeness levels increased acetone, dark berries, prune and sweet spice aroma, while red berries and herbaceous followed a decreasing trend with increased ripeness level.

Moreover, a large amount of descriptors did not follow an obvious increasing/decreasing trend during ripening. For instance, elderflower and eucalyptus peaked at R3, while the rose descriptor was just as prominent at the first ripeness level than at the last, and lowest at R3. With regard to the wine chemical compositional analysis, this reaction would be expected, since this was similar to compositional accumulation.

On the other hand, the taste descriptors displayed clear trends during ripening with increasing alcohol, astringency, body concentration and length with progressive ripeness level, and decreasing acidity. Nonetheless, principal component analysis was employed to assess vintage differences, site and the particulars in terms of evolution from red – dark fruit profile.

Table 5. Mean scores (%) for descriptive analysis with a trained panel of Pinotage wines of five ripeness levels, sites (A & B) and vintages (2015-2016) combined.

Selected Descriptors	R1	R2	R3	R4	R5
<b>Aroma</b>					
Acetone	12.51b	16.89ab	19.62a	21.97a	24.16a
Banana	39.24a	44.14a	44.14a	46.20a	47.99a
Cooked veg	10.15c	10.61c	26.89a	20.55ab	16.41b
Dark berries	24.48b	35.65ab	38.73a	39.88a	42.57a
Elderflower	17.77ab	16.22ab	19.68a	14.47b	12.47b
Eucalyptus	13.38a	11.42a	14.67a	13.49a	13.62a
Herbaceous	29.45a	19.55b	21.15b	18.53b	18.33b
Prunes	30.43b	35.53ab	32.69ab	35.56ab	38.02a
Red berries	35.29a	21.73b	16.55c	16.59c	16.26c
Rose	27.49a	26.10a	22.41b	27.64a	30.51a
Savoury	9.24c	12.78b	18.31a	16.05ab	15.10ab
Sweet spice	18.30b	19.28b	17.79b	21.61a	24.20a
Tobacco	18.66b	21.30a	19.87ab	20.82a	22.96a
Vanilla	33.16b	38.25a	34.53ab	39.03a	39.71a
<b>Palate</b>					
Acidity	66.12a	55.28b	48.51c	44.76d	40.49e
Alcohol	35.59e	47.47d	53.88c	60.33b	66.42a
Astringency	53.28c	61.11ab	60.64b	63.55a	66.86a
Body	37.16c	46.65bc	53.07b	57.10b	63.09a
Concentration	38.71d	48.83c	54.43b	58.63ab	63.84a
Length	37.04d	47.33c	53.40b	57.92a	62.87a

<sup>1</sup>Means followed by the same letter within a row do not differ significantly ( $p \leq 0.05$ )

Table 6. ANOVA results of mean scores in descriptive analysis of Pinotage wines as presented in Table 5.

Selected Descriptors	Vintage	Site	RL	V x S	RL x V	RL x S	RL x S x V
<b>Aroma</b>							
Acetone	**	<i>ns</i>	***	<i>ns</i>	<i>ns</i>	**	***
Banana	<i>ns</i>						
Cooked veg	***	<i>ns</i>	***	<i>ns</i>	<i>ns</i>	<i>ns</i>	***
Dark berries	<i>ns</i>	<i>ns</i>	***	<i>ns</i>	***	***	<i>ns</i>
Elderflower	<i>ns</i>	<i>ns</i>	***	<i>ns</i>	<i>ns</i>	<i>ns</i>	***
Eucalyptus	<i>ns</i>						
Herbaceous	**	<i>ns</i>	**	<i>ns</i>	*	*	***
Prunes	<i>ns</i>	<i>ns</i>	***	***	<i>ns</i>	<i>ns</i>	<i>ns</i>
Red berries	<i>ns</i>	<i>ns</i>	*	<i>ns</i>	***	*	<i>ns</i>
Rose	<i>ns</i>	<i>ns</i>	*	<i>ns</i>	<i>ns</i>	<i>ns</i>	***
Savoury	<i>ns</i>	**	**	<i>ns</i>	**	<i>ns</i>	***
Sweet spice	<i>ns</i>	<i>ns</i>	**	<i>ns</i>	**	<i>ns</i>	<i>ns</i>
Tobacco	<i>ns</i>	<i>ns</i>	***	<i>ns</i>	<i>ns</i>	*	***
Vanilla	**	<i>ns</i>	***	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
<b>Palate</b>							
Acidity	<i>ns</i>	<i>ns</i>	***	<i>ns</i>	***	**	***
Alcohol	<i>ns</i>	<i>ns</i>	***	***	***	<i>ns</i>	***
Astringency	<i>ns</i>	<i>ns</i>	***	*	<i>ns</i>	<i>ns</i>	***
Body	*	<i>ns</i>	***	<i>ns</i>	*	<i>ns</i>	***
Concentration	<i>ns</i>	<i>ns</i>	***	<i>ns</i>	***	<i>ns</i>	***
Length	<i>ns</i>	<i>ns</i>	***	<i>ns</i>	**	<i>ns</i>	***

*ns* non-significant ; \*  $p \leq 0.05$  ; \*\*  $p \leq 0.01$  ; \*\*\*  $p \leq 0.001$

Principal component analysis of individual vintages showed less coherency in loadings in 2015 compared to 2016 (Figs 2 & 3). However, in both vintages the separation of ripeness level was found to be along the first principal component (F1 56% 2015 and F1 84% 2016), separating wine samples from earlier (R1-R2) to later (R4-R5) ripeness levels, with R3 situated on the limit. Earlier ripeness levels associated with increased acidity, red berries and herbaceous characters, while later ripeness levels displayed increased body, concentration, alcohol (perception), length, dark berries and prunes. This evolution in profile was consistent for both sites and vintages. Interestingly, sites separated on F2 in 2015 (21%), but not in 2016 (6%). However, a noteworthy observation is the consistency of grouping of the descriptors that separated on the F2 axis in both vintages. High intensities, especially at R3, of cooked veg, elderflower, and eucalyptus and savoury notes, coincided with diminished intensities of rose, tobacco, vanilla and prunes at R3. This possibly rather points to the antagonistic effects of increased cooked veg (di-methyl sulphide) aromas (Escudero *et al.*, 2007; Bindon *et al.*, 2014) on other descriptors than an effect

of ripeness level *per se*. A standard adjustment of must nitrogen content was made 24h post yeast inoculation. Observations from fermentation curves (data not shown) suggest that fermentation had already progressed substantially for the R3 treatment by the time nitrogen content was adjusted in both vintages.

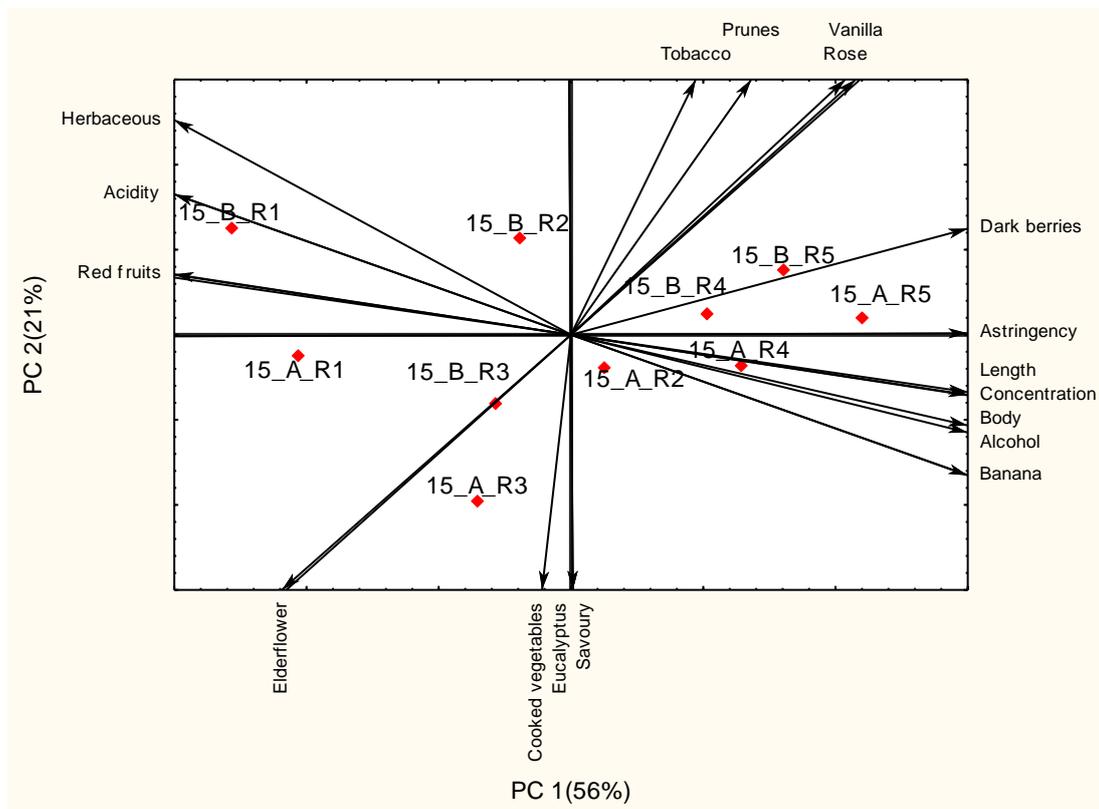


Figure 2. Principal component analysis of aroma and taste intensities from descriptive analysis (QDA) of cv. Pinotage wines from five ripeness levels (R1-R5), and two sites (A & B), for the 2015 vintage.

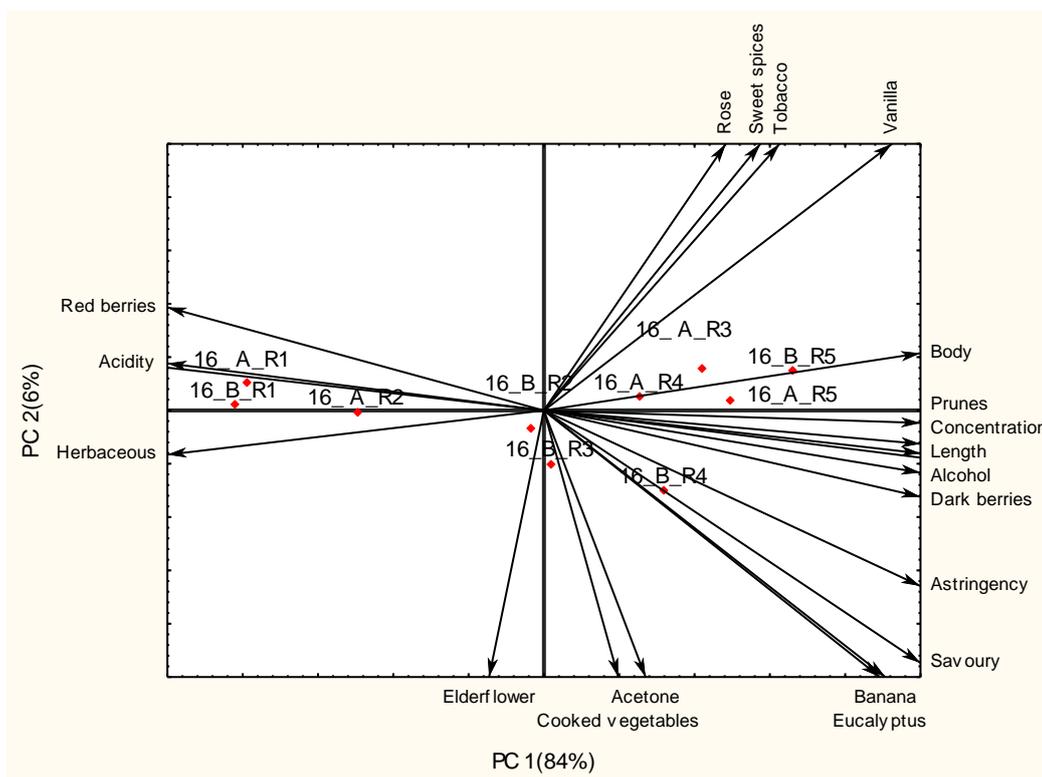


Figure 3. Principal component analysis of aroma and taste intensities from descriptive analysis (QDA) of cv. Pinotage wines from five ripeness levels (R1-R5), and two sites (A & B), for the 2016 vintage.

The results from the descriptive analysis confirmed the importance of taste as well as the descriptors associated with the various extremities, but also pointed towards high aromatic expression in ripeness levels R3-R4-R5. From the combination of the PCA plots (see  $R^2$  values in Addendum D Table 2) and ANOVA of the various descriptors it was clear that taste descriptors, in particular acidity, body, concentration, alcohol and length, to a large extent dictated the sensory response to ripeness level. Despite the large influence of vintage and site seen on compositional level, overall sensory profile did not vary significantly from vintage to vintage and by site. This places even more emphasis on the dictating effect of grape ripeness related changes with regard to choices of sugar and acidity levels. The coherency in terms of response over vintages was encouraging, yet a pertinent question was prompted regarding the large number of descriptors identified during analysis. As judges were prompted to rate the intensity of all the identified descriptors, the question was, which descriptors they would revert to if they had to use their own vocabulary.

### 6.4.3 Napping Experienced Panel

The Napping method allowed for the capturing of unique aroma (Fig 4) descriptors by panellists in wines of the 2017 vintage. Correspondence analysis plotted on a 2D plane, explained 77.09% of inertia (Dimension1 54.74% and Dimension2 22.35%). On the basis of relative distances plotted on a plane for each sample, the main effect (Dim1) regarding the aroma description was site, separating site A, characterised by candy, dark fruit and earthy descriptors, from site B which was characterised with descriptors of liquorice, smoky/soya, prune/jammy and cooked veg aromas. However, the wines from both sites shared many descriptors which were positioned close to or on the Dim1 axis (Addendum D Fig 2). Ripeness level separated wines in the second dimension, but the progression of attributes were less clear. Especially in site A, the aroma of the first four ripeness levels (R1-R4) was grouped closely on the Dim2 axis, with substantial distance from R5. In site B, ripeness levels were more distinguishable, but again ripeness levels did not follow separation in order of sequential harvesting. Here it seems as if the cooked veg descriptor was likely to have disrupted the expected response. In general, panellists were very sensitive to the cooked veg descriptor and often placed these samples at the extremities of the visualisation plane (data not shown). This is an important factor, as in descriptive analysis it appears to have a serious impact on the judges that may mask and even completely negate expected sensory outcomes (Escudero *et al.*, 2004).

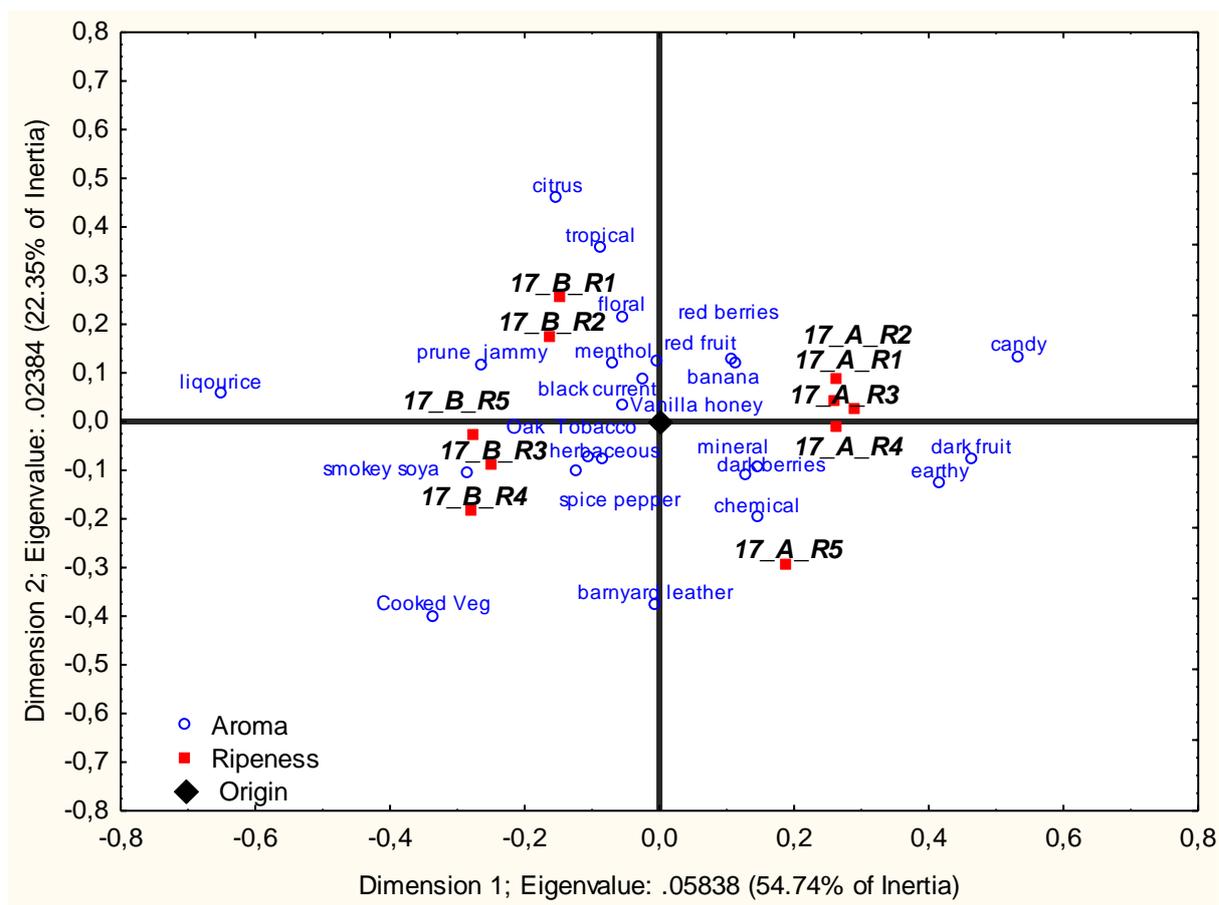


Figure 4. Correspondence analysis of aroma descriptors generated by Napping technique performed by an untrained panel of cv. Pinotage wines from five ripeness levels (R1-R5) and two sites (A & B), for the 2017 vintage.

Nonetheless, the correspondence analysis displayed a distinctive collection of ‘most’ prominent aroma attributes that could discern both site (A versus B), but also ripeness level (early *versus* late). The aroma response is summarised in Table 7 and is indicative of descriptors commonly encountered in the market place, that were significantly altered (See confidence ellipses, Addendum D Fig 2) by both site and ripeness level.

Table 7. Summary of Aroma descriptors derived from Napping analysis and Correspondence Analysis for Pinotage wines from two sites (A & B) for the 2017 vintage.

Site A		Site B	
Early (R1-R3)	Late (R4-R5)	Early (R1-R2)	Late (R3-R5)
Red berries	Mineral	Citrus	Oak/Tobacco
Red fruit	Dark berries	Tropical	Herbaceous
Banana	Dark fruit	Floral	Smokey/Soya
Candy	Earthy	Menthol	Spice/Pepper
	Chemical	Blackcurrant	Cooked Veg
		Vanilla/Honey	Barnyard/Leather
		Liquorice	

<sup>1</sup>Descriptors were derived from each quadrant of Correspondence analysis (CA), explaining 77.09 % of sample set inertia.

Napping results of taste descriptors were uniform due to significantly less descriptors that were used as well as greater consensus among panellists. Correspondence analysis explained 97.2% of inertia (Dim1 93.66% and Dim2 3.54%) for the six taste descriptors that were identified (Fig 5). The taste descriptors differed from that identified during training in DA, by the inclusion of both sweetness and bitterness, and exclusion of length and concentration.

The early ripeness levels were characterised by higher acidity, body and astringency, while later ripeness levels increased alcohol, bitterness and sweetness as previously reported in Cabernet Sauvignon and Merlot (Casassa *et al.*, 2013; Heymann *et al.*, 2013; Bindon *et al.*, 2014). This is in line with the expected response, as previous studies investigating these attributes individually *e.g.* found increases in sweetness perception with increases in alcohol (Zamora *et al.*, 2006) as well as increases in bitterness perception (Fischer & Noble, 1994; Fontoin *et al.*, 2008) and decreases in acidity. From the strong relationship seen in correspondence analysis, it would seem that increases in wine alcohol content were well perceived by panellists, as with DA analysis, and together with increases in wine phenolics and decreases in acidity along the ripeness gradient, provided a distinct discriminative axis between wines of different ripeness levels. Contrary to aroma, site played a diminished part in the reaction on taste, displaying a minor level of separation on Dim2 (3.54%) of site A and B. It would seem as if site A displayed more prominent relative alcohol, astringency and acidity descriptions, while site B was perceived with more body, bitterness and sweetness (all wines < 2 g/L sugar).

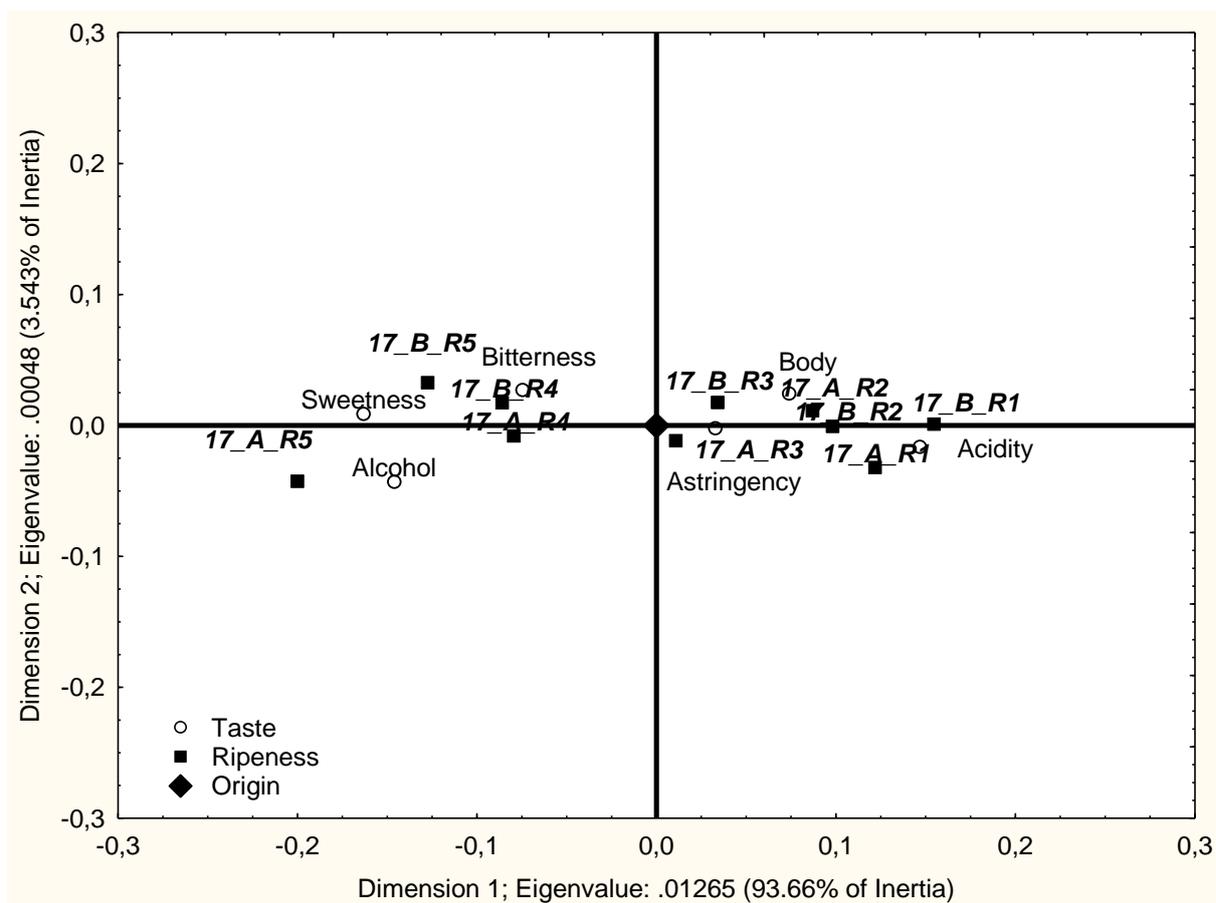


Figure 5. Correspondence analysis of taste descriptors generated by Napping technique performed by an untrained panel of cv. Pinotage wines from five ripeness levels (R1-R5) and two sites (A & B), for the 2017 vintage.

#### 6.4.4 Napping Expert Panel

Napping analysis conducted with Pinotage producers (experts) in 2017, combined both aroma and taste descriptors in order to ascertain if trends seen using QDA and Napping with experienced sensory panellists were comparable to those from producer perspectives (Fig 6a). By way of only allowing experts a maximum of five descriptors per wine, pertaining overall, aroma and taste, an attempt was made to understand which descriptors were of highest importance from a practical point of view. Correspondence analysis of data explained 72.54% of the inertia (Dim1 55.00%, Dim2 17.54%) and separated the wines according to ripeness level, displaying a progression of the sensory profile during ripening. Experts collectively used 36 unique descriptors to describe wines. Wines from early ripeness levels were perceived as lean, acidic, neutral and short, progressing to unripe, ripe and overripe associated descriptors (Table 8) along the ripeness level gradient (Dim1). Interestingly, individual ripeness levels were generally separated in the four quadrants of the CA plot. This was not the case for ripeness levels R1 and R2, which generally showed large degree of commonality in sensory profile (Fig 6b), but from R3 onward sensory profiles become more distinctive.



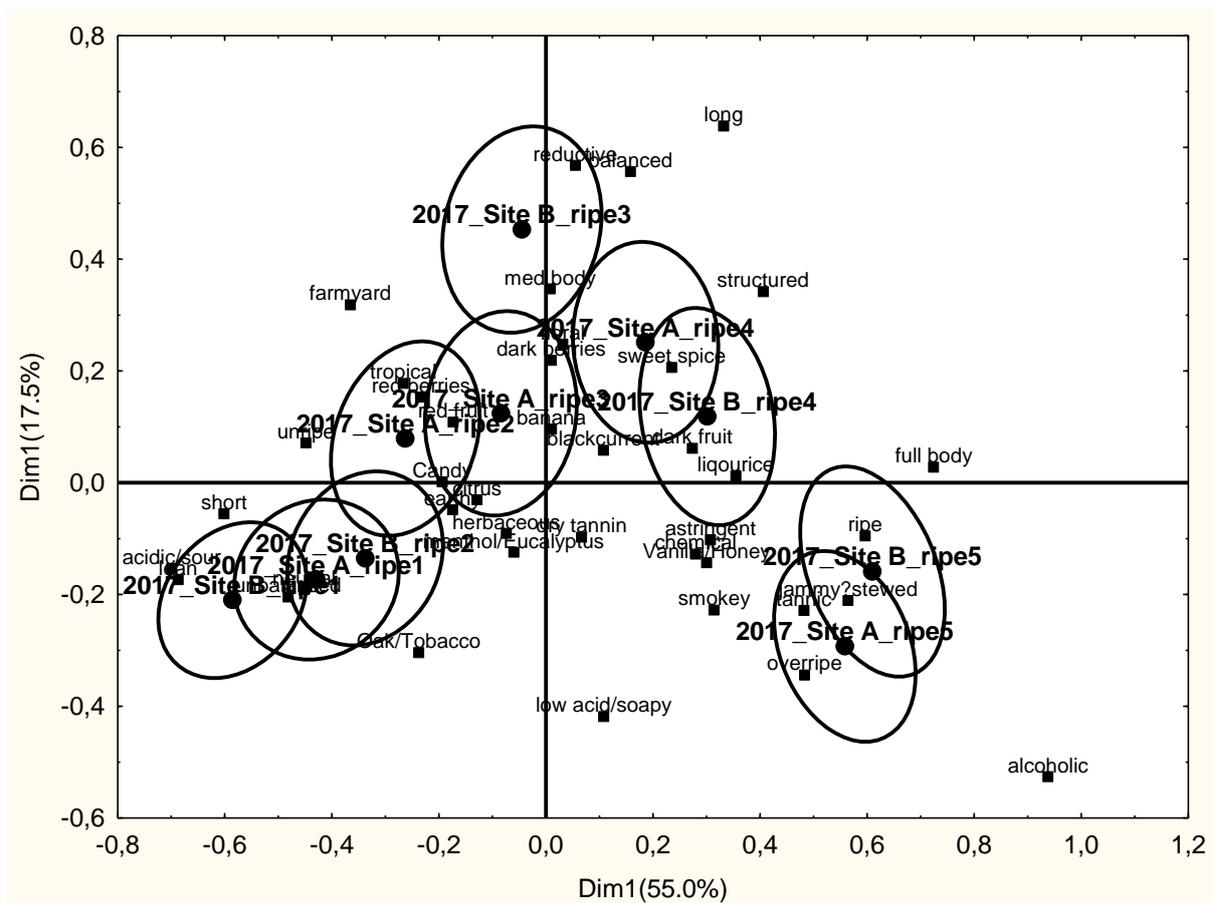


Figure 6b. Correspondence analysis of taste descriptors generated by Napping technique performed by wine experts for cv. Pinotage wines from five ripeness levels (R1-R5), and two sites (A & B), for the 2017 vintage. (Confidence ellipses 0.05)

The descriptors chosen by experts were grouped according to their prevalence in each of the quadrants (Table 8). From this it was clear that some ripeness levels were characterised by a greater number of descriptors (e.g. R4) than others (e.g. R2-R3). Nonetheless, there was a clear distinction in terms of stylistic descriptors in each category (ripeness level). From the data provided by the experts, R4 had more positive descriptors, while R1-R2 the most negative associated descriptors. According to the experts, the evolution during ripening in terms of aroma was from green/herbaceous to red fruit/tropical to complex/dark fruit/floral/spice to jammy/smoky/vanilla. In terms of taste, the change in descriptors for body was less clear. The evolution from neutral/lean/short to med bodied (R3-R4) and full-bodied were not accurately defined according to quadrants as the descriptors were placed on the axis perimeter. The data confirmed a stylistic change over the range of ripeness levels. In addition, the change seen in expert napping confirmed trends seen in other sensory testing modalities. Importantly, the expert Napping testing of a single session for each site, proved a useful rapid method, providing sound data.

Table 8. Summary of aroma and taste descriptors derived from Napping analysis with experts and Correspondence Analysis for Pinotage wines from two sites (A &amp; B) for the 2017 vintage.

Napping Expert Descriptors			
R1-R2	R2 - R3	R4	R5
Lean	Unripe	Medium bodied	Low acid/Soapy
Acidic/Sour	Red fruit	Full bodied	Tannic
Short	Tropical	Structured	Dry tannin
Neutral	Candy	Balanced	Astringent
Unbalanced	Farmyard	Long	Ripe
Oak/Tobacco		Reductive	Overripe
Menthol/Eucalyptus		Banana	Jammy/Stewed
Herbaceous		Dark berries	Smoky
Earthy		Sweet spice	Vanilla/Honey
		Floral	Chemical
		Liquorice	
		Blackcurrant	

<sup>1</sup> Descriptors were derived from each quadrant of Correspondence analysis (CA), explaining 72.54% of sample set inertia.

#### 6.4.5 Relating sensory response to compositional data

One of the biggest challenges when comparing large comparative data sets is the visual distortion due to the large number of variables. Therefore previous studies following a comparative approach (Cadot *et al.*, 2012; Bindon *et al.*, 2014) endeavoured to reduce the number of variables by removing perceived redundant components with similar reaction (co-correlation), components that were present with low odour active values (OAV specific concentration over putative odour threshold value) and components that do not differ significantly across samples. This enables the better visualisation of selected variables through simplified representation of comparative data sets. Yet, on the other hand literature presents many examples of components impacting sensory outcomes through multiple interactions, despite values below OAV (Escudero *et al.*, 2004, 2007; Peinado *et al.*, 2004; Pineau *et al.*, 2007; Demiglio & Pickering, 2008; Schelezki *et al.*, 2018; Wilson *et al.*, 2018). The major impact that the wine matrix can have on the perception of volatile and non-volatile components remains a major challenge for interpreting compositional/sensory data (Escudero *et al.*, 2007). Considering the possibilities of interaction as well as the possible collective contribution of components to a singular sensory descriptor, the full data set was visualised. The sensory data that was generated with descriptive analysis by the trained sensory panel (See Descriptive analysis – Trained Panel only for vintage 2015 & 2016), including the wine compositional data sets (See Chapter 5) from two vintages (2015 & 2016) as well as the two sites (A & B), were pooled in an attempt to include as much known variability as possible, to ascertain if ripeness level related changes were universal in the context of this study.

The individual factor map (IFM) generated by the MFA (Fig 7), indicated a separation of the various ripeness levels on the first dimension (Dim1 32.6%), displaying the progression of ripeness levels along the ripening gradient for both sites (R1-R5) as the main effect. Secondary to this, data points were separated in terms of vintage on the second dimension (Dim2 22.4%). This separation confirmed that in broad, wine compositional and sensory profile changes were consistent with regard to increased ripeness level, with noteworthy vintage effects.

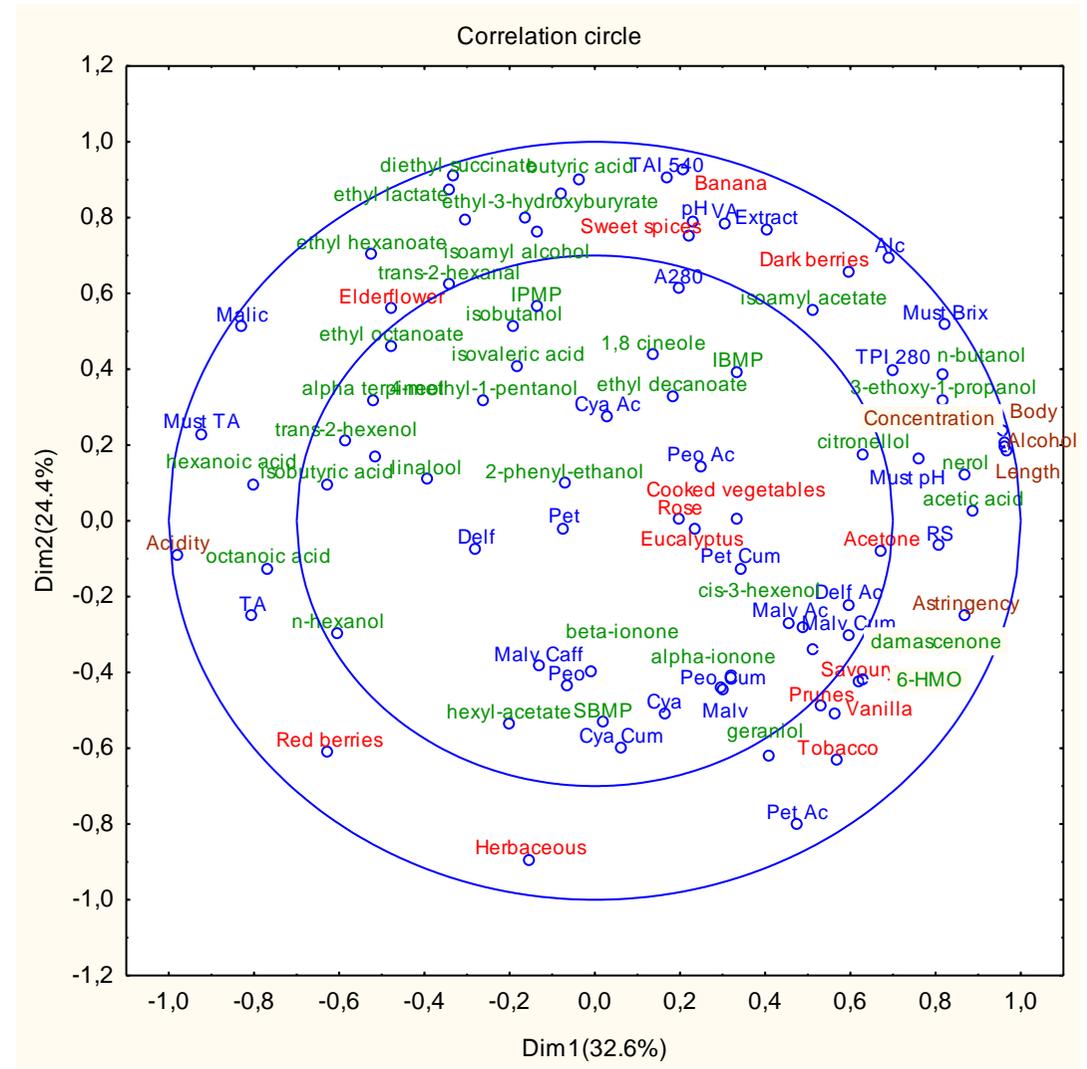
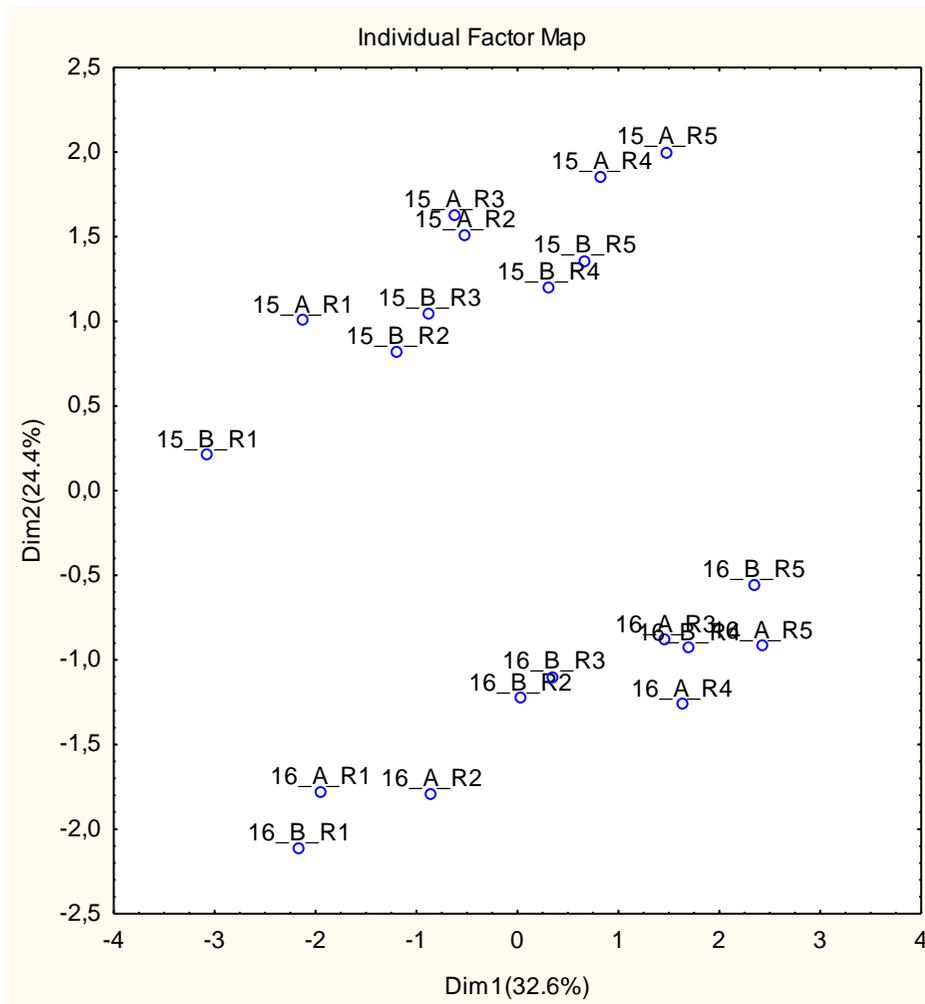


Figure 7. Multi factorial analysis, combining wine composition and sensory data obtained from descriptive analysis by a trained panel for cv. Pinotage wines from five ripeness levels (R1-R5) and two sites (A & B), for the 2015 and 2016 vintages. (Red =QDA Aroma, Brown = QDA Taste, Blue Wine chemical analysis, Green = Wine volatile analysis) (Outer correlation circle corresponds to  $R^2= 1$ , and inner circle to  $R^2= 0.7$ )

The correlation circle (PCA) displayed important associations of components and descriptors laying relatively close to one another, with the additional layer representing variables with better (outer circle) or worse (inner circle) correlation to separation viewed in the CA.

Wines of earlier ripeness levels were characterised by aroma descriptors of red berries, herbaceous and elderflower. The elderflower descriptor associated well with esters of short chain fatty acids, especially ethyl octanoate, known to impart green apple/floral aroma to wines. Ethyl octanoate is an important fermentation derived impact odorant of Pinotage wines (Marais *et al.*, 1981; Louw *et al.*, 2010). Red berry and herbaceous descriptors did not show distinctive association with specific compounds. Herbaceous aroma would be expected to correlate well with methoxyprazines (IBMP, SBMP and IPMP), but here only SBMP showed association with herbaceous aromas. Similarly, the red berries descriptor showed limited association with the volatiles included in the study. Hexyl acetate, together with n-hexanol were the compounds closest related to the red berries descriptor. Both these compounds were associated with early ripeness levels in Cabernet Sauvignon, similarly showing significant decline with fruit maturation (Bindon *et al.*, 2014). Hexyl acetate is also an ester that has been identified as important discriminator for Pinotage wines, contributing fruity/berry aromas (Marais *et al.*, 1981; Louw *et al.*, 2010). The relationship between hexyl acetate and n-hexanol is also likely as Dennis *et al.* (2012) proposed the formation of hexyl acetate from C6-alcohol precursors such as hexanol. From a general point of view aroma profile was dominated by C6 alcohols and aldehydes, short chain fatty acids and esters thereof, all of which have been implicated with a general red fruit profile (Bindon *et al.*, 2013, 2014). From the separation seen in the IFM during the early ripeness levels, the 2015 vintage displayed higher levels of fermentation-derived compounds compared to 2016, thereby displaying a more elderflower dominated early ripening profile in 2015 compared to 2016, which had better association to red berries and herbaceous notes.

Increased aromas of sweet spice, banana and dark berries as ripening progressed displayed good association with increased higher alcohols, esters and fatty acids. Importantly, the increasing dark berries and banana attributes were linked to isoamyl acetate that is a well-known fermentation odorant in Pinotage wine (Van Wyk *et al.*, 1979; Marais *et al.*, 1981; Louw *et al.*, 2010). Ripening also increased the aromas of tobacco, savoury, vanilla and prunes in association with increased norisoprenoids, specifically  $\beta$ -damascenone. Previous suggestions of the association of typical Pinotage prune aroma to  $\beta$ -damascenone was made, but not confirmed (Waldner & Marais, 2002). In this study, it seemed like a probable association as  $\beta$ -damascenone increased with ripeness level in close association with the prune descriptor.

The acetone attribute clearly also increased with increased ripeness level; this attribute is thought to be influenced by high levels of higher alcohols (De-la-Fuente-Blanco *et al.*, 2016). In this study, the increased perception of acetone was associated with increases in 3-ethoxy-propanol and n-butanol, but not with isobutanol and isoamyl alcohol. The interaction of isobutanol and isoamyl alcohol with ethyl and acetate esters most probably modulated the aroma to be perceived as spicy (sweet spice) rather than acetone/solvent/chemical (De-la-Fuente-Blanco *et al.*, 2016, 2017).

There were also aroma descriptors that were not strongly associated to ripeness level, that were located in the inner circle of the correlation circle (Fig 7). Specifically aromas of rose were not well correlated with ripeness level, but associated well with higher alcohol 2-phenyl ethanol, which also did not display particular response to ripeness level. Here it may seem that the rose aroma may be implicated with fermentation derivative, 2 phenyl ethanol, rather than monoterpenes such as linalool. The eucalyptus descriptor is thought to be brought about by the presence of the monoterpene 1-8 cineole, and is not necessarily related to the green aromas associated with low maturity grapes (Marais, 1983; Ribéreau-Gayon *et al.*, 2006b). In this study, despite a possible association between the eucalyptus descriptor and 1-8 cineole, there was limited relationship to ripeness level. Interestingly, although the monoterpenes geraniol and citronellol showed increasing trends with ripening, they were not associated with distinctive descriptors, most probably due to the fact that they were present in amounts well below their OAV. The cooked vegetable descriptor was not related to ripeness level, and did not clearly associate with any compositional parameters. This is to be expected as this aroma is thought to be related to di-methyl sulphide (Bindon *et al.*, 2014), which was not quantified here.

With regard to taste/palate descriptors there was a clear shift from an acidic profile to a more complex profile characterised by greater astringency, concentration, length, body, and alcohol perception. The acidity perception was associated with must titratable acidity (TA) and wine TA, but also the fatty acids octanoic and hexanoic acids. The latter two fatty acids decreased strongly with an increase in ripening and are fermentation derived components that have been associated with typical Pinotage young wine aroma (Louw *et al.*, 2010). These fatty acids are the precursors of ethyl esters (hexanoate and octanoate), which explains their rapid decrease with increased ripeness level (Ilc *et al.*, 2016). Nonetheless, they warrant further study to assess their potential as markers for early ripeness levels, specifically for Pinotage.

As expected, the perception of alcohol (burning sensation) increased with increased ripeness level in close association to increased must Brix and wine alcohol levels. The increased alcohol perception in wines was also closely associated with increased concentration (palate weight), length and body. Wine body is thought to increase through increased perceived sweetness

(Zamora *et al.*, 2006), decreased acidity and increased pH (Fischer & Noble, 1994). Accordingly, the body descriptor coincided with increases in residual sugar (RS), pH and phenolic content (IPT 280). Other palate descriptors, such as concentration and length, were seemingly closely related to the positive effects of increases in body and alcohol, most likely as they are closely related in definition. Concentration is often related to increased wine viscosity, through increased polysaccharides, glycerol, residual sugar and concentration of berry constituents, which may be linked to properties of wines of late maturity (Gawel, *et al.*, 2007; Casassa *et al.*, 2013; Heymann *et al.*, 2013). As expected, the perception of astringency also increased with increased ripening, but was not associated with specific compositional variables. This is likely, because astringency is not solely related to wine phenolic content, but also to wine acidity (Arnold *et al.*, 1980; Gawel, 1998). Particularly, investigations on the relationship of wine pH and perception of astringency have shown that decreased wine pH (increase in acidity) increased the perception of astringency (Fontoin *et al.*, 2008).

A general increase in individual anthocyanins as ripening progressed was not well associated with other sensory attributes as wine colour was not assessed during wine descriptive analysis. However, from the rapid descriptive analyses (Table 2) it is clear that colour intensities increased due to increases in ripening. Yet, with regard to total individual anthocyanin measurements (in wine), anthocyanins already peaked at R2 and remained constant or declined towards the last ripeness level (See Chapter 5), although spectrophotometric analyses showed increased densities from R1 to R5. This is probably due to increased amounts of stable tannin-anthocyanin bonds forming in the latter stages of ripening, due to the relatively greater abundance of tannins (Canals *et al.*, 2005; Del Llaudy *et al.*, 2008).

Overall, the sensory and compositional profile was dictated by ripeness level, displaying strong relationships with taste descriptors, related to associations with basic fruit chemistry, such as TA, pH, and Brix of the must. The aroma profile was also changed along the ripeness level gradient. Interestingly, the volatile composition of wines was also strongly influenced by vintage. For example the 2015 vintage displayed higher amounts of fermentation-derived esters and higher alcohols associated to elderflower, sweet spices and banana descriptors compared to 2016. Conversely, the 2016 vintage displayed higher amounts of norisoprenoids associated with prune, vanilla, tobacco and savoury descriptors. This response showed that within the boundaries of changes in ripeness level, there was also environmental influences that could modify the expected response. Nonetheless, the consistent response tested with a variety of sensory techniques confirmed a definite shift in sensory profile along the ripeness level. From a practical perspective the general response is summarised in Table 9. The summary is based on the

outcomes of the various sensory evaluations conducted on the wines over multiple vintages (three) and sites (two) and is not a predictive model derived from statistical analysis.

Table 9. Collective descriptor profiles for potential wines styles, compiled from sensory analyses of various ripeness levels for cv. Pinotage.

Potential wine styles				
R1	R2	R3	R4	R5
Under ripe	Ripe	Ripe	Ripe	Over ripe
Lean	Light bodied	Medium bodied	Full Bodied	Astringent/Bitter
Red fruit	Red fruit	Dark fruit	Prune	Jammy
Menthol/Eucalyptus	Floral(fresh)	Banana	Vanilla/Honey	Vanilla/Honey
Herbaceous	Herbaceous	Liquorice	Liquorice	Smoky
Neutral	Tropical	Red fruit	Sweet spice	Chemical
Short	Candy	Floral	Tobacco	Tannic
Unbalanced	Balanced	Balanced	Balanced	Unbalanced
High Acid	Med Acid	Med-Low Acid	Med-Low Acid	Low acid

## 6.5 CONCLUSIONS

The results of this study demonstrated the detailed changes in sensory profile, which can be expected with increased ripeness level for *Vitis vinifera* L. cv. Pinotage, during rapid sugar accumulation in a warm/dry Mediterranean climate. From the various techniques employed to assess sensory response, DA with a trained panel provided a detailed and objective dataset which was compatible with wine compositional data. Rapid techniques such as rapid DA and Napping provided similar separations with greater emphasis on holistic descriptors and valuable visual presentation. Especially the conjunctive descriptors generated from Napping using experts would be useful in communicating results on a practical level.

The general profile shifted from herbaceous/neutral to red fruit/floral to dark fruit/spice to jammy/acetone with clear distinction of under ripe (herbaceous/neutral at 21°B) and over ripe (jammy/acetone at 29°B) profiles. Importantly, the evolution of the sensory profile was well-defined considering the whole ripeness spectrum was completed in 21 days. On a compositional level, herbaceous notes were more closely related to C6 compounds than to methoxypyrazines, which were present in extremely low levels. Positive contributions by esters known to influence Pinotage varietal aroma, were for the first time placed into context of ripeness level, including ethyl octanoate (elderflower) in the red fruit/floral profile and isoamyl acetate (banana) in the dark fruit profile. Increases in the norisoprenoid  $\beta$ -damascenone with progressing fruit maturity were implicated in the prune aroma, along with tobacco, vanilla and savoury notes. This adds evidence to previous assumptions that it may be implicated as impact odorant in Pinotage wines. Moreover, increased ripeness level decreased acidity and increased body concentration, length, and alcohol perception. These descriptors were highly correlated with ripeness level and many displayed interrelated perception. The strong influence of the taste descriptors in combination with aroma, modulated by grape maturity, provided a good basis to identify potential wine styles according to grape ripeness level.

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6.7 ADDENDUM D

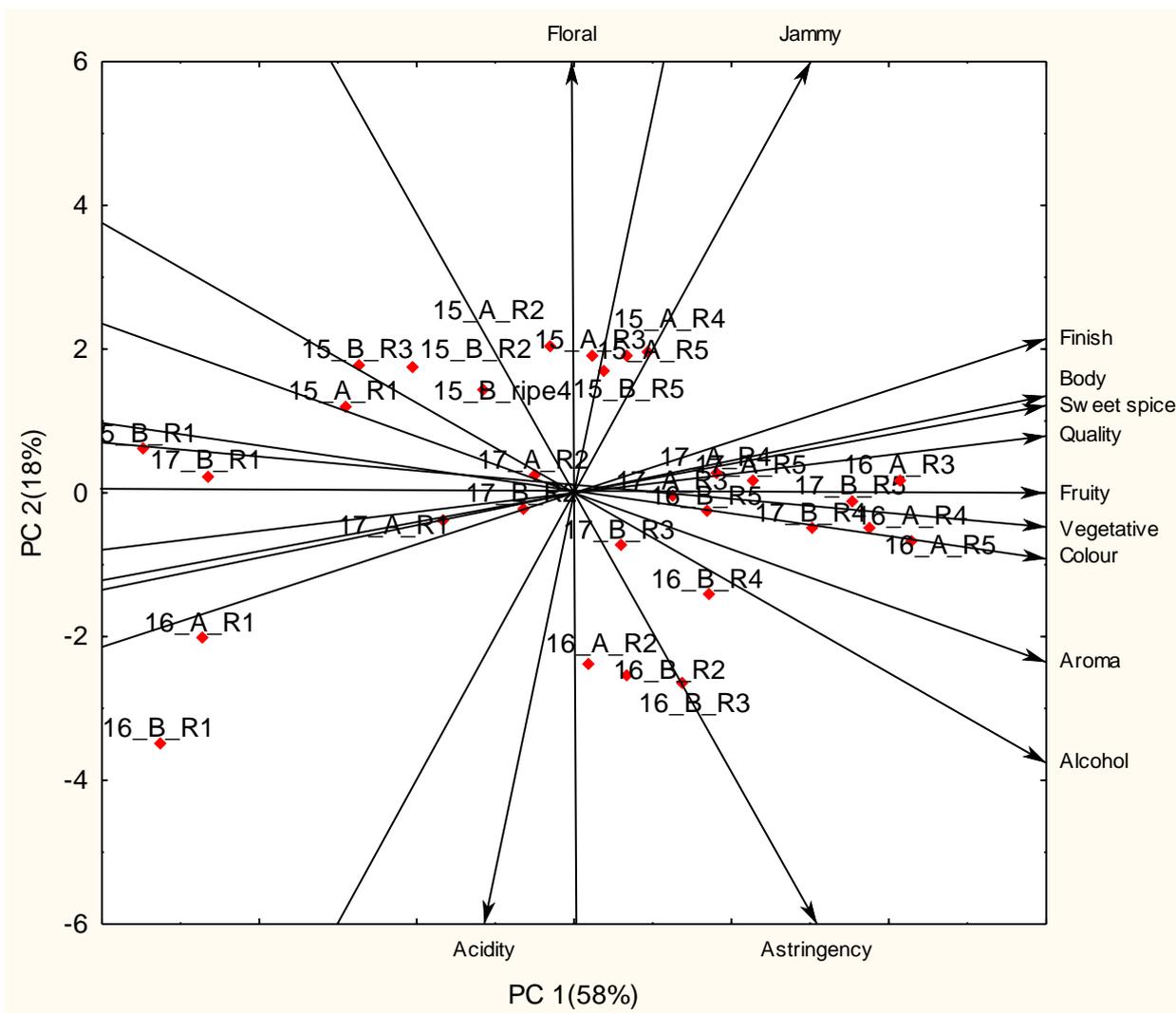


Figure 1 Principal component analysis of rapid descriptive analysis of cv. Pinotage wines from five ripeness levels (R1-R5), and two sites (A & B), for vintages (2015-17).

Table 1 R-square coefficients descriptors for PCA plots of trained descriptive analysis

2015		2016	
Descriptor	R <sup>2</sup>	Descriptor	R <sup>2</sup>
Body	0.985	Alcohol	0.996
Acidity	0.982	Length	0.995
Concentration	0.981	Concentration	0.994
Alcohol	0.972	Acidity	0.993
Length	0.957	Body	0.978
Astringency	0.949	Astringency	0.97
Rose	0.882	Dark berries	0.957
Elderflower	0.82	Red berries	0.95
Tobacco	0.775	Tobacco	0.903
Herbaceous	0.711	Cooked vegetables	0.866
Savoury	0.711	Herbaceous/mint	0.856
Red fruits	0.679	Vanilla	0.825
Cooked vegetables	0.679	Eucalyptus	0.823
Vanilla	0.658	Elderflower	0.802
Eucalyptus	0.621	Banana	0.746
Prunes	0.612	Sweet spices	0.658
Dark berries	0.578	Acetone	0.654
Banana	0.553	Prunes	0.625
Sweet spices	0.408	Savoury	0.616
Acetone	0.288	Rose	0.071

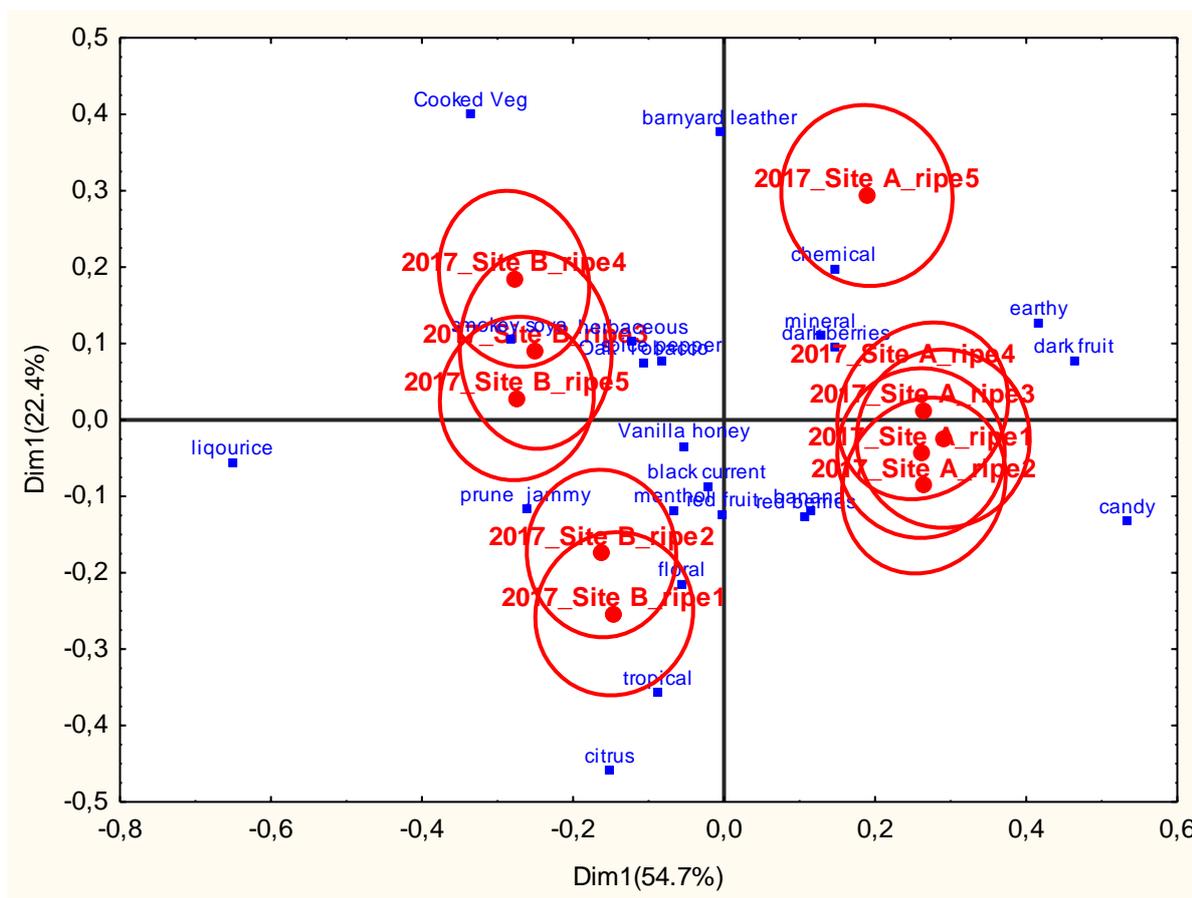


Figure 2 Correspondence analysis of aroma descriptors generated by Napping technique performed by an untrained panel of cv. Pinotage wines from five ripeness levels (R1-R5), and two sites (A & B), for the 2017 vintage. (Confidence ellipses 0.05)

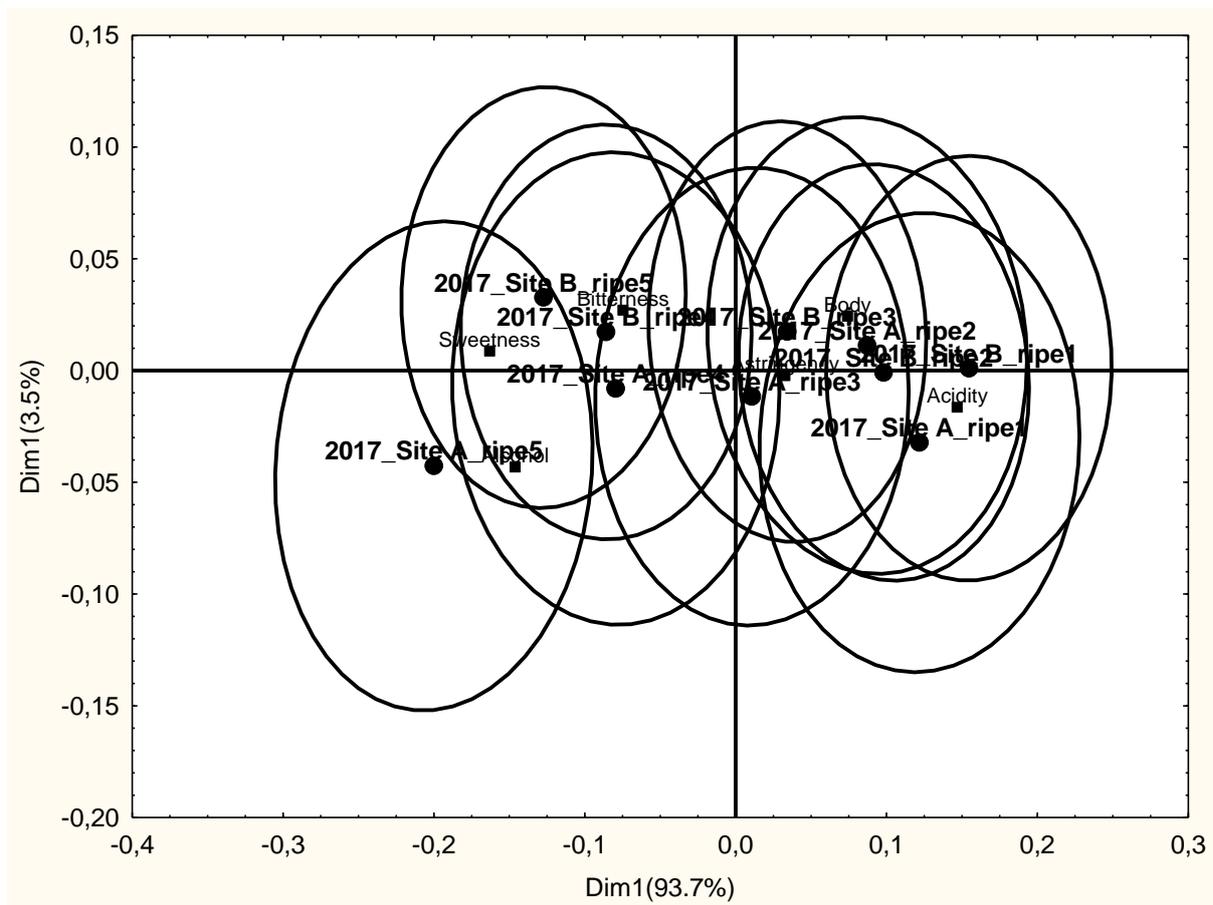


Figure 3 Correspondence analysis of taste descriptors generated by Napping technique performed by an untrained panel of cv. Pinotage wines from five ripeness levels (R1-R5), and two sites (A & B), for the 2017 vintage. (Confidence ellipses 0.05)

## Chapter 7

### General discussions and conclusions

#### 7.1 INTRODUCTION

This study investigated the impact of grape ripeness level on grape- and wine composition and resulting wine sensory profiles of *Vitis vinifera* L. cv. Pinotage. In general, literature on the extent of grape ripeness level interaction with composition and sensory profiles is limited to results from a single vintage (Bindon *et al.*, 2013, 2014) and those with sparse detail regarding important seasonal and *terroir* related factors (Casassa *et al.*, 2013; Heymann *et al.*, 2013; Sherman *et al.*, 2017), such as climate, soil, grapevine water status and bunch microclimate (Koundouras *et al.*, 2006; Van Leeuwen & Seguin, 2006; Keller *et al.*, 2016), all of which are known to interact with ripeness level (Hunter *et al.*, 2004, 2010).

Nonetheless, the relationship between Pinotage grape and wine sensory profiles remains undocumented. Pinotage has unique varietal characteristics, *e.g.* early maturation and rapid sugar accumulation. Ripeness level evaluation necessitates field study, as this will allow information to be related to previous works in other varieties, such as Cabernet Sauvignon (Canuti *et al.*, 2009; Bindon *et al.*, 2013, 2014; Heymann *et al.*, 2013) and Shiraz/Syrah (Nadal & Hunter, 2007; Guidoni & Hunter, 2012; Hunter *et al.*, 2014; Hunter & Volschenk, 2018). Compared to other varieties, Pinotage has shown many differentiating attributes in terms of wine volatile composition (Marais *et al.*, 1979; Waldner & Marais, 2002; Louw *et al.*, 2010) and phenolic and anthocyanin composition (Rossouw & Marais, 2004; van der Merwe *et al.*, 2012). These differentiating wine attributes are yet to be placed in context of grape ripeness level.

#### 7.2 OVERVIEW OF RESEARCH

This study characterised the grape- and wine composition of Pinotage, including measurements of basic fruit/wine chemistry, phenolic compounds and aroma/volatile compounds associated with five grape ripeness levels of *ca.* 21, 23, 25, 27 and 29 °Brix, for three consecutive vintages and two vineyard sites (rootstock/soil). This information was used to interpret wine sensory profiles in order to propose practical harvest guidelines.

All vineyard, compositional and sensorial data were generated using previously published methods. Vineyard measurements were based on the methodologies for field experimentation proposed by Hunter *et al.* (2014), for vineyard characterisation of physiology, vegetative and reproductive growth as well as the quantification of bunch microclimate. Furthermore, apart

from classical ripening indices, grape compositional analyses included the quantification of whole berry phenolic content with spectrophotometric methods (Vivas *et al.*, 1994; Ojeda *et al.*, 2002) as well as individual and total anthocyanin contents by means of HPLC (Guidoni & Hunter, 2012). Grape berry aroma potential was assessed by direct acid hydrolysis of grape berry material and subsequent HS-SPME and GC/MS analyses as detailed in Yuan & Qian (2016). Analyses led to the successful identification of 20 released and free volatile compounds and quantification with authentic standards. An acid hydrolysis method was chosen, due to it being better representative of expected changes in wines compared to enzymatic methods (Loscos *et al.*, 2009). Enzymatic methods generally produce higher yields of released compounds, but relate poorly to wine volatile contents (Francis *et al.*, 1992; Loscos *et al.*, 2009; Hampel *et al.*, 2014). Routine wine analyses were performed by a commercial wine laboratory (Vinlab, Stellenbosch), while wine phenolic and colour indexes were determined using established methods (Somer, 1968; Ribéreau-Gayon *et al.*, 2006). Anthocyanin profiling of wines was performed similar to that of grapes (Guidoni & Hunter, 2012). Due to the large variation in concentration and complexity, wine volatile compounds were assessed using two separate methodologies for groups of compounds. The esters and alcohols were analysed by liquid-liquid extraction as reported by Louw *et al.* (2010), while the low-concentration volatiles (norisprenoids, terpenes and methoxypyrazines) were extracted by HS-SPME (Panighel & Flamini, 2014), followed by GC/MS detection as described in De Vries *et al.* (2016).

Sensory analysis was conducted on experimental wines utilising a variety of sensory techniques in order to explore and assess the profiles and consistency of ripeness-related sensory responses. Sensory evaluations included rapid descriptive analysis with an untrained panel, which provided sound general descriptive data (quantitative). Moreover, descriptive analysis carried out by a trained panel provided a rich lexicon of aroma and taste descriptors which aided ripeness level profiling (Lapalus, 2016). A rapid profiling technique called Napping was also utilised in the final year of the study (Dehlholm *et al.*, 2012). Napping analysis with both experienced and expert panels, as described by Pagès (2005), was used as alternative to descriptive analysis and provided comparable profiles, albeit without intensity scores.

Quantitative vineyard, grape and wine data representing three consecutive vintages was subjected to analysis of variance (ANOVA) to establish significant differences among grape ripeness levels, sites and vintages. A combination of multivariate statistical analyses, including Discriminant Analysis (DA), Principal component analysis (PCA), Partial least squares regression (PLS) and Multi-factorial (MFA) analyses were used to identify associations between the measured variables and differentiation of the grape and wine samples as a function of ripeness level.

### 6.3 CONCLUSIONS

Seasonal differences in rainfall and temperature had a major impact on cv. Pinotage grapevine performance under the experimental conditions. Grapevines grafted to drought tolerant rootstocks adapted to warmer and drier conditions, demonstrating sustained WUE under higher seasonal water deficits. From a ripening perspective, reduced vegetative growth in drier vintages increased solar irradiance in the bunch zone, while leaf senescence led to the decreased capacity of the canopy during late ripening stages. Despite this, Pinotage grapevines managed to ripen fruit (21 – 29°B) within a short timeframe (21 days). Moderate pre-véraison water constraints experienced by the plants, and rapid sugar accumulation led to stable berry weight during early ripeness levels (R1-R4), decreasing significantly towards the last ripeness level as a result of dehydration.

Berry compositional analysis displayed an increasing trend for berry phenolics and anthocyanin accumulation up to R4 (peak), after which phenolic composition generally declined towards R5. This decline in phenolic potential, despite the presumed ‘concentration’ due to berry size reduction, highlighted the detrimental effect of excessive ‘hang time’ and over ripeness. Grape aroma potential demonstrated minor shifts along the ripeness gradient, with a large degree of commonality between profiles of sequential harvests. A shift in profile along the ripeness gradient was related to a decrease in greenness-associated compounds (especially C6 - compounds), whereas the positive related compounds that are known to increase during ripening (monoterpenes and norisprenoids) did not show increases. The reason for this remains unclear, but may be related to the high temperatures and high sunlight incidence that may have induced compound degeneration during ripening.

Wine composition did not mirror grape compositional data and demonstrated that the winemaking process (extraction and fermentation) transforms the grape substrate to a large extent. Wine phenolic content and colour indexes increased from R1-R5, despite the decreased grape phenolic potential of R5. The reaction of wine volatiles to ripeness level is complex, as they differ regarding metabolic origin (yeast-derived *versus* grape-derived). Generally speaking, wines of later ripeness levels were characterised by decreased greenness-associated compounds (C6-compounds, methoxypyrazines and fatty acids) and increased positive associated compounds (norisoprenoids, monoterpenes and higher alcohols). In addition, decreases in many volatile components were demonstrated at the final ripeness level, suggesting that over ripe grapes may have a detrimental effect on volatile composition. From wine volatile compositional data it is clear that in this study direct acid hydrolysis of grape components did predict wine volatile composition well. In light of the large array and influence of surrounding factors and

matrix effects, micro scale fermentations may be a way forward in order to better predict wine volatile composition during maturity assessment.

A shift in wine aroma and taste descriptors and intensities thereof along the ripeness level gradient, confirmed ripeness level as the major impacting factor among the variables studied (ripeness level, site and vintage). From the various techniques employed to assess sensory response, QDA with a trained panel provided a detailed and objective dataset, which correlated well with wine compositional data. Rapid techniques such as Napping provided similar separations with greater emphasis on holistic descriptors and valuable visual presentation. Especially the conjunctive descriptors generated from Napping using experts would be useful in communicating results on a practical level. However, due to the fact that no quantitative data was generated with the Napping approach, overlaying quantitative compositional analysis was not possible. Rapid DA with an untrained panel proved useful as screening method and provided adequate analysis for separating ripeness levels. The wine quality score generated in this untrained analysis provided insight into judge preferences. Comparable qualitative scores for wines from (23-29°B) provided insight into the acceptance of different styles by consumers and warrant further investigation through consumer testing.

The general aroma profile shifted from herbaceous/neutral to red fruit/floral to dark fruit/spice to jammy/acetone with clear distinction of under ripe (herbaceous/neutral at 21°B) and over ripe (jammy/acetone at 29°B) profiles. Importantly, the evolution of the sensory profile was well-defined considering the whole ripeness spectrum was completed in only 21 days, providing ample evidence that the development in flavour is not exclusively related to extended “hang time”, but involves complex ripening-related processes.

On a compositional level, herbaceous notes were more closely related to C6 compounds (green leaf volatiles) than to methoxypyrazines, which were present in extremely low levels. Positive contributions of esters known to influence Pinotage varietal aroma, were for the first time placed into context of ripeness level, including ethyl octanoate (elderflower) in the red fruit/floral profile and isoamyl acetate (banana) in the dark fruit profile. Increases in wine of the norisoprenoid  $\beta$ -damascenone with progressing fruit maturity were implicated in the prune aroma, along with tobacco, vanilla and savoury notes. This adds evidence to previous assumptions that it may be implicated as impact odorant (prune/plum) in Pinotage wines.

Finally, the results of this study demonstrated the detailed changes in sensory profile, which can be expected with increased ripeness level for *Vitis vinifera* L. cv. Pinotage, during rapid sugar accumulation in a warm/dry Mediterranean climate. Novel information with regard to the links between grape ripeness level, grape- and wine composition and sensory profile has been put

forward. Particularly encouraging is the extent of possible sensory profiles that materialised during the short maturation period.

#### 7.4 LIMITATIONS OF THE STUDY

The results demonstrated in this study should be viewed as applicable in context of the growing environment, specifically Pinotage grapevines grown on a sloped *terroir* (3°), with north western aspect, trained to a VSP (Vertical Shoot Positioned) trellis in clayey soil, and characterised with moderate to high summer temperatures. Different results, especially regarding timing and extent of compositional amplitudes may be obtained in vineyards grown in different *terroirs*, as observed with vintage and/or site variation.

Additional information pertaining more detailed phenolic profiles, especially tannins (oligomeric phenolic compounds) would have increased the ability to link palate/taste descriptors, such as body, astringency and bitterness perception, to specific ripeness levels. Nevertheless, some correlation was observed between levels of spectrophotometric phenolic indices and sensory perception. In addition, details pertaining must nitrogen and amino acid content in relation to ripeness level would have been valuable in the interpretation of yeast metabolism derived volatiles, such as esters en alcohols.

Grape aroma potential through acid hydrolysis did not show good predictive fit to wine volatile composition, mainly due to the large contribution of fermentation-derived aromas. Much future work is needed in order to place grape compositional data in context to that which is expected in wines. Moreover, berry heterogeneity remains a major challenge in grape compositional studies. Despite the fact that berries of essentially differed ripeness levels are added together in a practical winery situation, on a scientific level it presents a large source of variation especially during sensitive measurements and analyses. While reducing berry heterogeneity through a sorting technique would certainly aid data interpretation, the results would not allow for direct application in practice. The solution would likely be to have a control (naturally heterogeneous) and sorted sample set for each measurement repetition, but will soon become unmanageable if multiple analyses and samplings are undertaken. This remains one of the major limitations of applied viticultural studies, yet deserves due mention due to its formidable impact.

#### 7.5 STRENGTHS OF THE STUDY

This is the first study illustrating the multi-layered effect of grape ripeness level on Pinotage wines under different, complex *terroir* (soil and climate) conditions and genotype combinations, across multiple vintages. Grape and wine composition were evaluated in the context of grapevine morphology and physiological functioning within a characterised soil x climate environment. A

large body of grape and wine compositional data was related to common wine sensorial profiles and identified key impacting components for further investigation. Elaborative methodology was applied to explore sensory profiles, exposing clearly distinguished wine styles. Novel information regarding changes in Pinotage grape aroma potential and wine volatile composition was generated. Especially information regarding potential grape aroma composition in relation to ripeness level was of interest. Despite the challenging matrix, compounds could be identified that displayed consistent trends along ripening. For instance, the volatile phenol guaiacol was well correlated to ripeness level (decrease along ripening), adding from another chemical group (volatile phenols) to those that have already been proposed to be sensitive to ripeness level (e.g. methoxypyrazines, monoterpenes and norisprenoids).

The large array of measurements undertaken in this study provide a well-developed and solid context for reasoning the effect of ripeness level on the various grape and wine compositional spheres. The impacting role of grape ripeness level on grape and wine composition was shown convincingly. In particular, the study demonstrated that grape ripeness level can be utilised by viticulturists and winemakers alike to introduce unique wine styles into portfolios.

## 7.6 RECOMMENDATIONS

The results of the study suggest harvesting Pinotage at different ripeness levels would allow the producer to elaborate different wine styles (Table 1). In particular the differences obtained between wines of the harvest window from 23 – 27°B can be of significant interest to the producer. Wines made from the 21°B level were marked by herbaceous characters, high acidity and low perceptions of positive mouthfeel (body, concentration and length, astringency) and may be described as neutral, short and lean. On the contrary, wines of the 29°B level were marked by low acid, overripe flavours and jammy and chemical notes. Importantly, the decreased potential for wine quality at the last ripeness level could be related to the vineyard condition *via* decreased canopy functionality, berry dehydration (raisining), displaying decreased colour and phenolic content, as well as decreased grape aroma and wine volatile composition. Therefore injudicious, delayed ripening may have detrimental effects on wine quality. This is especially important for the successful elaboration of Pinotage wines, because of its relatively short maturation period, necessitating prompt decision making.

In addition, the harvesting decisions between ripeness levels 23°B to 27°B, allow for elaboration of differential styles within a +/- 11 day harvest window (situation dependent). This would allow for a progression in style from a light- to medium- to full bodied style wine, with flavours progressing from red fruit to dark fruit to dried fruit/spice profiles (Table 1).

Table 1 Potential wine styles as proposed from sensory data

Potential wine styles				
21°B	23°B	25°B	27°B	29°B
Under ripe	Ripe	Ripe	Ripe	Over ripe
Lean	Light bodied	Medium bodied	Full Bodied	Astringent/Bitter
Red fruit	Red fruit	Dark fruit	Prune	Jammy
Menthol/Eucalyptus	Floral(fresh)	Banana	Vanilla/Honey	Vanilla/Honey
Herbaceous	Herbaceous	Liquorice	Liquorice	Smoky
Neutral	Tropical	Red fruit	Sweet spice	Chemical
Short	Candy	Floral	Tobacco	Tannic
Unbalanced	Balanced	Balanced	Balanced	Unbalanced
High Acid	Med Acid	Med-Low Acid	Med-Low Acid	Low acid

Although this progression in flavours was stable along the vintages and sites tested in this study, extrapolation should be done with caution. The specific cultivation practices in addition to soil type and other *terroir* conditions, primarily climatic factors, may mediate the effect of ripeness level on Pinotage wine stylistic progression. These parameters exert an independent and mostly indirect effect on Pinotage grape berry ripening wine qualitative factors and need to be considered in attempts to manage/manipulate wine style in the vineyard. However, the relative ease with which this can be tested at producer level, by harvesting selected portions of grapes at various targeted levels (23-27°B) and vinifying separately, allows for the assessment of the given *terroir* potential and the extent to which stylistic changes are possible.

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