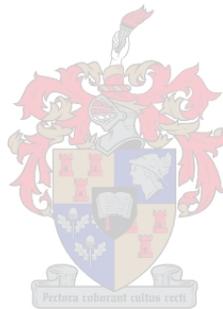


Site and vintage response of malic and tartaric acid in *Vitis vinifera* L. cv's Cabernet Sauvignon and Sauvignon blanc

by

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Declaration

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Summary

Acids are one of the major components that originate largely from the berry, that are found in wine, and that influence the sensory perception. The presence of organic acids in adequate concentrations in the grape berry, of which tartaric- and malic acid are the main organic acids present, is important as this determines the potential of a must to produce a good and stable wine.

The effect of temperature on the organic acid content of the must is widely discussed with higher temperatures in general being associated with lower quantities of organic acids present in the juice, and lower temperatures during ripening associated with higher quantities, specifically in the case of malic acid.

Due to the topographical diversity of the Stellenbosch Wine of Origin district and the closeness of the ocean and the occurrence of sea breezes, the mesoclimate differs greatly over short distances. Sixteen sites, consisting of eight Sauvignon Blanc and Cabernet Sauvignon sites respectively, were selected from a broader terroir study site network. Three vintages with complete climatic datasets were selected for vintage comparisons. Climate in the study area was monitored on differing scales, and data from a weather station network, as well as from mesoclimatic dataloggers within the sites were available.

The available data was firstly compared to determine the variability of the data, not only between the two climatic scales, but also between the sites. Different climate classification indices and parameters available in literature were thereafter compared and evaluated for the best representation in this area. The Huglin index was found to be a better representation regarding the thermal climatic indices. Due to the great differences between temperatures noted for the mesoclimatic loggers and the nearest automatic weather station, the use of mesoclimatic logger data was preferred, and is advised in future studies where this scale of data is available.

Malic and tartaric acid has a definite synthesis period up until véraison, after which the content of tartaric acid remains constant in the berry and the content of malic acid decreases until harvest due to mainly respiration. The temperature data was therefore separated in a synthesis period from flowering to véraison, and a ripening period from véraison to harvest.

In this study, clear differences were firstly seen in the climate as expected, not only between sites per vintage, but in addition between vintages and between vintages per site. The phenological differences between the sites could be largely attributed to the differences in temperature as phenology and temperature was found to be highly correlated in this study.

Differences in the ripening parameters were noticed in addition to the contents of the organic acids between sites, although no definite contribution of temperature was shown to affect the contents of these compounds at either véraison or harvest. These differences may be attributed to other factors such as the soil water content and the canopy architecture. In addition, these factors all contribute in differing percentages to the differences found in the contents per site.

It was found though that temperature can be used as an indicator of the organic acid content in the grape berry, considering that the temperature data is available on a mesoclimatic scale, separated in a synthesis and period of degradation, and the number of hours within the temperature thresholds are determined. Differences seen in the organic acid contents can however not only be attributed to the differences in topography and the temperature as discussed in this study.

Opsomming

Sure is belangrike druiwkomponente wat grootliks hul oorsprong in die korrel het, in die wyn voorkom, en die sensoriese persepsie van die wyn beïnvloed. Die voorkoms van organiese sure in genoegsame konsentrasies in die korrel, waarvan wynsteensuur en appelsuur die hoof organiese sure is, is belangrik aangesien dit die potensiaal van die sap om 'n goeie en stabiele wyn te produseer, bepaal.

Hoe temperatuur die inhoud van organiese sure in die druiwesap affekteer is gereeld onder bespreking, met hoër temperature in die algemeen geassosieer met 'n laer inhoud van organiese sure, terwyl laer temperature geassosieer word met 'n hoër inhoud van organiese sure in die sap, veral in die geval van appelsuur.

As gevolg van die topografiese diversiteit van die Stellenbosch Wyn van Oorsprong distrik, asook die nabyheid van die oseaan met die gepaardgaande voorkoms van die seebries, verander die mesoklimaat aansienlik oor klein afstande in hierdie area. Vir die studie was sestiën wingerde, wat bestaan het uit agt Sauvignon Blanc en agt Cabernet Sauvignon wingerde, geselekteer vanuit 'n groter terroir studie. Verder was drie seisoene, met volledige klimaatsdatastelle, geselekteer vir die vergelyking van data tussen die seisoene.

Klimaat was op verskillende skale binne die studie area gemonitor en data van 'n weerstasie netwerk, sowel as van mesoklimaat dataversamelaars binne die wingerde, was beskikbaar. Die beskikbare datastelle was vergelyk, asook geëvalueer, om die mees verteenwoordigende datastel vir die area te bepaal. Met die oorweging van die termiese indekse was daar gevind dat die Huglin indeks beter verteenwoordigend van die area was. Verder, as gevolg van die groot verskille wat gevind is tussen die temperature gemeet met die mesoklimaat dataversamelaars en die naaste outomatiese weerstasie, was daar besluit dat die gebruik van die mesoklimaat data verkies is en is dit ook aan te beveel vir die gebruik in toekomstige navorsing indien die tipe data beskikbaar is.

Wynsteen- en appelsuur het beide 'n definitiewe sintese periode tot en met véraison, waarna die hoeveelheid wynsteensuur in die korrel relatief konstant bly en die hoeveelheid appelsuur afneem hoofsaaklik as gevolg van respirasie. Die temperatuur data was dus verdeel in 'n periode van sintese vanaf blom tot en met véraison, en 'n rypwordingsperiode vanaf véraison tot en met oes.

In hierdie studie was daar eerstens groot verskille waargeneem in die klimaat soos wat daar verwag is. Hierdie verskille was nie net waargeneem as tussen die seisoene nie, maar ook tussen die wingerde binne 'n seisoen. Die fenologiese verskille tussen die wingerde wat ook waargeneem is, kon hoofsaaklik aan die verskille in die temperatuur toegeskryf word en 'n goeie korrelasie tussen temperatuur en fenologie is opgemerk.

Merkwaardige verskille in die rypwordingsparameters, asook in die inhoud van die organiese sure, was waargeneem, alhoewel die bydrae van temperatuur op die inhoud van hierdie komponente by véraison of oes nie as definitief getoon is nie. Dit kan toegeskryf word aan die bydrae van ander faktore, soos byvoorbeeld die grondwaterinhoud en die lowerargitektuur, op die inhoud van hierdie komponente. Die addisionele faktore dra egter in verskillende persentasies by tot die verskille waargeneem tussen die wingerde.

This thesis is dedicated to my mother, Engela Coetzee, who insisted that we had the best education possible and ensured the best opportunities for us in the world.... and for teaching us to think and question everything in life.

Biographical sketch

Zelmari Anél Coetzee was born in Cape Town on 15 October 1977 and spent her first years in Mpumalanga, first in Ogies and thereafter Witbank. After starting her school career in Witbank, her father was transferred to Pretoria where she first attended Wonderboom Primary School and thereafter the Afrikaans Hoër Meisiesskool where she completed high school. Her family has a long history in Stellenbosch as this is where her parents and grandparents lived and her two brothers were both born, and she therefore decided to return here and study Viticulture and Oenology at the Stellenbosch University. During her practical training at Kanu Wine Estate under Teddy Hall, a clear passion for Viticulture was noticed and after completing her studies in 2000, she worked for an American cruise line company to save money for a postgraduate degree, specialising in Viticulture. In 2004, after completing her HonsBScAgric in Viticulture, she was appointed as a technical assistant researcher at the Stellenbosch University and is currently still in this position.

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Chapter I: Introduction and project aims

1.1 Introduction

Acids are major compounds found in the wine that originate largely from the berry (Boulton et al., 1998; Coombe & Iland, 2005) and plays a vital role in the organoleptic and aesthetic character of the wine (Boulton, 1980; Terrier & Romieu, 2001). Acids furthermore influence the physical, biochemical and microbial stability of the wine (Volschenk et al., 2006).

In wine, organic acids quantitatively dominate the acid composition (Ribéreau-Gayon, 1968; Jackson, 2008a), with L-tartaric acid (TA) and L-malic acid (MA) contributing to more than 90% of the total acids found in grape berries and wine (Winkler, 1962). Tartaric acid, the stronger and more stable acid (Dry & Coombe, 2005), is the main organic acid determining the suitability of the grapes for winemaking as it controls the juice pH (DeBolt et al., 2007). In comparison, MA play a role in malolactic fermentation (MLF) (Dry & Coombe, 2005) during which it is converted to the smoother tasting lactic acid (Jackson, 2008a).

Tartaric acid and MA are secondary products of sugar metabolism and primarily synthesized in the berry (Watson, 2003). Although these two acids are similar in structure, they have differing origins with TA originating from L-ascorbic acid (DeBolt et al., 2007) and MA being an intermediate in grapevine metabolism (Ruffner, 1982a, 1982b). Malic acid and TA are actively synthesized up until véraison (Ruffner, 1982a, 1982b), after which the content of TA remain relatively stable in the berry (Ruffner, 1982a; DeBolt et al., 2004) and the MA content decrease due to metabolism of MA through different pathways (Sweetman et al., 2009).

The effect of climate, in particular temperature, on the metabolism of MA and TA, and the subsequent breakdown of MA after véraison, is well studied and understood (Winkler, 1962; Kliewer, 1964; Kliewer & Lider, 1970; Buttrose et al., 1971; Ruffner et al., 1976; Ruffner, 1982a, 1982b; Coombe, 1987; Iland, 1989; Kanellis & Roubelakis-Angelakis, 1993; Van Leeuwen et al., 2004). Although temperature has been found to have little or no effect on the TA content of the berries (Kliewer, 1964; Buttrose et al., 1971; Coombe, 1987; Terrier & Romieu, 2001), the MA content reveal great changes according to the seasonal climatic differences (Winkler, 1962; Coombe, 1987; Van Leeuwen et al., 2004).

The concept of terroir has been described by Seguin in 1988 (as cited in Van Leeuwen & Seguin, 2006) as an interactive ecosystem which includes the climate, soil and the vine. Prior to this publication, in 1986, Seguin (also cited in Van Leeuwen & Seguin, 2006) included human factors in the definition of terroir as no vineyard exist where intervention of mankind is not present. Although the effect of climate, soil and the cultivar on berry development and berry composition cannot be separated, Van Leeuwen et al. (2004) found that the effect of climate was indeed the greatest, after which soil and cultivar followed.

Climate has been described by several authors as having a major influence on berry composition with temperature determined as the main component of climate affecting the berry composition (Coombe, 1987; Jackson & Lombard, 1993; Marais et al., 1999; Jones & Davis, 2000; Happ, 2007; Hunter & Bonnardot, 2011).

The regional climate of the Stellenbosch Wine of origin district is described as Mediterranean with mild, wet winters and warm dry summers (Bonnardot et al., 2002). This region is a complex study area though due to the diverse topography (Carey et al., 2008) resulting in topoclimatic

variability. In addition to the topography, the closeness of the Atlantic ocean, resulting in the occurrence of a sea breeze during the ripening period (Bonnardot et al., 2001), also influences the climatic variability of this region. The position of a vineyard in the landscape (the distance from the ocean, aspect and openness of the landscape) influences the intensity of the effect of the sea breeze on the daily temperature kinetic (Bonnardot et al., 2002).

Climate is mainly monitored on three different scales, namely a macroclimatic- (regional and mainly long term), mesoclimatic- (vineyard area or districts and shorter periods of time) and a microclimatic (climate within or surrounding the plant, mainly monitored in minutes and seconds) scale (Bonnardot et al., 2004). The choice of the scale of monitoring depends on the data that is available, the area of interest and the aim of the study.

Different methods to define the climate exist ranging from summated indices like the Huglin- and Winkler indices (Amerine & Winkler, 1944; Tonietto & Carbonneau, 2004), the use of mean temperature values (Prescott, 1965; Smart & Dry, 1980; Tonietto & Carbonneau, 2004; Van Leeuwen et al., 2004) and more recently the use of optimal hour thresholds (Hunter & Bonnardot, 2011). As single parameter classification methods usually lack representation of an area, a complete vintage or mostly a specific cultivar, several models including multiple criteria also exist (Smart & Dry, 1980; Tonietto & Carbonneau, 2004; Van Leeuwen et al., 2004).

In this study, macro- and mesoclimatic data was available and therefore focused on the use of different climatic scales and different climatic classification methods to determine if temperature was the main driver in our study of the organic acid content at *véraison* and harvest in Sauvignon Blanc and Cabernet Sauvignon grapes.

1.2 Project aims

The aim of this study was therefore to study the effect of the topoclimatic variability between sites across vintages, but mainly the variability of the temperature, on the content of malic- and tartaric acid in Sauvignon Blanc and Cabernet Sauvignon. For this study, the project aims were accordingly divided into two parts:

- i. To determine the comparative effects of vintage on the organic acid content of Sauvignon Blanc and Cabernet Sauvignon grapes from the Stellenbosch Wine of Origin District at *véraison* and harvest.
- ii. To determine the range of malic- and tartaric acid contents in grapes of Cabernet Sauvignon and Sauvignon Blanc from the Stellenbosch Wine of Origin District at two key phenological stages and within three vintages by investigating the relationship between the climatic characteristics of vineyards with the tartaric- and malic acid contents of the grapes.

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Chapter II: Literature study

2.1 Introduction

Acids are one of the major compounds that originate mainly from the grape berry and is also found in the wine and that influence its sensory perception (Boulton *et al.*, 1998; Coombe & Iland, 2005). The presence of organic acids in adequate concentrations in the grape berry is important as this determines the potential of a must to produce a good and stable wine (Boulton *et al.*, 1998). Wine pH, an important indicator of grape quality, together with flavour and colour, indicates the strength of these acids as well as the presence of other ions that are present in the wine. The three main factors influencing wine pH include the ratio of malic to tartaric acid, the total amount of acid present in the wine and the quantity of potassium (K^+) (Conde *et al.*, 2007).

There are at least 27 free organic acids present throughout the grapevine (Kliewer (1966). The dicarboxylic acids, namely malic- (MA) and tartaric acid (TA), contribute from 68% to 92% of the total acids found in the berries and leaves (Jackson, 2008a). In turn, the percentage contribution of MA and TA to the total juice volume is 0.2 to 1% and 0.3 to 1.5%, respectively (Winkler, 1962).

Other organic acids present in the wine are amino acids (1 to 3 g/L) and several other to a lesser extent such as acetic-, galacturonic-, pyruvic-, α -keto-glutaric acid, *etc.* (Boulton *et al.*, 1998). The organic acids present in grapevines are mainly intermediates of metabolic pathways operating in the grape berries (Winkler *et al.*, 1974; Ruffner, 1982b; Kanellis & Roubelakis-Angelakis, 1993).

The occurrence of this number of organic acids, including all the intermediates of glycolysis, the Krebb's and glyoxylic acid cycles and the shikimic acid pathway, suggests that several metabolic cycles are involved. Malic acid and TA are secondary products of sugar metabolism, primarily synthesized in the berry and to a lesser extent in the leaves. No conclusive evidence exists of transport of either of MA or TA from the leaves to the fruit (Watson, 2003).

The biochemical pathways for the formation and degradation of MA and TA are in fact unrelated despite the two acids being structurally similar (Ruffner *et al.*, 1983a; Conde *et al.*, 2007), The origin of TA metabolism has recently been shown to be L-ascorbic acid (DeBolt *et al.*, 2007) and not the oxidative metabolism of sugars (Loewus & Stafford, 1958) as previously stated whereas the origin of MA is as a central intermediate in grapevine metabolism (Ruffner, 1982a, 1982b).

The concentration of these acids and their corresponding salts vary according to variety, season, location, cultural conditions and the state of maturity (Kliewer *et al.*, 1967; Watson, 2003). Furthermore, climatic conditions during berry development have an very strong effect on the ratio of the content of MA, TA and K^+ (Terrier & Romieu, 2001), with temperature being the main exogenous factor affecting the acid content (Ruffner, 1982a; Kanellis & Roubelakis-Angelakis, 1993).

In immature berries, MA and TA are synthesized with a subsequent decline in their concentration during ripening (Hardy, 1968). During ripening, the TA content per berry however remains relatively constant whereas the malic acid is rapidly degraded due to an increase of the supply of respiratory substrates from acid degradation as opposed to carbohydrate breakdown (Possner *et al.*, 1983; Ruffner *et al.*, 1983a). Additional possibilities responsible for the observed decrease in acid concentrations are noted by Ruffner *et al.* (1983a) and Kanellis & Roubelakis-Angelakis (1993) as: the dilution in acid concentration as berry volume increases, the inhibition of acid synthesis

coinciding with an increase in MA degradation, and the transformation of acid to sugar. The TA to MA ratio at harvest varies considerably according to the grape variety (Ruffner, 1982a; Kanellis & Roubelakis-Angelakis, 1993).

The rate of the biosynthesis and degradation of organic acids are mainly influenced by environmental factors as pathways are mainly enzymatically activated. Although the chemical intermediates of TA biosynthesis have been identified, identifying the corresponding enzymes has been unsuccessful (Conde *et al.*, 2007; Martínez-Esteso *et al.*, 2011). Information on the effect of in particular light and temperature on the biosynthesis and degradation of MA, referring mainly to enzyme activity, is widely researched and discussed (Kliewer & Lider, 1970; Ruffner & Kliewer, 1975; Ruffner *et al.*, 1976; Ruffner, 1982b; Sweetman *et al.*, 2009).

2.2 The role of acid in wine

Organic acids play a vital role in the production of quality wines as they ultimately determine the organoleptic and aesthetic character perceived in the wine. In addition, both the shelf-life and ageing potential is influenced since the physical, biochemical and microbial stability of the wine is determined by the levels of wine acidity (Volschenk *et al.* 2006, Terrier & Romieu 2001, Boulton 1980).

Organic acids control the pH of the wine quantitatively and dominates the acid composition (Ribéreau-Gayon, 1968; Jackson, 2008a). As the condition of grapes, microbiological activity (*i.e.* *Botrytis cinereae*) and changes during winemaking may cause changes in the balance of acids, both the juice and wine acidity needs to be considered (Boulton *et al.*, 1998). Tartaric acid and MA are both important in wine making with TA being the more stable and stronger acid while MA play a large role in malolactic fermentation (MLF) (Dry & Coombe, 2005).

Tartaric acid is economically the most important acid as it is the main organic acid controlling the juice pH and therefore determining the suitability of the grapes for winemaking. Furthermore, through addition of TA during vinification, oxidative and microbiological spoilage can be minimized and thus improving the organoleptic and ageing potential of the completed wine (DeBolt *et al.*, 2007).

Tartaric acid is in general present in a constant average concentration in the juice of around 5 to 10 g/L (Ruffner, 1982b), whereas MA juice concentrations fluctuate mostly according to the climatic growing region. These fluctuating MA juice concentrations may prove detrimental to winemakers during the winemaking process, as unbalanced wines can be produced. Kanellis & Roubelakis-Angelakis (1993) noted that wines can either be tart with a low sugar content, or in contrast, 'flat' with either a high or low sugar content, all depending on the preceding climatic conditions.

Malolactic fermentation is used as a biological deacidification method and is performed on most red and some white wines. During this secondary fermentation performed by lactic acid bacteria (LAB), MA, a dicarboxylic acid, is converted to lactic acid which is a smoother tasting monocarboxylic acid (Jackson, 2008a). In wine, TA remains microbiologically inert and is not oxidised during fermentation (DeBolt *et al.*, 2007).

In wine, TA is found mainly in the form of potassium bitartrate and these crystals have also been observed in grape berries. Due to the instability of potassium bitartrate at lower temperatures and

in the presence of ethanol, finished wines are stabilised for crystallisation during storage by cold stabilisation (Boulton *et al.*, 1998).

2.3 Accumulation of malic- and tartaric acids in the berry during growth and ripening

Berry growth consists of two successive sigmoid cycles (Coombe, 1960, 1973, 1992; Coombe & Iland, 2005) which are separated by a lag phase (Coombe, 1973). The first cycle represents berry formation or cell division and during the second cycle the berry enlarges or cells expand that culminates in ripening.

The curve division can vary between two and four phases with the duration and the actualisation varying according to the cultivar and environmental conditions (Coombe, 1973; Kanellis & Roubelakis-Angelakis, 1993). Temperature does not only have an effect on sugar and acid levels in the grapes, but also has a significant bearing on berry growth, particularly in the first stage of berry development (Ruffner *et al.*, 1976).

During stage I (Fig. 2.1) berries actively accumulate organic acids, but small quantities of sugar (Dokoozlian & Kliwer, 1996). Stage II is in fact the slowing part of stage I, ending as ripening starts (Coombe & Iland, 2005). The second cycle (stage III) commences with berry softening, berry colouring and a renewed increase in size and is termed *véraison* (Kanellis & Roubelakis-Angelakis, 1993; Coombe & McCarthy, 2000). Sugar and colour accumulates rapidly in the berry during stage III, with a sharp decline in the total organic acid concentration (Dokoozlian & Kliwer, 1996). The pattern of decline of titratable acidity corresponds with the sharp decline in the MA content (Crippen & Morrison, 1986).

Accumulation patterns of MA and TA differ during the sigmoidal growth. Despite TA being intensively accumulated during rapid cell division following anthesis, MA is present in concentration two- to three fold prior to the start of ripening (Crippen & Morrison, 1986; Terrier & Romieu, 2001).

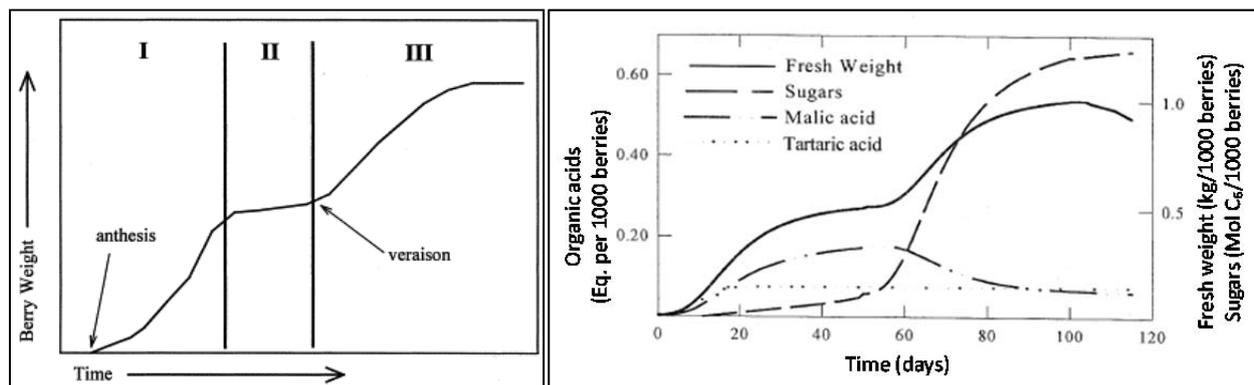


FIGURE 2.1

The three major developmental stages as noted by Coombe (1973) to the left, illustrating the double sigmoidal curve of berry development. The figure to the right illustrates the typical changes of the content of malic- and tartaric acid as well as sugar in the grape berry during development. The change in fresh mass is also indicated (Terrier & Romieu, 2001).

2.4 Malic acid

L-malic acid, of which the chemical structure of the molecule is shown in Fig. 2.2, is known to be an active intermediate in grape metabolism and the only high-proportion organic acid actively metabolised during ripening (Sweetman *et al.*, 2009). It has a significant role in certain anabolic reactions such as dark fixation of carbon dioxide and acid catabolising processes connected to fruit ripening (Ruffner, 1982b).

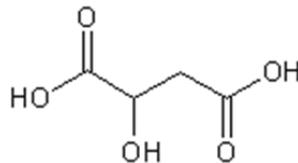
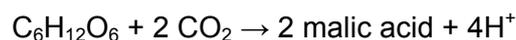


FIGURE 2.2

The chemical structure of L-malic acid (www.chemspider.com; accessed 10 October 2010).

Malic acid is accumulated in pre-*véraison* grapes mainly by the metabolism of sugars translocated to the grape berry, broken down to glucose and fructose, which in turn enters glycolysis for use in respiration (Sweetman *et al.*, 2009).



EQUATION 2.1

The metabolism gain of malic acid through respiration as reported by Terrier & Romieu (2001).

Although it has yet to be determined if MA is exclusively synthesized *in situ*, it is a fair assumption as Hunter & Ruffner (2001) found that phloem disruption by girdling did not influence the MA content in the grapes at any stage of development. Additionally, enzymes involved in the synthesis of MA in the grapes are present and active in the berries (Hawker, 1969).

Malic acid metabolism is dependent on the physiology of the fruit, the isoforms in which the enzymes are present and in addition the subcellular compartmentalisation of the enzymes, and the availability of the substrates (Martínez-Esteso *et al.*, 2011).

Sucrose is the main precursor of MA in grape berries and utilised via glycolysis, the oxidative pentose phosphate pathway and β -carboxylation (Ruffner *et al.*, 1976; Ruffner & Hawker, 1977; Kanellis & Roubelakis-Angelakis, 1993).

No correlation between MA accumulation and the activity of phosphoenolpyruvate carboxylase (PEPC) as well as acid decrease and the activity malic enzyme (ME) was detected in grape berries (Hawker, 1969), which indicates that neither of these pathways are the main pathways of synthesis or breakdown of MA and different synthetic and catabolising pathways are present in the grape berry which will be discussed hereafter.

2.4.1 Malic acid synthesis

Refer to Fig. 2.3 for the complete synthesis pathway of L-MA inside the grape berry. The principal synthetic pathways will be discussed below.

2.4.1.1 Glycolysis

Carbon dioxide is assimilated into MA in the grapevine and young berries by the C₃-mechanism (Ruffner & Hawker, 1977; Ruffner, 1982b) mediated by ribulose-1,5-biphosphate to form the primary product of phosphoglycerate (Kanellis & Roubelakis-Angelakis, 1993). Results from research by Ruffner *et al.* (1976) suggest that the acid-sugar interrelationship might be mediated by the rate and direction of carbon flow in glycolysis before and after the onset of ripening.

Martínez-Esteso *et al.* (2011) found eight enzymes active in glycolysis which slightly decreases in abundance during the early stages of development and sharply decreases at the end of the growth period. This is in concurrence with the inhibition of glycolysis at *véraison* as found by Ruffner & Hawker (1977).

The regulatory site in glycolysis is thought to be the nearly irreversible reactions catalysed by phosphofructokinase which is active in the conversion of fructose-6-phosphate to fructose-1,6-biphosphate (not shown in Fig. 2.3), and pyruvate kinase which plays a role in the conversion of phosphoenolpyruvate (PEP) to pyruvate as indicated in Fig. 2.3 (Ruffner & Hawker, 1977; Martínez-Esteso *et al.*, 2011).

As MA was found to be the main labelled product in the fruit after ¹⁴CO₂-assimilation in the light and the dark, the lack of diurnal acidity fluctuation and CO₂-compensation points rule out the existence of an active C₄- or Crassulacean acid metabolism (Ruffner, 1982b).

2.4.1.2 β-carboxylation of phosphoenolpyruvate (PEP) by phosphoenolpyruvate carboxylase (PEPC)

Lakso & Kliewer (1975a) initially stated that the essentially irreversible reaction of β-carboxylation of PEP and pyruvate to form MA is the most likely pathway of MA synthesis in grape berries, which in turn was later shown to not be the case by Ruffner *et al.* (1984).

Phosphoenolpyruvate is β-carboxylised to MA via oxaloacetate (OAA) (Ruffner *et al.*, 1983b) by PEPC and malate dehydrogenase (MDH) (Hawker, 1969; Ruffner & Kliewer, 1975; Lakso & Kliewer, 1975a; Ruffner, 1982b; Ruffner *et al.*, 1983b) of which the activity is inhibited by the presence of MA (Lakso & Kliewer, 1975a).

Phosphoenolpyruvate and pyruvate ratios, the products of glycolysis, were highest during the green stage of berry growth. Thereafter, PEP tends to accumulate while the pyruvate concentration decreased.

Phosphoenolpyruvate carboxylase activity increases during stage one of berry development in green berries, thereafter decreasing to around 50% as *véraison* approaches and subsequently recovering to some degree (Ruffner *et al.*, 1976). This coincides with the findings of Lakso & Kliewer (1975a) as they confirmed a 50% inhibition of PEPC in the presence of MA. The activity of PEPC after *véraison* however remains at low activity throughout berry ripening (Hawker, 1969; Lakso & Kliewer, 1975a).

2.4.1.3 β-Carboxylation of pyruvate by malic acid enzyme (ME)

The activity of ME is highest in very young berries, showing a decrease as *véraison* approaches (Lakso & Kliewer, 1975a). Post-*véraison*, ME activity increases temporarily after which the activity decreases with the advancement of berry ripening (Hawker, 1969). Under cooler conditions, the activity of ME nearly doubles post-*véraison* and remains at maximum values nearing maturity (Ruffner *et al.*, 1976).

During the acid accumulation phase of berry development, a high ME activity is observed indicating an alternative biosynthesis of MA through the carboxylation of pyruvate (Kanellis & Roubelakis-Angelakis, 1993) possibly also in the cytosol by nicotinamide adenine dinucleotide phosphate (NADP)-ME (Martínez-Esteso *et al.*, 2011). Ruffner *et al.* (1984) did, however, indicate through experimentation that this reaction does not essentially contribute to MA synthesis in the grape.

The activity of ME is possibly regulated in grape berries by succinate, a product of the tricarboxylic acid (TCA)-cycle, as it activates the enzyme at low MA concentrations and inhibits the enzyme at high concentrations (Lakso & Kliewer, 1975a), therefore indicating that succinate may be an effector for MA synthesis. Little is known about the presence of succinate in grape berries though.

2.4.1.4 The tricarboxylic acid (TCA) cycle (oxidative pentose phosphate pathway)

The role of the TCA cycle is mentioned in numerous publications as a pathway for the conversion of glucose to MA, but is seldom elaborated on (Kanellis & Roubelakis-Angelakis, 1993; Sweetman *et al.*, 2009). In particular, it is noted for its role in the breakdown of MA (Terrier & Romieu, 2001; Famiani *et al.*, 2005; Sweetman *et al.*, 2009).

According to Kanellis & Roubelakis-Angelakis (1993), intermediates of the TCA cycle account for some of the additional organic acids found in the grape berry. Furthermore, MA that is formed via fumerase in the mitochondria as part of the TCA cycle (step not shown in Fig. 2.2) may be extracted for accumulation in the vacuole instead of being converted to OAA by mitochondrial malate dehydrogenase (MDH) (Sweetman *et al.*, 2009).

2.4.2 Malic acid synthesis in grapevine leaves

In very young tissue, MA accumulates steadily after a lag phase after and the highest level is reached when the lamina is approximately one fourth of its full length. MA concentration shows a tendency to increase steadily as leaves age (Kliewer & Nassar, 1966). No change in MA synthesis or in MA remetabolisation is evident when the leaf is photosynthetically active (Ruffner, 1982b).

Variation in the content of MA in the leaves may be attributed to the balanced activity of synthesizing-, dissimilatory- or transport mechanisms (Lakso & Kliewer, 1975b; Ruffner *et al.*, 1976). Furthermore, light altered the content of MA in the leaves whereas the TA content remained unchanged (Stafford, 1959; Morrison & Noble, 1990; Hunter *et al.*, 1991).

In the leaf, MA is a primary product in the C₄-decarboxylic acid pathway of photosynthesis in which CO₂ is assimilated. This serves as a transport vehicle for CO₂ from the mesophyll to the bundle sheath compartments of the leaf and this is reflected in the diurnal acidification or deacidification rhythm in mostly succulent tissues implementing crassulacean acid metabolism (Ruffner, 1982b). The coarse control of MA metabolism and regulation is not found in the leaf tissue as the seasonal acid and sugar fluctuating pattern is not exhibited (Ruffner *et al.*, 1976).

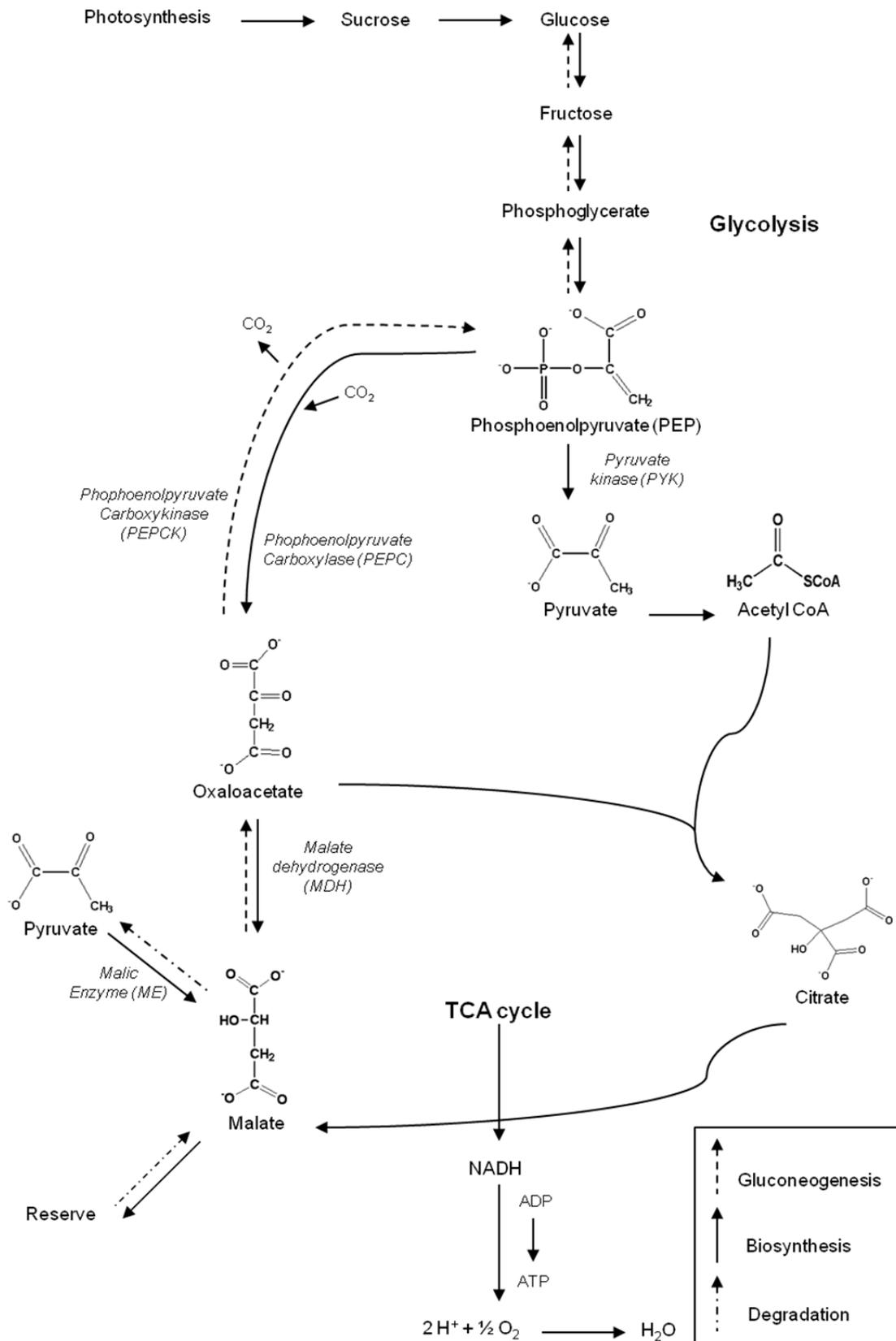


FIGURE 2.3

The pathways of malic acid inside the grape berry as described by Eggers (2006) and Volschenk *et al.* (2006).

2.4.3 The fate of malic acid during ripening

Malic acid accumulates in the first six weeks of berry development with a substantial decrease in concentration after *véraison* (Ruffner & Hawker, 1977; Ruffner, 1982b; Terrier & Romieu, 2001; Sweetman *et al.*, 2009). After *véraison*, MA is released from the vacuole and catabolised through different pathways including the TCA-cycle, respiration, gluconeogenesis, amino acid interconversions, ethanol fermentation and included in the production of complex secondary compounds such as anthocyanins and flavonols (Sweetman *et al.*, 2009). Work done by Crippen & Morrison (1986) showed a delay in the decline of MA in the juice fraction of the berry versus the pulp and therefore supports the findings of Ruffner (1982b) and Terrier & Romieu (2001) that MA is translocated from the vacuole to the cytoplasm for catabolism. As sugars are accumulated and glycolysis inhibited during ripening (Ruffner & Hawker, 1977; Ruffner *et al.*, 1983a), it is likely that MA is used as the source of carbon in the mentioned pathways.

It is clear that there are two metabolically different pools of MA present in the grape berry (Ruffner, 1982b), namely a rapidly equilibrated metabolic pool and, on the other hand, a slow equilibrated storage pool (Lakso & Kliewer, 1978). Upon inhibition of the glycolysis pathway, the intermediates of glycolysis and the Krebs cycle are consumed and in turn replenished from the MA reserve pool (Kanellis & Roubelakis-Angelakis, 1993).

2.4.3.1 Respiration

The acid decrease during ripening is mostly attributable to the consumption of MA (Ruffner & Hawker, 1977) by the respiration of pyruvate by the NADP-dependent malic enzyme (Hawker, 1969; Lakso & Kliewer, 1975b; Ruffner *et al.*, 1976). The malic enzyme has a pronounced specificity for the L(-) form of MA (Ruffner *et al.*, 1984) which occurs naturally in grapes and indicates a regulatory property of the enzyme (Kanellis & Roubelakis-Angelakis, 1993).

With higher temperatures, the supply of respiratory products is switched from simple carbohydrate degradation to acid breakdown (Kanellis & Roubelakis-Angelakis, 1993), therefore increasing the respiratory rates of MA (Ruffner, 1982b). This substrate shift is steered by the relative size of the sugar and MA pools, which in turn is directed by changes in vacuolar compartmentation (Terrier & Romieu, 2001). The release of MA from the vacuole into the cytoplasm at *véraison* does not induce an increase in the respiration rate, but may be necessary to maintain the respiration rate as sugars are redirected to the vacuole (Sweetman *et al.*, 2009). Hawker (1969) did however note that the increased rate of MA through respiration after the onset of ripening may be due to a change in the permeability of membranes and the consequent contact of MA with MAE and pyruvate decarboxylase.

More CO₂ per molecule of O₂ consumed is released through respiration of MA in comparison with respiration of starch and sugar in ripening climacteric fruit, therefore it could be considered a better source of “fuel” (Sweetman *et al.*, 2009).

2.4.3.2 Gluconeogenesis

In experimentation with specifically labelled tracers, it was demonstrated that a reversal of carbon flow occurs during berry ripening (Ruffner *et al.*, 1976; Ruffner, 1982b). In green berries, no transformation of ¹⁴C malate to sugar was observed suggesting glycolytic flow towards pyruvate. Ripening berries show discernible gluconeogenic activity though (Ruffner & Hawker, 1977). The gluconeogenic metabolism of MA is initiated with the oxidation of MA to OAA, followed by

decarboxylation and subsequently by reversed glycolysis (gluconeogenesis) to yield hexose (Kanellis & Roubelakis-Angelakis, 1993) as seen in Fig. 2.3.

Cytoplasmic malate dehydrogenase (Martínez-Esteso *et al.*, 2011) catalyses the oxidation of MA to OAA and phosphoenolpyruvate carboxykinase (PEPCK) the decarboxylation of OAA to PEP (Hawker, 1969; Ruffner & Kliewer, 1975; Sweetman *et al.*, 2009). PEPCK shows 25 to 50% of the activity of that of PEPC during berry development (Lakso & Kliewer, 1975a) and catalyses the reversible reaction of the conversion of OAA to PEP with the use of ATP and the production of CO₂ and ADP (Lakso & Kliewer, 1975a).

Phosphoenolpyruvate carboxykinase showed the highest activity during rapid sugar accumulation and remains at maximum levels during ripening whereas PEPC activity drops rapidly in this period, thereby changing the direction of the cycle towards sugar accumulation and a consecutive acid decrease (Ruffner *et al.*, 1976). Although PEPCK display distinct regulatory properties, Ruffner & Kliewer (1975) found that the activity of the enzyme was not influenced by temperatures of up to 40°C.

Gluconeogenesis does not contribute to more than 5% of the transformation of MA to sugar though, assuming that no fresh MA is formed during this period (Kanellis & Roubelakis-Angelakis, 1993). As MA is mainly metabolized via respiration, gluconeogenesis should not be seen as an important pathway for hexose accumulation (Ruffner & Hawker, 1977), but rather considered as a mechanism between the two groups of compounds by mediating the flow of carbon (Kanellis & Roubelakis-Angelakis, 1993).

No direct correlation between temperature and gluconeogenesis has been found, however the influence of the ripening stage on gluconeogenesis was found to be more pronounced than that of temperature (Ruffner, 1982b).

2.4.3.3 Activity of the malic acid enzyme (ME)

As noted before, ME has a pronounced specificity for L (-) form of MA that naturally occurs in grape berries (Possner *et al.*, 1981; Ruffner *et al.*, 1984), and is capable of an absolute selective reverse C₄-decarboxylation reaction to pyruvate. This reaction is favoured when energetic considerations as well as the allosteric regulation of the enzyme are taken into account (Possner *et al.*, 1981; Ruffner *et al.*, 1984). Despite this favouritism, ME activity is still detected during the acid-accumulation phase of berry development (Ruffner *et al.*, 1984). However, Ruffner *et al.* (1976) observed a maximum activity of the ME accompanying a distinct decrease in the MA content from the onset of ripening. This coincides with the theory of Sacher in 1973 as cited by Possner *et al.* (1981) that membrane permeability increases at the start of ripening, allowing the leaking of MA out of the vacuole into the cytoplasm, therefore flooding it with ME substrate.

Malic enzyme is found either in the cytosol as NADP-dependent ME (NADP-ME), or in the mitochondria in a nicotinamide adenine dinucleotide (NAD)-dependent form (NAD-ME) (Sweetman *et al.*, 2009). NADP-ME found in the cytosol may take part in degradation of MA during ripening which was confirmed with an increase in NADP-ME measured during ripening by Ruffner *et al.* (1984). The activity of NAD-ME has not yet been detected in grapes, but the presence of the NAD-ME protein was observed by Famiani *et al.* (2000) with immunohistochemical work, therefore indicating a possible role of NAD-ME in MA degradation (Sweetman *et al.*, 2009).

2.4.3.4 The tricarboxylic acid (TCA) cycle (oxidative pentose phosphate pathway)

When MA enters the TCA cycle, it is firstly converted to OAA and thereafter to acetyl coenzyme A (acetyl-CoA) by either ME or PEPCK, whereafter acetyl-CoA is condensed to citrate and back to MA with the release of two CO₂ molecules (Famiani *et al.*, 2005). This reverse reaction can be seen in Fig. 2.3.

Cycle intermediates are constantly withdrawn from the cycle for use in other processes, therefore a constant carbon injection is needed into the cycle. Part of the MA released from the vacuole at *véraison* into the cytoplasm may be transported to the mitochondria and fed into the TCA cycle to maintain this respiratory flux in the cells (Sweetman *et al.*, 2009).

(Ruffner *et al.*, 1976) found a noticeable increase in PEPCK in the flesh of grape berries after the onset of ripening which suggested that it may function in the catabolism of MA and citrate. Indirectly pyruvate is also an important respiratory fuel as acetyl-CoA, an intermediate necessary for the functioning of the TCA cycle, is metabolised from pyruvate by pyruvate dehydrogenase, or in turn pyruvate is created by the decarboxylation of MA by malic enzyme activity either in the cytosol or the mitochondria (Sweetman *et al.*, 2009). Several forms of ME exist in the grape berry, with NADP-ME being proposed as playing a role in the catabolism of malic and citric acid in the flesh of ripening berries (Ruffner, 1982b).

2.5 Tartaric acid

Ruffner (1982a) pointed out that even though the occurrence of TA in higher plants is relatively unusual in commercially cultivated fruit, grapes can accumulate TA in substantial amounts. Contrary to other cultivated fruit where MA and citric acid accumulation are more common, grapes accumulate significant quantities of L-TA (refer to the chemical structure in Fig. 2.4) which remains in the berry during ripening (Watson, 2003; DeBolt *et al.*, 2006; DeBolt *et al.*, 2007).

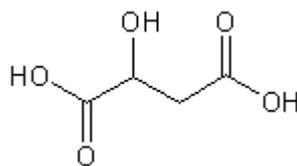


FIGURE 2.4

The chemical structure of L-tartaric acid (www.chemspider.com; accessed 10 October 2010).

The biosynthetic origin is only partly understood (Loewus, 1999) and even though the nature of the chemical intermediates have been established, data regarding the identification of the enzymes responsible is scarce and attempts unsuccessful (Conde *et al.*, 2007). Loewus (1999) did comment that the direct conversion of D-glucose to L-ascorbic acid produce only carboxyl-labeled TA from L-[1-¹⁴C]ascorbic acid as produced in grape tissue.

In the family Vitaceae, TA is present as an optically active L-(+)-stereoisomer (Wagner *et al.*, 1975). Contrary to most organic acids, the metabolic origin of TA is situated outside of the oxidative metabolism of sugars and originates with L-ascorbic acid (Conde *et al.*, 2007) which in turn is synthesised from glucose (Loewus, 1999). Tartaric acid is mainly stored as an insoluble calcium salt i.e. calcium tartrate (Ruffner, 1982a; Storey, 1987) and calcium oxalate (Hardie *et al.*, 1996) with a portion stored in the vacuoles in the free acid form (Kanellis & Roubelakis-Angelakis,

1993). Ruffner (1982a) concluded that K^+ is not in any way correlated with the TA contents in grapes at any stage in development, which was previously proposed by Dokoozlian & Kliewer (1996).

The biosynthesis of TA is limited to the period from post-anthesis up to *véraison* (Coombe & McCarthy, 2000; Watson, 2003; DeBolt *et al.*, 2006; Conde *et al.*, 2007). Thereafter, the concentration of TA decreases due to an increase in the berry volume, but the TA content in the berry remains relatively constant. The two main pathways of TA synthesis are indicated in Fig. 2.5.

2.5.1.1 Synthesis of ascorbic acid

According to Cruz-Rus *et al.* (2010), several biosynthetic pathways for ascorbic acid might function in plants. Wheeler *et al.* (1998) determined the following pathway as demonstrated in Fig. 2.5 part A for the biosynthesis of L-ascorbic acid in plants (Loewus, 1999; DeBolt *et al.*, 2006).

The conversion of D-glucose-6-phosphate to guanosine diphosphate (GDP)-D-mannose and GDP-L-galactose has been well defined and the enzymes identified and specified in Fig. 2.4 part A.

L-galactose is derived from GDP-L-galactose which in turn is derived from GDP-D-mannose by GDP-D-mannose-3,5-epimerase, a poorly characterized enzyme (Wheeler *et al.*, 1998). GDP-L-galactose is hydrolysed in two steps to produce L-galactose, but the presence of L-galactose-1-phosphate as an intermediary compound remains speculative.

L-galactose is oxidized in position C1 by L-galactose dehydrogenase resulting in L-galactono-1,4-lactone and finally oxidised by mitochondrial L-galactono-1,4-lactone dehydrogenase to form L-ascorbic acid.

Melino *et al.* (2011) found ascorbic acid accumulation and its catabolites coincide with the photosynthetic rate of *Vitis vinifera* berries and leaves. This can be attributed to the light dependency of ascorbic acid accumulation being linked to its functioning in the chloroplast where it is found in varying concentrations.

2.5.1.2 Biosynthesis of tartaric acid from ascorbic acid

Three pathways for the formation of TA is known, of which two originate from L-ascorbic acid and one from D-gluconate (Saito & Kasai, 1984; Loewus, 1999).

The first pathway occurs in Vitaceae and experimentation showed that the main step for the formation of TA was the cleavage of L-ascorbic acid between C4 and C5 and the C4 fragment consequently converted to TA (Wagner *et al.*, 1975; Loewus, 1999) as can be seen in Fig. 2.5 part B. The residual two-carbon compound is most probably glycoaldehyde (Conde *et al.*, 2007) which is recycled back to a hexose phosphate (Williams *et al.*, 1979; Loewus, 1999).

In the second pathway, L-ascorbic acid is cleaved between C2 and C3 which yields oxalic acid and L-threonate and in turn converted in plants of the Geraniaceae to TA in the leaves (Loewus, 1999; DeBolt *et al.*, 2006). DeBolt *et al.* (2004) found that in intact berries of *Vitis vinifera* both OAA and TA is formed through ascorbic acid catabolism, suggesting that in grapevine both pathways are functional.

Radio-isotope tracer studies revealed the direct pathway of the catabolism of ascorbic acid to TA and is illustrated in Fig. 2.5 part B. DeBolt *et al.* (2007) and Kanellis & Roubelakis-Angelakis (1993) indicated that ascorbic acid is converted to 2-keto L-idonic acid, successively reduced to L-idonic

acid and oxidized to 5-keto-D-gluconic acid. Later literature substituted 2-keto L-idonic acid with 2-keto-L-gluconic acid as indicated in Fig. 2.5 part B (Conde *et al.*, 2007). The penultimate step includes the cleavage of 5-keto-D-gluconic acid between C4 and C5 yielding a four-carbon compound, L-threo-tetruronate, and a two-carbon compound which is proposed to be glycoaldehyde. L-threo-tetruronate is finally oxidised to L-TA.

To date, the only enzyme isolated in the second pathway is L-idonate dehydrogenase, responsible for the conversion of L-idonic acid to 5-keto-D-gluconic acid as indicated in Fig. 2.5 part B (DeBolt *et al.*, 2006; Martínez-Esteso *et al.*, 2011).

2.5.2 Tartaric acid synthesis in the leaves

According to Stafford (1959), *Vitis vinifera* falls in a group of plants that has the highest concentration of TA in the leaves of 20 to 200 $\mu\text{moles/g}$ fresh weight of the leaf, and a cell sap pH that ranges from 3.0 to 3.8.

Tartaric acid is only synthesized at a substantial rate in leaves up to a certain size and stage of development (Ruffner, 1982a), with the quantity fluctuating rapidly around a constant value between the older and younger leaves (Peynaud & Maurié, 1958). As mentioned by Ruffner *et al.* (1976), the peculiar pattern of seasonal fluctuations as seen in the berries are not exhibited in the leaf tissue.

Tartaric acid is segregated in the salt form (calcium tartrate) in the idioblasts that is grouped in the intercostal section of fully differentiated leaves. The occurrence of these tartrate cells are more frequent in the serrated tips of the leaves than in the rest of the lamina, although the MA concentration is equal in both (Ruffner, 1982a).

The intensive TA formation in young and growing tissue under hot conditions may be ascribed to the increased production of glucose via the hexose monophosphate course as TA is believed to be oxidised from glucose (Ruffner *et al.*, 1976).

2.5.3 The fate of tartaric acid during ripening

Tartaric acid biosynthesis occurs during the early part of berry growth and the content per berry remain stable during the ripening period (Ruffner, 1982a; Crippen & Morrison, 1986; Iland & Coombe, 1988; DeBolt *et al.*, 2004). The resistance of the crystalline forms towards metabolizing enzymes attributes to the inertness of TA during ripening (Iland & Coombe, 1988).

The influence of light, hour of the day, age, plant tissue and cell compartment on the ascorbic acid content in plants are well known (Cruz-Rus *et al.*, 2010). The effect of these factors on the level of ascorbic acid and ultimately TA in the grape berry and the leaves, are later discussed in Section 2.6. in greater detail.

Although TA content remains relatively stable during ripening, a decrease in the content in warmer areas can be ascribed to TA being utilised for respiration. Gerber (1897) investigated the effect of temperature on the respiratory quotient of organic acids and found that when temperature was above 35°C, TA was respired, whereas MA was respired at 30°C (Kliwer, 1964). These findings have been noted in numerous references, but to date have not yet been proven scientifically.

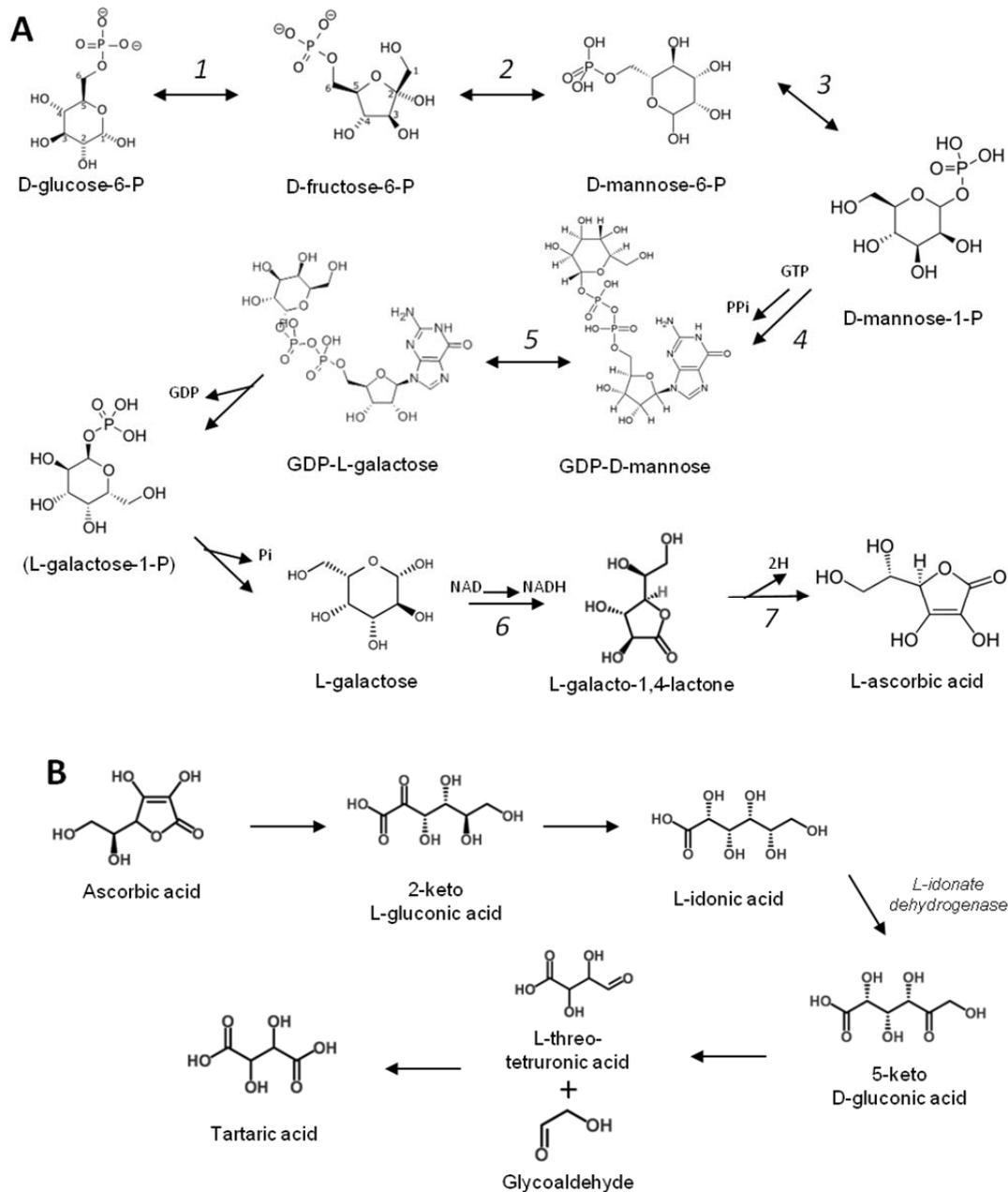


FIGURE 2.5

A - The Smirnov–Wheeler pathway proposed for L-ascorbic acid biosynthesis in higher plants. Cited by (Loewus, 1999) from Wheeler *et al.* (1998). The enzymes identified are: 1 - hexose phosphate isomerase; 2 - phosphomannose isomerase; 3 - phosphomannose mutase; 4 - GDP-D-mannose pyrophosphorylase; 5 - GDP-D-mannose-3,5-epimerase; 6 - L-galacto dehydrogenase; 7 - L-galactono-1,4-lactone dehydrogenase.

B - The proposed pathway for tartaric acid formation from ascorbic acid in the grapevine as adapted from Loewus (1999) as adapted by DeBolt *et al.* (2007) indicating the presence of L-idonate dehydrogenase. The presence of this enzyme provide evidence of a biochemical component in this pathway (Conde *et al.*, 2007) which is also seen as the rate-limiting step in the pathway (DeBolt *et al.*, 2006). The precise nature of the reaction step of ascorbic acid to 2-keto-L-gluconic acid is unknown.

2.6 Distribution of malic- and tartaric acids within the berries and the vine

2.6.1 Compartmentation of malic- and tartaric acid in the cell

Hawker (1969) and Ruffner (1982b) hypothesised that grape berry acidity changes are driven by compartmentation, and this has since been confirmed by research (Terrier & Romieu, 2001).

It is widely accepted that organic acids are transported via secondary transport into the vacuole by an electrochemical gradient generated by two proton pumps, and that this is possibly the rate limiting step for the complete pathway of MA synthesis and storage (Terrier & Romieu, 2001). They also indicated that with an increase in berry K^+ , there was a corresponding increase in MA concentration and a reduction in TA. This would explain the rapid blockage of TA accumulation during a high rate of MA synthesis during berry development.

Intense vacuolar enlargement due to the mass storage of MA, TA and citric acid as major osmotic causing a nine-fold increase of the pericarp cells between anthesis and ripening (phase I) (Terrier & Romieu, 2001; Volschenk *et al.*, 2006). MA is stored in the vacuole and used as a source of energy post-*véraison* as the glycolytic pathways is severely inhibited (Kanellis & Roubelakis-Angelakis, 1993).

2.6.2 Localisation of malic- and tartaric acid in the berry

The differences in organic acid content between the skin and the flesh are significant, with MA prior to *véraison* accumulating substantially in the flesh. In contrast, TA is mainly found in the berry skin (Coombe & Iland, 2005).

2.6.2.1 Malic acid

Prior to *véraison*, MA is found at an increasing gradient towards the centre from the periphery of the berry (Possner & Kliewer, 1985). At the onset of *véraison*, the accumulation of MA in the skin occurs at a more rapid rate than any other fruit part (Possner & Kliewer, 1985). Consequently, MA is predominantly found in the skin in comparison to the pulp as well as the more commonly found organic acid in the skin (Storey, 1987).

During the onset of ripening following a lag phase of MA breakdown (Possner & Kliewer, 1985), the content of MA falls rapidly with a simultaneous drastic increase of mainly glucose and fructose (Ruffner *et al.*, 1976; Ruffner, 1982b).

2.6.2.2 Tartaric acid

Unlike MA, TA is predominantly found in the berry pulp at levels which are three to four times higher than that of MA (Storey, 1987). During berry development, the concentration of TA remains relatively constant in the pulp and the area around the seeds. On the other hand, whilst the concentration of TA is initially higher in the skin and adjacent flesh, a steady decrease throughout berry development ultimately results in berry skin levels considerably lower than found in the pulp (Possner & Kliewer, 1985).

In contrast to MA breakdown after the onset of ripening, the TA content remained constant with a decrease in TA concentration due to the dilution effect of an increase in berry volume (Possner & Kliewer, 1985).

Biomineralisation (crystal formation) is present in leaves, roots, fruits, seeds and stems in *Vitis* and results in two crystalline forms, namely raphides and druses. Raphides, which are bundles of needle like crystals of calcium tartrate (Hardie *et al.*, 1996) are found in idioblasts distributed throughout the exocarp, whereas druses are star-shaped crystal aggregates (of calcium oxalate (Hardie *et al.*, 1996) which, in contrast, are found within cells of the berry endocarp as seen in Fig. 2.6 (DeBolt *et al.*, 2004). Even though K^+ is present at high levels in the berry skin, crystalline deposits of K^+ were not observed and is mainly found in the soluble form in the vacuoles of hypodermal cells (Storey, 1987).

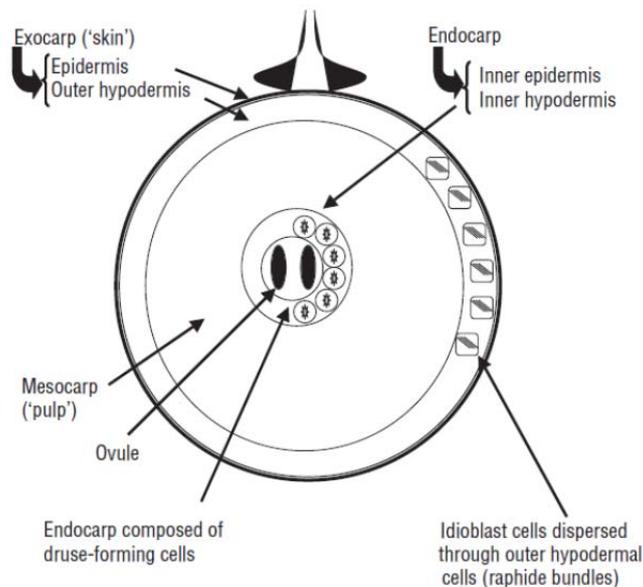


FIGURE 2.6

A schematic diagram illustrating the distribution of raphide and druse crystals within the berry tissue (DeBolt *et al.*, 2004).

2.6.3 Localisation of malic- and tartaric acid in the plant

As noted before in Section 2.5.2, Stafford (1959) reported that both TA and MA were observed in the *Vitis vinifera* leaves at high quantities.

In the month following budburst, TA and MA levels increased two- to four-fold coinciding with the rapid increase of the leaf area. During this period TA is rapidly synthesised to high levels in immature leaves where after the content is stable (Kliewer & Nassar, 1966; Morrison & Noble, 1990). Despite an increase in MA production in immature leaves, it is found at levels lower than that of TA, with the content steadily increasing during the growing period (Kliewer & Nassar, 1966; Morrison & Noble, 1990). Towards the end of the season in autumn, TA levels in the leaves decreases three-fold and MA seven-fold as leaves become senescent (Kliewer & Nassar, 1966), coinciding with an increase of the levels in the wood suggesting that the TA and MA is translocated to the woody tissue (Kliewer, 1966; Ruffner, 1982b).

2.6.4 Translocation of malic- and tartaric acid from the leaves to the berries

Literature regarding the translocation of MA and TA from the leaves is scarce, with available literature presenting contradictory results and no definite conclusions. In addition, where conclusions have been made, very often supporting evidence is lacking.

Initially, Winkler (1962) concluded from statements made from other authors (papers cited within this publication but unavailable), that even though some authors did find that acids are translocated from the leaves to the berries, other authors found that both organic acids were mainly synthesized in immature grape berries, not excluding the possible translocation of the organic acids from the leaves to the berries though.

Using ^{14}C incorporation studies by other authors, Skene & Hale (1971) concluded that immature grape berries are capable of synthesizing MA and TA, either from sugar or from CO_2 - fixation products from the leaf, or present in the berry. More recently, Sweetman *et al.* (2009) remarked that it has not yet been determined yet whether MA is exclusively synthesised *in situ*, or if a certain percentage is synthesised in other tissues and transported to the berry, even though the potential for organic acid translocation from the leaves to the fruit was already suggested in 1968 (Ribéreau-Gayon). However, there is no evidence that indicates that either MA or TA is transported from the leaves to the fruit (Watson, 2003).

According to Sweetman *et al.* (2009) and Ribéreau-Gayon (1968) it is mainly sucrose as precursor that is transported from the leaves to the berries, broken down to glucose and fructose which in turn enter in glycolysis for the use in respiration. This occur pre-*véraison* and enables synthesis of MA in the berry.

Lobit *et al.* (2006) concluded that MA has to be synthesised in the grape berry itself, as the pH of the xylem sap ranges between pH 5 and pH 6, and is higher than pH 7 in the phloem sap therefore, these values are higher than the pK of the weakest MA ($pK=5.2$), and consequently cannot be transported except as a conjugated base with a cation e.g. K^+ .

2.7 Factors affecting the concentration of malic- and tartaric acid in the berry

Berry composition predominantly affects the wine quality, which in turn is affected by how and under what conditions the berry develops (Coombe & Iland, 2005). The effect of environmental factors on the TA content is less obvious (Boulton *et al.*, 1998), whereas the rate of MA breakdown is mainly determined by cultivar, rootstock, nutritional status and temperature (Terrier & Romieu, 2001). However, the variation as instigated by environmental factors is minimal in comparison to the variation triggered by berry development (Terrier & Romieu, 2001).

2.7.1 Physiological factors

The MA to TA ratio at harvest differ considerably according to the grape variety (Kanellis & Roubelakis-Angelakis, 1993), with varieties having a higher TA to MA ratio more favourable for the production of wine, especially in warmer wine regions (Buttrose *et al.*, 1971). Interestingly to mention is *Gora chirine* (*Vitis vinifera* cv L.), which, as an exception to the rule, naturally contains five times less acid than other cultivars. As compensation for the loss in osmotic potential, glucose is stored precociously before ripening (Terrier & Romieu, 2001).

Phenology plays an important role and it was shown on Cabernet franc in the Loire valley that early anthesis indicated an early *véraison* (Barbeau *et al.*, 1998). These earlier vintages were synonymous with positive correlations for sugar and anthocyanins. However, early vintages were negatively correlated with MA. An early budburst did not however indicate an earlier *véraison* as also found by Jones & Davis (2000) for the other phenological events. Jones & Davis (2000), in addition, noted the correlation between the phenological dates, with a phenological event being correlated with the preceding event.

Conradie *et al.* (2002), however, noted that subtle climate differences does have an effect on grapevine phenology which in turn has an effect on wine chemical parameters as ripening is either delayed or advanced and sugar/acid/pH balances affected. Temperature is still used as the most important parameter in plant phenology modelling (Due *et al.*, 1993; Jones & Davis, 2000; Chuine *et al.*, 2003; Jones, 2003; García de Cortázar-Atauri *et al.*, 2010; Parker *et al.*, 2011). The duration of the various phenological stages also influences the climatic temperature regimes the grapevine is exposed to, therefore influencing either the rate of synthesis or degradation of the organic acids. Jones & Davis (2000) found that acid levels for Cabernet Sauvignon and Merlot noir showed large positive correlations with the main phenological indicators and indicating a higher relative acidity when the stages as delayed.

According to Van Leeuwen & Seguin (2006), expressing terroir optimally through the precocity of the grapevine with varieties that are suited to the climatic conditions, will ensure that ripeness will be obtained by the end of the growing season, i.e. reaching ripeness under the local climatic conditions.

2.7.2 Environmental factors

Altered environmental factors may delay (Hardie & Considine, 1976; DeBolt *et al.*, 2008) or accelerate (Coombe & Iland, 2005) ripening, or shorten the ripening process. Consensus have not yet been reached whether the best quality wines are produced in a shorter or a longer ripening season, but according to Gladstones (1992) grapes that ripened rapidly and early favoured the compositional balance and ensures desirable flavour characteristics. He furthermore noted that an early harvest time reduces the risk of losing quality due to unfavourable environmental conditions. Van Leeuwen *et al.* (2004) noted that the vintage has the strongest influence on total acidity as well as pH of the grape juice, with the cultivar and the soil type influence noticed to a lesser extent. This is mainly due to the variability of MA between vintages and according to the cultivar as the influence is not that noticeable for TA.

Grapes at harvest may however respond to a desired composition if the vegetative and reproductive period of development of the grapevine is adapted to the local conditions (Parker *et al.*, 2011).

2.7.2.1 Temperature

The acid content of berries are mainly affected by temperature (Kliewer, 1964; Kliewer & Lider, 1970; Buttrose *et al.*, 1971; Ruffner *et al.*, 1976; Coombe, 1987a; Iland, 1989; Kanellis & Roubelakis-Angelakis, 1993; Van Leeuwen *et al.*, 2004). The effect of air temperature on the composition of the berry in relation to berry size, soluble solids, [MA], pH and titratable acidity however vary according to duration of exposure and stage of berry growth (Crippen & Morrison, 1986).

Variations in heat summation during berry development can be related to the seasonal variations in the organic acid and sugar concentrations at harvest (Winkler, 1962; Buttrose *et al.*, 1971). Viticulturists are particularly interested in the negative correlation between acid concentrations and high seasonal temperatures (Ruffner *et al.*, 1976). He further found that lower acidity levels at maturity of grapes grown at a higher temperature are due to acid breakdown rather than a reduction in MA accumulation and Buttrose *et al.* (1971) mentioned that it may be the result of an increase in the rate of respiration of the berries.

Lakso & Kliewer (1975b) and several other authors (Kliewer & Lider, 1970; Buttrose *et al.*, 1971) noted that the negative correlation for MA accumulation has been demonstrated and that an *in vitro* temperature close to 20°C is optimal for MA accumulation. This temperature indeed resulted in a higher acid content in grapes at maturity, therefore confirming the presence of higher levels of MA in cooler wine growing regions. Furthermore, no significant effect was seen on the concentration of MA with a decrease of temperature for 10 days from 30°C to 20°C either prior to, or after the lag phase (Fig. 2.1). In comparison, a temporary increase in the temperature from 20°C to 30°C during the same period resulted in a minor but consistent decrease in the MA content towards the end of ripening (Buttrose *et al.*, 1971). A great number of days with temperatures above 30°C during flowering and véraison produce grapes with a lowered total acidity (Jones & Davis, 2000).

In addition, a daytime temperature ranging between 20°C and 25°C (Kliewer, 1964; Kliewer & Lider, 1970; Lakso & Kliewer, 1975b; Ruffner *et al.*, 1976) and a night time temperature of approximately 15°C (Kliewer & Lider, 1970; Hunter & Bonnardot, 2011) during the pre-véraison period are seen as optimal temperatures for MA synthesis. Kobayashi *et al.* (1967) found that differences in day- and night temperatures, especially low night temperatures increased the yield and berry quality.

Where grapes are exposed to solar radiation, the use of a degree day index as an assessment tool for viticultural climates provides limited information on fruit temperatures (Smart and Sinclair, 1976). This is also applicable for the same daily temperatures. In an area such as Stellenbosch in the Western Cape Coastal region where the sea breeze is a prominent occurrence (Bonnardot, 1997), ambient temperature cannot be seen as a reliable indicator of berry temperatures. Bergqvist *et al.* (2001) likewise noted that temperature differences between exposed and shaded berries may be less distinct in cooler rather than in warm climates.

Oliveira (1998) stated that thermal time and plant growth and plant development is proportional. Temperature does therefore not only influence berry composition, but also the phenology of the grapevine as discussed earlier in this section.

2.7.2.2 Light

It has to be noted that light and temperature cannot easily be separated (DeBolt *et al.*, 2008) as the temperature of grape berries is linearly related to the level of incident radiation, where direct sunlight has a greater heating potential than diffused light (Smart & Sinclair, 1976). Higher temperatures and higher respiration rates usually found in light exposed berries are in general attributed to lower MA levels (Lakso & Kliewer, 1978; Dokoozlian & Kliewer, 1996; Bergqvist *et al.*, 2001).

DeBolt *et al.* (2006) and Kliewer & Schultz (1964) showed that maximum levels of TA formation was obtained where berries were fully exposed to sun. During the first six weeks of berry development, the rate of MA formation in the berry does not respond to a change in light intensity,

after which, up to *véraison*, an increase in light exposure induced an increase in MA levels in comparison to bunches with a lower light exposure (DeBolt *et al.*, 2008).

Post-*véraison*, MA shows a sharper decrease in content with an increase in light intensity as opposed to no influence seen in the accumulation of MA prior to *véraison* (DeBolt *et al.*, 2008). The activation of metabolic enzymes by light was given as a possible explanation and Famiani *et al.* (2000) had similar results.

Crippen & Morrison (1986) suggested that in a situation where the temperature was controlled during an increase in light exposure, no significant changes in the final levels of total acidity in grape juice was noticed.

Therefore, light exposure has a direct (light quantity and quality) and indirect (temperature mediated) influence on berry composition (Bergqvist *et al.*, 2001).

The berry size of shaded berries is increased in relation to exposed berries, thereby decreasing the TA and MA concentration in the berry as a consequence of dilution. Where higher levels of MA is found in shaded berries can however be attributed to lower berry temperatures (Smart, 1992) as well as a decrease in light (Melino *et al.*, 2011).

2.7.2.3 Water

The availability of water to the grapevine, either as rainfall or irrigation, affects the berry composition through grapevine water status. This, in turn, depends on the climate, soil and the training system (Van Leeuwen *et al.*, 2004; Van Leeuwen & Seguin, 2006). The seasonal timing of the water deficit is, however, indicative of the response of the grapevine (Ojeda *et al.*, 2001; Deloire *et al.*, 2004; Roby & Matthews, 2004).

The influence of grapevine water status on the organic acid content is mainly indirect though, through influencing the physiological responses of the plant (e.g. phenology), the vegetative response (e.g. vigour) and the reproductive responses (e.g. berry size).

High water availability can also induce vigorous conditions, which firstly influences the berry volume, and secondly the canopy micro climate through shading (temperature and light exposure). It has to be noted though that the berry volume does not influence the MA or TA content of the berries, but the TTA and the pH though dilution. Shading though can contribute to a higher MA content due to lower berry temperatures (Smart, 1992).

2.7.3 Management practices

Management practices, enveloping the human influence, has to be considered in any studies regarding grapevine as “no vineyard exists without the intervention of mankind” (Van Leeuwen & Seguin, 2006). Understanding the growth phases of the plant, i.e. phenology, is beneficial in determining the optimal timing of cultural and chemical practices (Jones & Davis, 2000).

Viticultural management practices mainly alter the microclimate of the vine, which influences the environmental factors as mentioned previously in Section 2.6, and in turn alters the physiology of the vine (Smart *et al.*, 1985; Smart, 1992). Therefore, management practices i.e. pruning, trellising, canopy management, etc. influence the foliage density and orientation which in turn significantly affect the canopy microclimate (Smart & Dry, 1980). A distinct relationship is found between

microclimate and grape composition and ultimately the wine quality (Van Zyl & Van Huyssteen, 1980; Coombe, 1987b; Hunter *et al.*, 2004).

Canopy management is one management tool used to alter the vine to avoid within-canopy shading and poor ventilation (Smart, 1992). Trellising, leaf removal and pruning strategies all alter the light interception of the bunches and therefore influence the levels of organic acids found in the berries.

Terrier & Romieu (2001) and Smart *et al.* (1985) cited that in general, higher MA concentrations can be found where conditions lead to vigorous vines and high yields. Therefore, altering the light and temperature as a result of a dense canopy, the MA content at harvest may be altered.

In the case of TA, even though it was found by Melino *et al.* (2011) that ascorbic acid accumulation in the berry is light dependent as higher sun exposure resulted in higher levels, it was also found the conversion of ascorbic acid to TA is driven by factors that are not responsive to light. In addition, the accumulation of oxaloacetate was also not responsive to light throughout berry development.

Altering of the microclimate through canopy manipulation might be overwhelmed by the influence of the macroclimate. This is especially noticeable in warmer areas. The effect of canopy manipulation on the microclimate probably is more pronounced in cooler climate areas (Van Zyl & Van Huyssteen, 1980).

Management practices therefore needs to be judiciously planned and performed to create a favourable microclimate towards berry composition. The timing of canopy management furthermore needs to be adapted to the physiological cycle of the grapevine and the pursued grape composition and wine style.

2.8 The role of berry cation levels

Potassium (K^+) is the most abundant cation in the berry skin and pulp, with the concentration in the skin being almost four times greater than in the pulp (Storey, 1987). The concentration of calcium (Ca^{2+}) is the second most abundant in the berry skin, but the levels are insignificant compared to those of K^+ . The storage of K^+ in the berries persist during ripening (Terrier & Romieu, 2001). Magnesium (Mg^{2+}) and sodium (Na^+), and anions such as chloride (Cl^-), phosphor (P^-) and sulphur (S^-) are also present at very low concentrations (Storey, 1987). K^+ concentrations in berries are mainly dependant on the growing conditions, rootstock and the competition of developing canes and leaves relating to transport (Boulton *et al.*, 1998).

Storey (1987) also mentioned that in the early stages of berry development prior to ripening, K^+ is preferentially localised in the sub-epidermal cells near the vascular bundles. Terrier & Romieu (2001) concluded that this may be the reason for the presence of high concentrations of unsalted free acids at a cellular level.

As mentioned previously in Section 2.5.5, tartaric salts of both Ca^{2+} and K^+ are found in the grape berry and leaf tissues of *Vitis* in the form of raphide and druses crystals due to biomineralisation (i.e. crystal formation) (DeBolt *et al.*, 2004). Although the composition of druses (calcium oxalate) is clear (Hardie *et al.*, 1996), the composition of raphide crystals are still under discussion (DeBolt *et al.*, 2004). The presence of calcium oxalate raphides were reported in 1914 to be present in grapevine tissue, but Winkler (1962) later described the raphide crystals composition as that of

potassium hydrogen tartrate (KHT). However, Ruffner (1982a) noted that this conclusion could have been derived from the abundant presence of both K^+ and TA in the berries. Ruffner (1982a) further concluded that the main mineral element is Ca^{2+} , with Storey (1987) confirming the composition of the raphides found in skins as that of calcium tartrate. Several studies concluded though that the composition of crystals differ between species of *Vitis* (DeBolt *et al.*, 2004).

The influence of the presence of K^+ in the berry seems to be greater towards MA than TA as it seems that K^+ modify the effect of temperature on the MA concentration in grapes (Hale, 1977).

TA however does not occur in the crystalline form with K^+ or Ca^{2+} in the berry due to compartmentation of the cations and the compounds. During crushing, K^+ and Ca^{2+} can however precipitate with TA as the cell walls are broken (Keller, 2010).

2.9 Conclusions

L-malic and L-tartaric acid are two structurally similar dicarboxylic organic acids but differing synthesis pathways and accumulation patterns. The origin of MA is known and the synthesis pathways completely understood, but there is still some uncertainty regarding the pathways and the enzymes present in TA synthesis. MA and TA are both synthesized *in situ* in the grape berry as well as leaves, although there is no scientific evidence indicating translocation of either acid between the two organs.

MA and TA are actively synthesized following flowering until around *véraison*, after which the TA content stays relatively constant and the MA content decreases mainly due to respiration. The content and the ratio of these two acids in grapes are important as it indicates the suitability of the grapes for vinification and ultimately predicts the quality of the wine as it determines the perceived organoleptic and aesthetic character of the wine. The acidity of the wine furthermore influences the physical, biochemical and microbial stability of the wine.

Several factors influence the MA and TA content in the grape berry. These include climatic factors such as temperature which is the main climatic factor contributing to the differences in the content of these acids. Although temperature is mainly driven by the climate, canopy manipulation can also alter the microclimate of the grapevine with subsequent changes not only the temperature, but also the light interception of the grape bunches. This, in turn, has an additional influence on the berry temperature. The influence of temperature on the content of MA is more pronounced than the influence on TA. Consequently, the thermal thresholds regarding MA synthesis and degradation are well reported. There is a clear phenological turning point at which synthesis of MA and TA ceases, namely *véraison*, after which degradation of MA commences. There are many factors that influence the final MA and TA content though, either acting in conjunction with temperature, of which the interactions are intricate or acting individually.

As certain factors are quantifiable and definable, like the site x cultivar response, it is essential to understand the reaction of certain varieties and the response of the organic acids to these terroirs. This will ensure that the grapes ripen optimally or that the knowledge is possessed to make the correct decisions regarding the management practices to avoid correcting the composition of the must in the cellar.

2.10 Literature cited

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Chapter III: Materials and methods

The study formed part of a greater terroir research project that was conducted in the Stellenbosch Wine of Origin district from 2004 until 2010. From the wider project, three seasons (2006/2007, 2008/2009 and 2009/2010) were selected in order to investigate the effect of the temperature on the malic and tartaric acid content of grape berries at *véraison* and harvest in two cultivars (Cabernet Sauvignon and Sauvignon blanc).

3.1 Area of interest

The study area of the Stellenbosch Wine of Origin district is situated in the Cape Winelands District Municipality of the Western Cape, South Africa and is situated around 34° S; 19° E. According to recent statistics of the South African Wine Industry Information and Systems (SAWIS), Stellenbosch is the main winegrowing region in South Africa with approximately 17.1% of the total vines planted in this district. Regarding cultivar, Sauvignon Blanc (SB) represents 35.6% of all the grapevines planted and Cabernet Sauvignon (CS) 35.7% of the grapevines planted (www.sawis.co.za, accessed 12 November 2012).

The district is further divided into the wards of Banghoek, Bottelary, Devon Valley, Jonkershoek Valley, Papegaaiberg, Polkadraai Hills and Simonsberg-Stellenbosch (refer to Fig. 1 and Fig. 2 in the addendum).

This Stellenbosch area is of interest due to the complex topography inducing significant mesoclimatic variance across the viticultural area (Carey, 2001; Bonnardot *et al.*, 2012). Furthermore, the sea breeze effect, as described by Bonnardot *et al.* (2001), results in variation in the temperature in this region, depending on the penetration of the breeze into the study area. This variation furthermore varies in degrees of interference in temperature as dry air from the land, due to thermal convection, combine with the moist air of the sea breeze (Carey, 2005). The regional climate is described as Mediterranean (Bonnardot *et al.*, 2002) with mild, wet winters and warm dry summers.

3.2 Site selection and plot layout

In 2003, 60 commercial SB and CS vineyards were initially identified from questionnaires completed by industry. The vineyards were selected mainly according to the rootstock (101-14 and Richter 99 for CS, Richter 99 and Richter 110 for SB), age of the vines (10 to 15 years), depth of soil preparation (without a plough bank), trellis system (a 4- or 5- wire hedge system) and being rain fed. As most vineyards in Stellenbosch are irrigated, such vineyards had to be included later to complete the selection.

In 2005, the vineyard selection was further narrowed down to a final set of 30 vineyards. From these, 16 vineyards (eight SB and eight CS) were extracted for this project (Table 3.1), initially on the basis of the availability of complete data sets. A further criterion for vineyard selection was the presence of a mesoclimatic datalogger within the vineyard. Missing vegetative and reproductive

information is probably due to vineyard management actions completed on the experimental plots by the producers before sampling, harvesting or pruning. The position of the selected vineyards within the Stellenbosch Wine of Origin District is indicated in Fig. 3 in the addendum and will be referred to as sites from here on. Each site was assigned a coding consisting of an abbreviation of the location of the vineyard and the cultivar so ensure anonymity of the vineyards discussed. These abbreviations will be used from here on when referring to the specific sites.

Three research plot positions, with each plot consisting of 10 consecutive healthy vines, were determined with the use of multispectral images obtained in 2004 for certain of the vineyards. The Ratio Vegetation Index (RVI) was calculated per image with the use of OrthoView™ (software supplied by the imaging service provider) to enable interpretation of the vigour heterogeneity within the vineyards. Three locations of similar vigour were determined from these images and pinpointed as the plot positions. Where multispectral images were not available, plots were located diagonally across the vineyard, visually selecting areas of similar vigour.

TABLE 3.1

A list of the experimental sites selected from the original database including general information on the sites. Site codes ending in CS indicate Cabernet Sauvignon sites and ending in SB, Sauvignon Blanc sites.

Site code	Slope (%)	Aspect	Elevation (m)	Distance from ocean (km)	Planting year	Rootstock	Row direction	Planting distance	Size (ha)	Irrigation
ADWSB	20	SSE	200	16.3	1994	R 99	E-W	2.7 m x 1.2 m	3.1	Drip
AMPSB	26	SSW	270	18.8	1991	R 99	NW-SE	2.7 m x 1.2 m	0.8	Drip
DEHSB	14	WSW	410	25.5	1993	R 110	N-S	2.7 m x 1.2 m	3.6	Dryland
HEKSB	17	NNE	230	9.4	1993	R99	SE-NW	2.7 m x 1.2 m	4.4	Drip
LACS	13	NW	240	27.3	1995	R 99	E-W	2.5 m x 1.0 m	3.5	Dryland
LEICS	15	SW	160	20.0	1989	R 99	NNW-SSE	2.4 m x 1.2 m	4.7	Micro
LIBSB	6	NNW	120	14.8	1992	R 110	E-W	1.4 m x 2.74 m	5.9	Drip
MMCS	27	S	240	20.6	-	-	E-W	2.8 m x 1.2 m	1.1	Dryland
MOISB	11	N	360	16.2	1996	R99, R110	N-S	2.5 m x 1.2 m	2.3	Drip
MOCS	27	W	210	23.0	1995	101-14	N-S	3.0 m x 1.2 m	1.5	Dryland
RECS	11	S	210	23.3	1992	R 99	E-W	1.2 m x 3.0 m	2.5	Drip
RUCS	9	SW	385	24.4	1990	R 99	N-S	2.5 m x 1.5 m	0.8	Drip
RUSB	32	S	470	25.0	1995	R 99	E-W	1.0 m x 1.5 m	0.3	Drip
SWCS	3	W	155	23.9	1991	R 99	SE-NW	1.0 m x 2.7 m	3.0	Drip
SWSB	4	N	155	24.2	1990	R 99	NNE-SSW	1.0 m x 2.7 m	1.8	Drip
WATCS	17	NE	170	17.8	1998	101-14	N-S	2.75 m x 1.4 m	16.0	Micro

Refer to the general site descriptions in the addendum for further terrain descriptions and the positioning of the plots within the vineyards.

Some vegetative and reproductive measurements done are noted as an overall indication of mainly the vigour differences between the vineyards and a possible indicator of quality.

The yield to pruning mass index (Ravaz, 1903) was determined by dividing the total yield per vine (kg) by the total pruning mass per vine (kg). The vines were harvested by hand and the yield per vine was determined by weighing the total number of main bunches per vine harvested. The mean bunch mass per vine was determined by dividing the yield per vine by the number of bunches counted per vine at harvest. The pruning mass per vine was determined by weighing the pruned canes per vine with a hanging balance scale.

TABLE 3.2

The yield to pruning mass index vigour classification for topped vines according to Smart & Robinson (1993).

	Vigour		
	Low	Moderate	High
Index value	> 12	5 to 10	< 3

Point quadrat measurements (PQ) were performed per plot within the site as developed by Smart & Robinson (1993). A thin steel rod was inserted 50 times into the canopy across the extent of the 10 vines, noting any contact with leaves (B), bunches (T) and gaps (G). From these measurements the total percentage of gaps (total G count/50), the leaf layer number (total B count/50), the percentage shaded leaves (internal B count /total B count) and the percentage of shaded bunches (internal T count/total T count) were determined.

The vineyards score card (Smart & Robinson, 1993), adapted for use in South Africa (Archer, 2002), was used as a general indicator of the potential of the vines to produce quality grapes and focused on scoring of the vegetative and reproductive aspects (Table 3 in the addendum). Although this method is a broad and general way of vineyard assessment, it is an easy and rapid way of assessment of vineyards and serves as a general indication of differences within vineyards.

3.3 Phenology

The date of the beginning of each phenological stage was determined visually for every plot. These phenological stages were defined according to the E-L phenological system developed by Eichhorn & Lorenz (1977), and adapted by Coombe (1995). For the purpose of this study, the budburst date was determined at E-L 4 (green tip; first leaf tissue visible), anthesis (flowering) at E-L 23 (17 to 20 leaves separated; 50% caps off), and *véraison* at E-L 35 (berries begin to colour and enlarge). Grapes were considered ready for harvest (E-L 38; berries harvest-ripe) when berries reached a total soluble solid (TSS) concentration of 23 °Brix so that the time of harvest for all the plots was standardised according to one variable. In addition, the date of bunch closure for berry sampling was determined as E-L 31 (berries pea size, 7 mm diameter) to E-L 32 (beginning of bunch closure, berries touching) and ripening as E-L 36 (berries with intermediate Brix values). Refer to Fig. 4 in the addendum for the complete phenological classification system.

3.3.1 Precocity indices (*indices de précocité*)

The precocity indices were initially used by Barbeau *et al.* (1998b) for Cabernet franc in the Loire valley to define the terroir and is used to characterise the phenological attributes. The number of days until anthesis and *véraison* were determined from the 1st of September.

The precocity index of flowering (iP_f) as is calculated as:

$$iP_f = 100 \times \left[1 + \frac{F_m - F_j}{F_m} \right]$$

EQUATION 3.1

The equation to determine the precocity index of flowering per site (iP_f) as developed by Barbeau *et al.* (1998a) where F_m represents the mean date of flowering for all the studied sites ($n=16$) and F_j the date of flowering of the site.

The precocity index of *véraison* (iP_v) is calculated in a similar fashion with the dates of *véraison* inserted in the equation as for flowering.

The precocity index for the cycle (iP_{cy}) is calculated as:

$$iP_{cy} = iP_f + 100 \times \left[(V_m - F_m) - (V_j - F_j) \right] / (V_m - F_m)$$

EQUATION 3.2

The equation to determine the precocity index for the cycle per site (iP_{cy}) as developed by Barbeau *et al.* (1998a) where iP_f represents the precocity index of flowering, V_m and F_m the mean dates of *véraison* and flowering respectively for all the study sites ($n=16$), and V_j and F_j the date of *véraison* and flowering respectively for the site.

3.4 Sample handling

3.4.1 Sampling

Berry sampling was performed at the four phenological stages according to Coombe (1995) of E-L 31-32, E-L 35, E-L 36 and E-L 38. Sampling was performed early in the morning to avoid sampling and transportation of the berries at high temperatures. Due to the number of plots within the project, sampling of all the relevant blocks were performed over a one week period for both cultivars.

One hundred and fifty berries were harvested from random bunches across the 10 vines per plot, taking care not to damage the berries. The berries were selected from the top, middle, bottom and back of the bunches to ensure a representative sample.

The berries were placed into transparent plastic jars for transportation to minimise damage and kept in a cool box to minimise temperature fluctuations.

3.4.2 Sample preparation

One hundred berries per sample were randomly selected from the initial sample and the volume and mass determined (see analyses protocols). The sample was thereafter recombined and

divided in 3 x 50 berries samples. One sample was frozen at -20°C for the determination of K⁺ by BEMLAB (Pty) Ltd (PathCare Group) (SANAS accreditation ISO/IEC 17025) (vintage 1) and by the Central Analytical Facilities (CAF) housed at the Department of Soil Science, Stellenbosch University (vintage 2 and 3). Fresh berries of the second sample were sent to an independent laboratory (Koelenhof Laboratory Services, Stellenbosch) for the analyses of the malic- and tartaric acid. The final fresh sample was analysed for TSS, pH and the total titratable acidity (TTA) in-house according to the methods of the DVO. Fresh samples were prepared and analysed on the day of sampling.

During the period of *véraison*, samples for the determination of the malic- and tartaric acid were frozen at -20°C for a period not exceeding two weeks. According to García *et al.* (2011), the effect of storage on the fresh and frozen samples' malic acid values are insignificant ($R^2 = 0.95$) with an increase of no more than 10% after one month of storage. On the other hand, tartaric acid contents between fresh and frozen samples has a low correlation ($R^2 = 0.61$) with a decrease of up to 35% noticed after one month of storage. It was found that the effect of variety on the magnitude of the malic- or tartaric acid is more significant than the duration of storage. This is due to the initial differences in the average grape composition as determined by the genotype.

3.4.2.1 Sample preparation for analyses of technological ripeness

The berry samples were placed into a glass beaker and lightly pulsed with a handheld kitchen blender to ensure that the berry skins were broken and the seeds not damaged. Thereafter, the samples were centrifuged at 16°C for five minutes at 6000 rpm (Sorvall RC 6 Superspeed Centrifuge with a SLA-1500 rotor, Thermo Scientific, Waltham, Massachusetts, USA) so that clear grape juice, as specified by the OIV (OIV-MA-AS1-02), could be obtained. The clear supernatant was decanted for further analyses of the TSS, pH and the TTA.

3.4.3 Analyses protocols

3.4.3.1 Physical analyses: mass and volume

The 100 berries were weighed on a mass balance (UWE JW-100, UWE, Xindian City, Taiwan) and this mass noted. Thereafter, berry volume was determined using volume displacement at room temperature. The berries were placed in a 1 L volumetric cylinder containing 300 mL of distilled water. The berries' volume was calculated by subtracting the initial volume from the final volume and noted. To determine the mass and volume per berry, the mass and volume was divided by the number of berries used in the analyses. After determination of the berry volume, the berries were drained and lightly dried with tissue paper.

3.4.3.2 Analyses of technological ripeness: TSS, pH and TTA

The supernatant was brought to a temperature of around 25°C and firstly analysed for TSS using a digital handheld refractometer (Atago PAL-1 Pocket Refractometer, Atago, Tokyo, Japan). To determine the TTA and pH, 50 mL of the supernatant was transferred by means of a pipette into a 150 mL glass beaker. Where 50 mL of juice was not available, the volume was adjusted to 50 mL with distilled water and the volume of the juice noted. The pH and TTA were determined through sodium hydroxide titration with a Metrohm titrator and sample changer (785 DMP Titrino with a LL-Unitrode Pt1000 F P, Metrohm AG, Herisau, Switzerland). The conversion of the TTA for the adjusted juice volumes are automatically computed by the Metrohm.

3.4.3.3 Organic acid analyses

The fresh samples of 50 berries were sent to an independent laboratory for sample preparation using enzymatic kits and analyses by the UV-spectrophotometry method on the same day as sampling.

The supernatant was prepared by weighing 1 g of the homogenised sample in a 10 mL centrifuge tube and extracted in 10 mL 1N hydrochloric acid (HCl). Thereafter, the solution was extracted on a rotary shaker for one hour, centrifuged and the supernatant decanted. The phenolic compounds were removed by filtration of 1 mL of the supernatant with the addition of 0.1 mg Polyvinylpyrrolidone E1202 (PVPP) through a 0.45 micron sterile filter.

3.4.3.4 Malic acid

The filtered extract was diluted 1:50 with distilled water and the L-malic acid content was determined according to the protocol of the L-malic acid enzymatic kit (Boeringer Mannheim/ R-Biopharm Enzymatic bioAnalyses, Darmstadt, Germany). The absorbance was read in a 1 cm glass cuvette at 340 nm (Secoman Anthelie Advanced Spectrophotometer, Ales, France).

3.4.3.5 Tartaric acid

The L-tartaric content of the supernatant was determined by reading the absorbance in a 1 cm glass cuvette at 500 nm. The sample was prepared from the filtered extract according to the protocol as prescribed by the L-tartaric acid colorimetric enzymatic kit (Isitec lab SEPPAL, Montauban, France).

3.5 Temperature monitoring

Temperature was monitored on both a macroclimatic- (regional/location) and mesoclimatic spatial scale (site specific). In addition, data will be handled on a temporal scale as specified in Fig. 3.1.

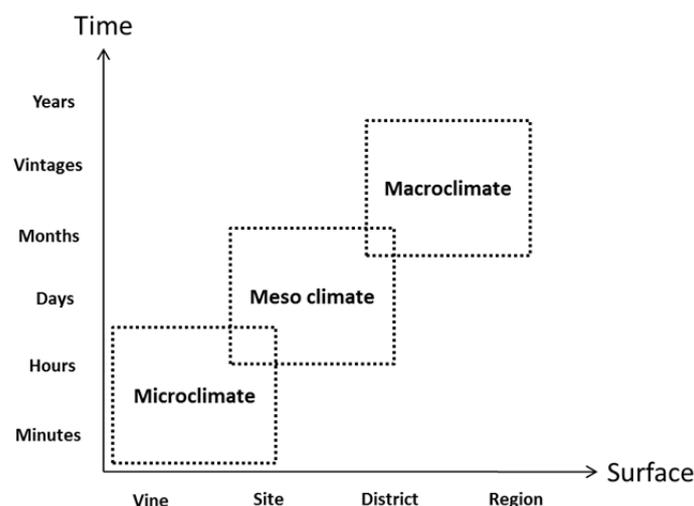


FIGURE 3.1

An indication of the spatial and temporal scale of climatic monitoring as used in the study (Bonnardot *et al.*, 2004).

Refer to Fig. 5 in the addendum for the location of the automatic weather stations (AWS) in relation to the dataloggers. Table 3.3 indicates the distance from the GPS position of the AWS to the GPS position of the datalogger in the site. The altitude, aspect and distances were calculated with the use of Quantum GIS 1.7.4.-Wroclaw (QGIS) software.

3.5.1 Datalogger network monitoring

Twenty TinyTag[®] Ultra internal dual channel (temperature and relative humidity) dataloggers (Gemini Data Loggers (UK) Ltd., Chichester, United Kingdom) were installed in 20 topographically representative sites across the study area. The placement of these loggers were determined according to the guidelines provided by the World Meteorological Association (Ehinger, 1993). The loggers were installed inside a radiation gill screen to avoid direct sunlight radiation and ensure an ambient temperature and relative humidity measurement is taken as air movement around the sensor is assured.

From the network, 16 sites, which had complete datasets, were selected for the study.

Temperature and relative humidity were logged hourly (at the end of each interval) and the daily mean, minimum and maximum data calculated using STATISTICA[®] software.

TABLE 3.3

A list of the AWS assigned per experimental site, indicating the distance of the weather station from the site, and topographical descriptors of aspect and altitude.

DATALOGGER NETWORK			AWS NETWORK			Distance from weather station (km)
Site code	Logger altitude	Logger aspect	Weather station code	Station altitude	Station aspect	
LACS	237	NW	Els	177	NW	2.060
LEICS	154	SW	Bon	150	SSE	4.856
MMCS	234	SSW	Bon	150	SSE	4.902
MOCS	203	WSW	Niet	148	SW	3.258
RECS	206	SSE	Rem	148	SW	2.460
RUCS	385	SW	TheI	423	N	1.737
SWCS	157	NNW	Groen	122	W	6.019
WATCS	176	NNE	Bon	150	SSE	2.676
ADWSB	210	SSE	Bon	150	SSE	0.355
AMPSB	261	S	Goed	235	N	1.099
DEHSB	408	SW	TheI	423	N	4.190
HEKSB	233	N	Alto	225	NW	1.058
LIBSB	116	NNW	Niet	148	SW	5.314
MOISB	352	NNW	Goed	235	N	2.040
RUSB	467	S	TheI	423	N	2.923
SWSB	157	NNW	Groen	122	W	6.114

Due to technical problems in the 2007/2008 vintage with the activation of the TinyTag[®] loggers, no datalogger network data is available for this period and this vintage was therefore excluded from the study.

3.5.2 Automatic weather station (AWS) network monitoring

Hourly and daily data was collected from seven AWS (MCSystems, Cape Town, South Africa) of the network run by the Institute for Soil, Climate and Water (ISCW) of the Agricultural Research Council (ARC). The AWS are installed and maintained according to the standards of the World Meteorological Organisation (WMO) (Ehinger, 1993).

For the study, the AWS closest to the installed datalogger was selected for comparison of the indices (refer to Table 3.3). Due to the topographical diversity of the area, in some instances, the AWS in the vicinity of the logger with a similar terrain, and not necessarily the closest, was selected for the study. Refer to Fig. B in the addendum for the positioning of the dataloggers in relation to the AWS.

3.5.3 Climatic indices

Climatic indices were calculated both on a macroclimatic (monthly, seasonal) and a mesoclimatic (daily and hourly) temporal scale for the datalogger and AWS network.

The Winkler (Amerine & Winkler, 1944) and Huglin (Tonietto & Carbonneau, 2004) climatic indices are the most commonly used indices and were calculated from the daily climatic data collected from the datalogger- and the AWS network. Both indices are based on a summation method and calculated according to a base temperature of 10°C. This temperature has been widely accepted for grapevine (*Vitis vinifera* L.) as little vegetative growth occurs below this temperature (Branas, 1946; Kirk, 1986; Zorer *et al.*, 2011), though the base temperature is likely cultivar specific (Branas, 1946; Jones *et al.*, 2010).

In addition, mean monthly temperature classifications were selected as used in climatic classifications by Smart & Dry (1980) and Tonietto & Carbonneau (2004), among others.

Due to incomplete datasets, three seasons were selected from the complete project database for this study. Datalogger data is missing for all experimental plots for 10 days (12 September 2006 to 21 September 2006) during vintage one. Missing temperature data could be attributed to faulty weather stations and technical problems with dataloggers.

3.5.3.1 Winkler index

The Winkler growing degree day index (WI) was calculated according to the method originally developed by Winkler (1962) and adapted for the South African growing season by Le Roux (1974). The calculation (equation 3.3) is based on the summation of growing degree days (temperature units above a base temperature of 10°C) over a seven month period and can be classified into five regions as seen in Table 3.4.

$$WI = \sum_{31.03}^{01.09} T_{mean} - 10^{\circ}\text{C}; \geq 0$$

EQUATION 3.3

The calculation of the Winkler index as adapted by Le Roux (1974) where T_{max} represents the daily maximum temperature and T_{min} the daily minimum temperature measured.

TABLE 3.4

The classification of the Winkler index for grape growing regions as adapted for South African condition by Le Roux (1974).

Region	Degree days
I	<1389 degree days
II	1389-1666 degree days
III	1667-1943 degree days
IV	1944-2220 degree days
V	>2220 degree days

3.5.3.2 Huglin index

The heliothermic climatic index of Huglin, better known as the Huglin index (HI), was developed by Huglin in 1978 and later classified into classes of viticultural climates by Tonietto & Carbonneau (2004) (Table 3.5). The index is, as in the case of the WI, based on a summation of heat units above a base temperature of 10°C. Included in the equation is a coefficient that expresses the mean day length in relation to the latitude which, for South Africa where the latitude is ≤ 40°00', is equal to 1 (as seen in Equation 3.4). Unlike the WI, the HI is calculated over a six month period from the mean and maximum daily temperatures from the 1st of October to 31 March (Tonietto & Carbonneau, 2004) where:

$$HI = \sum_{31.03}^{01.10} \frac{(T_{mean} - 10^{\circ}C) + (T_{max} - 10^{\circ}C)}{2} d; \geq 0$$

EQUATION 3.4

The calculation to determine the Huglin index for the Southern hemisphere where T_{mean} represents the mean daily temperature, T_{max} the maximum daily temperature and d the length of the day coefficient (Tonietto & Carbonneau, 2004), where $d=1$ for South Africa.

TABLE 3.5

Heliothermal index classes for viticultural climates as classified by Tonietto & Carbonneau (2004).

Classification	Acronym	Class interval
Very warm	HI+3	> 3000
Warm	HI+2	> 2400 ≤ 3000
Temperate warm	HI+1	> 2100 ≤ 2400
Temperate	HI-1	>1800 ≤ 2100
Cool	HI-2	>1500 ≤ 1800
Very cool	HI-3	≤ 1500

3.5.3.3 Fresh night index (FNI)

The fresh night index, also known as the cool night index, is calculated as the mean minimum temperature for the final month of ripening (Tonietto & Carbonneau, 2004). This index is relevant mainly regarding grape and wine colour and aroma, notably towards the synthesis of secondary metabolites *i.e.* polyphenols (Tonietto & Carbonneau, 2004).

In the Southern hemisphere, March is specified in literature as the relevant month for calculation of the FNI. However, since grape harvest occurs in February for SB and early March for CS in Stellenbosch, the FNI was calculated for February as it represents the final month of ripening in this region. The fresh night index classes are noted in Table 3.6.

TABLE 3.6

Fresh night index classes for viticultural climates as classified by Tonietto & Carbonneau (2004).

Classification	Acronym	Interval of the mean minimum monthly temperature (°C)
Very cool nights	CI + 2	≤ 12
Cool nights	CI + 1	> 12 ≤ 14
Temperate nights	CI – 1	> 14 ≤ 18
Warm nights	CI – 2	> 18

3.5.3.4 Mean January temperature (MJT)

The calculation of MJT as a climatic index was introduced by Smart & Dry (1980) for Australian viticultural regions, as this is the warmest month of the growing season in the majority of their regions. As for the MFT, the value is calculated as the mean of the mean daily temperatures, however data from January, rather than February, is used for this project.

3.5.3.5 Mean February temperature (MFT)

Smart & Dry (1980) used the calculation of the mean temperature for the warmest month, a method originally suggested by Prescott (1965), to classify thermal viticultural areas. The value is calculated as the mean of the mean daily temperature. In the Stellenbosch Wine of Origin region, February is the warmest month in the growing season and therefore used in the calculation (De Villiers *et al.*, 1996). In addition, this is the final month of ripening of the majority of the sites used within the study.

3.5.3.6 Optimal hours summations according to thresholds

The total number and percentage of optimal hours were determined by summing the number of hours between or above temperature thresholds for the pre-*véraison* (1 November to 31 December) and post-*véraison* (1 January to 28 February) period. For the percentage of the optimal hours, the number of hours were divided by the total number of hours per period of time ($n = 732$ for pre-*véraison*; $n = 708$ for post-*véraison*).

As MA is synthesized up to *véraison*, the diurnal temperature threshold for optimal MA synthesis was indicated for the period from anthesis to *véraison* as between 20 and 25°C between the hours

of 06:00 and 18:00 (Kliewer, 1964; Kliewer & Lider, 1970; Lakso & Kliewer, 1975b; Ruffner *et al.*, 1976), and night temperature of around 15°C between 18:00 and 06:00 (Kliewer & Lider, 1970; Hunter & Bonnardot, 2011). The lower temperature threshold for MA respiration during the post-*véraison* period is noted as 30°C (Winkler, 1962).

3.6 Statistical analyses

All statistical analyses were performed using STATISTICA® data analysis software system version 10 and multivariate analyses with LatentiX® data analytical software version 2.11.4859. GIS data was compiled with the use of ESRI® ArcMap™ 10.0 software.

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Chapter IV: Inter-vintage variability of climate and grape malic and tartaric acid contents of commercial Sauvignon blanc and Cabernet Sauvignon grapes in the Stellenbosch Wine of Origin District

4.1 Introduction

The Stellenbosch Wine of Origin district is regarded as a complex study area due to its topographical variation and its closeness to the ocean, which results in a varied environment (Carey *et al.*, 2008). Identification and characterisation of diverse environments for viticulture at different scales are of great importance for the South African Wine Industry and a focal point of South African viticultural research (Carey *et al.*, 2008).

Van Leeuwen *et al.* (2004) undertook studies on the influences of soil, climate and grapevine cultivar on grapevine performance and discovered that the impact of the soil and climate on grapevine behaviour and berry composition was greater than that of the cultivar, most likely mediated through the influence of these factors on the grapevine's water status. Climate seemed to be the main driver of grapevine physiology and, ultimately, performance, although the effect of the soil cannot be excluded (Conradie *et al.*, 2002) and the interaction of climate and soil was recently described by Rogiers & Clarke (2013).

The regional climate of Stellenbosch is classified as Mediterranean (Bonnardot *et al.*, 2002), with mild, wet winters and warm, dry summers, therefore reducing the influence of rainfall on ripening during the growing season. Variability between vintages includes differences in temperatures during the growing and ripening period. Winkler (1962) initially noted that variations in heat summation during berry development can be related to seasonal variations in the organic acid and sugar concentrations, specifically at harvest.

The influence of temperature on berry composition, including the influence on the organic acid content, is well researched and documented according not only to heat summation indices, but also according to thermal thresholds (Winkler, 1962; Kliewer, 1964; Kliewer & Lider, 1970; Buttrose *et al.*, 1971; Lakso & Kliewer, 1975b; Ruffner *et al.*, 1976; Coombe, 1987; Iland, 1989; Jackson & Lombard, 1993; Dokoozlian & Kliewer, 1996; Jones & Davis, 2000; Spayd *et al.*, 2002; Conde *et al.*, 2007; Happ, 2007; Hunter & Bonnardot, 2011). Biological systems are driven mainly by temperature, as the enzymes responsible for the reactions within these systems are activated by specific temperatures and perform optimally within certain temperature thresholds (Kliewer, 1964; Lakso & Kliewer, 1975b; Sweetman *et al.*, 2009).

Temperature differences determine differences in thermal accumulation during a growth season, and thus influence the phenological differences between vintages (Jones & Davis, 2000; Jones, 2003). Thus the period of the growth and ripening stages is changed and, with this, the ambient temperature experienced by the plant during each stage.

This chapter will focus mainly on the climatic diversity between vintages and the associated differences in the content of tartaric acid (TA) and malic acid (MA) in the berries at particular phenological stages. It must be emphasised that many other factors, both physiological and

environmental, play a role in the content of these organic acids in the berries, as discussed in Chapter II. Due to the extent of the study, temperature was focused on because, according to extensive research completed on this topic, it is the main climatic driver of the content of TA and MA in grape berries (Kliewer, 1964; Kliewer & Lider, 1970; Buttrose *et al.*, 1971; Ruffner *et al.*, 1976; Coombe, 1987; Iland, 1989; Spayd *et al.*, 2002; Van Leeuwen *et al.*, 2004).

4.2 Project aim

The aim of the project was to determine the comparative effects of vintage on the organic acid content of Sauvignon Blanc and Cabernet Sauvignon grapes from the Stellenbosch Wine of Origin district at *véraison* and harvest.

4.3 Results and discussion

4.3.1 Data

The study periods (hereafter referred to as vintages with a seasonal code) were:

- Vintage 1 (V1): 1 September 2006 to 31 March 2007;
- Vintage 2 (V2): 1 September 2008 to 31 March 2009;
- Vintage 3 (V3): 1 September 2009 to 31 March 2010.

Each vintage includes data from seven automatic weather stations (AWSs) and 16 Tinytag[®] dataloggers, except for V2. All climatic parameters calculated from the datalogger data of the “LACS” site for vintage 2 were excluded from the statistics due to exceptionally low values in comparison with the complete dataset and in relation to the nearest automatic weather station. This can be attributed to a faulty sensor during the period of observation.

4.3.2 Climate monitoring

In general, climate is studied on three spatial scales – a macroclimatic scale, which describes the regional climate, a mesoclimatic scale, describing smaller areas, e.g. vineyards, and finally a microclimatic scale, describing the plant canopy or the direct surrounding area (Bonnardot *et al.*, 2004). For this study, both macroclimatic and mesoclimatic spatial and temporal datasets were available, as indicated by Bonnardot *et al.* (2004) (refer to Fig. 3.1).

For the purpose of this study, “locality” refers to an area inside the region represented by the AWSs and the dataloggers assigned to the AWSs (please refer to Chapter III, Table 3.3), therefore indicating a sub-region within the main study region. “Site” refers to the experimental vineyard and therefore represents the data spatially on a mesoclimatic scale.

“Regional data” refers to data obtained from the complete AWS or datalogger network, representing the seasonal indices or monthly averages for the Stellenbosch Wine of Origin district. The differences in values relating to the origin of the data from either the AWSs or the dataloggers will be discussed later in this chapter.

“Mesoclimatic data” refers to the hourly or daily data obtained from a specific AWS or datalogger, thus representing the immediate surrounding area.

Please refer to Table 1 in the addendum for a complete dataset of the climatic parameters per vintage, indicated per region and per site, as well as the delineation of the localities.

From Table 4.1, indicating the macroclimatic parameters calculated for both the AWS and datalogger networks, it can be seen that there were notable differences within the dataset, not only between vintages for the climatic parameters that were calculated, especially the mean monthly parameters, but also between the data sources.

When considering mainly the values of the datalogger network in Table 4.1, it can be seen that the values of the thermal indices (WI and HI) do not differ significantly between vintages. The mean monthly temperature parameters (FNI, MJT and MFT) do differ significantly, however, suggesting that the climate during the months indicated by the parameters drives the climatic differences noticed between the vintages. Although the differences are indicated as not significant for the thermal index values of the AWS network, the differences between vintages are apparent when considering the absolute values.

It can furthermore be noted from Table 4.1 that there is a clear difference between the values calculated using data from the AWS and the datalogger network, from 70 to 170 growing degree days (GDD) for the Winkler index (WI) and 160 to 290 thermal units for the Huglin index (HI). This can be attributed mainly to the housing of the sensors. The AWS temperature sensors were housed in a Stevenson screen as prescribed by the World Meteorological Organization (WMO), whereas the datalogger sensors were housed in a Gill screen, also known as a radiation screen, deemed as accurate by the WMO. When the notable differences in temperature were observed, Dudley Roswell of the Institute for Soil, Climate and Water of the Agricultural Research Council in Stellenbosch was contacted. He confirmed the variation in values between the two housing units and ascribed this to the fact that the measurements taken in a Gill screen are more responsive to extreme measurements. The area around the sensor is heated and cooled much quicker, considering that less air movement is required to affect the sensors due to the size of the housings, therefore logging higher maximum and lower minimum values. This further explains the great difference between the HI per sensor, as the maximum hourly temperature (T_{max}) per day is included in the HI equation. The variation in the maximum temperatures between sites is discussed later in this chapter. This phenomenon has also been reported by Bonnardot *et al.* (2004) and is shown in Fig. 4.1.

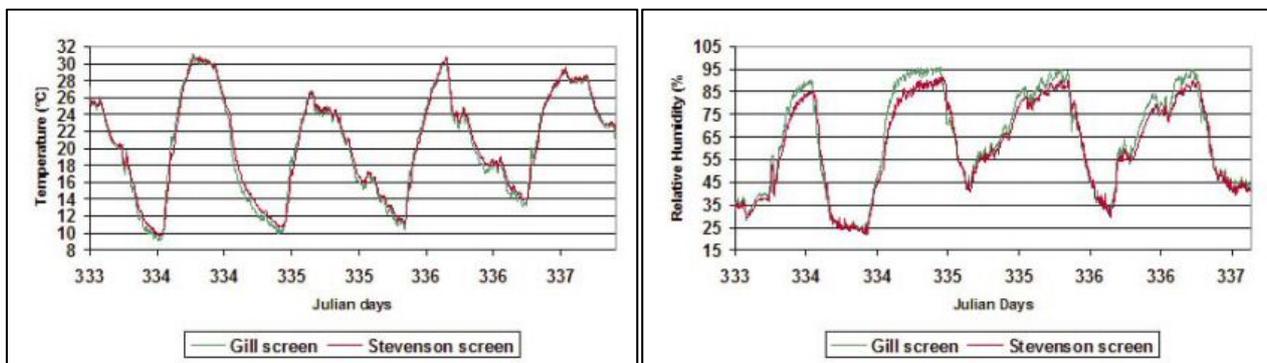


FIGURE 4.1

Graphs indicating the difference in temperature (left) and relative humidity (right) as recorded by the same sensors under a Gill screen and a Stevenson screen over a four-day period (Bonnardot *et al.*, 2004).

The extent of the standard deviation (SD) values in Table 4.1, mainly of the WI and the HI in both networks, further illustrates the variation in temperature across the study area, which can mainly be attributed to the topographical diversity.

TABLE 4.1

Macroclimatic parameters per vintage for V1 (2006/2007), V2 (2008/2009) and V3 (2009/2010) for the Stellenbosch Wine of Origin district, calculated from the datalogger and automatic weather station networks. Values are indicated as mean values, followed by the standard deviation. Values designated by different letters per climatic parameter per row and per network differ significantly ($p \leq 0.05$)

	Datalogger network			Automatic weather station network (n = 7)		
	V1 (n = 16)	V2 (n = 15)	V3 (n = 16)	V1	V2	V3
WI ¹	2 034 ± 67 a	1 997 ± 59 a	2 039 ± 82 a	1 959 ± 52 b	1 884 ± 53 a	1 966 ± 60 b
HI ²	2 595 ± 131 a	2 644 ± 102 a	2 626 ± 122 a	2 328 ± 58 b	2 357 ± 62 ab	2385 ± 72 a
FNI ³	14.9 ± 0.5 a	16.0 ± 0.6 b	15.70 ± 0.6 b	15.9 ± 0.8 a	16.9 ± 0.6 b	16.7 ± 0.6 b
MJT ⁴	23.2 ± 0.5 a	21.6 ± 0.5 c	22.23 ± 0.5 b	22.5 ± 0.3 a	21.0 ± 0.3 c	21.7 ± 0.2 b
MFT ⁵	21.2 ± 0.6 c	23.2 ± 0.3 a	21.98 ± 0.5 b	20.8 ± 0.3 c	22.5 ± 0.1 a	21.8 ± 0.2 b

¹ WI: Winkler index calculated according to equation 3.1 (Amerine & Winkler, 1944)

² HI: Huglin index calculated according to equation 3.2 (Tonietto & Carbonneau, 2004)

³ FNI: The fresh night index calculated for February (Tonietto & Carbonneau, 2004)

⁴ MJT: Mean January temperature (Smart & Dry, 1980)

⁵ MFT: Mean February temperature (Smart & Dry, 1980)

In Fig. 4.2, moreover, the diversity of the landscape is emphasised by the extent of the values of the SD, and to a lesser extent by the min-max values per locality, indicating the variation in the temperature as measured by the dataloggers. This is a further indication that care has to be taken that the data is truly representative of the site when using AWS data referring to a larger study area in a highly variable landscape. Unfortunately it is not possible to install AWSs to be representative of a mesoclimatic level, but the installation of temperature dataloggers is a reality and the same care should be taken as for the AWS that the logger is installed in a representative location within the site.

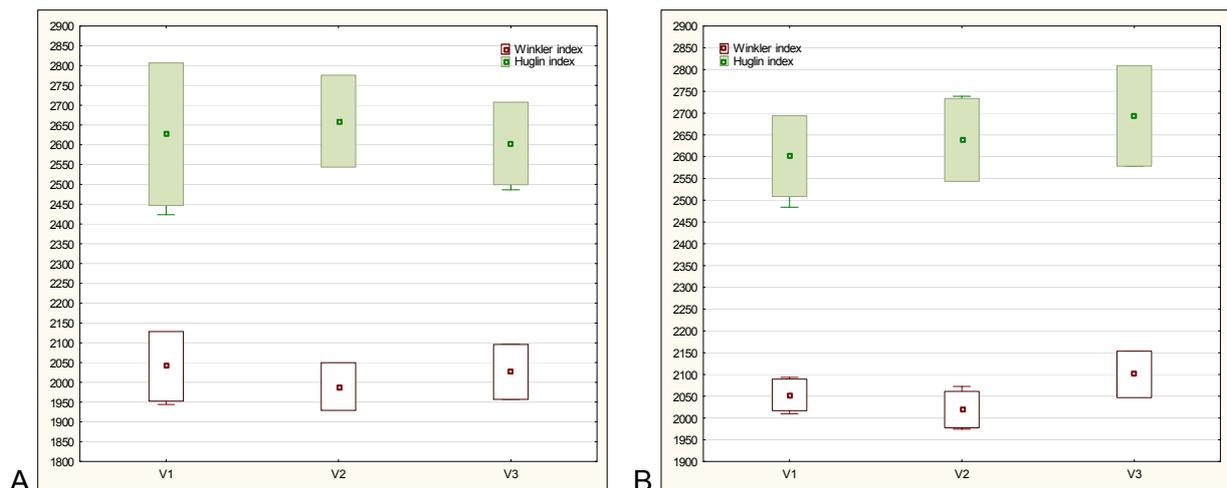


FIGURE 4.2

Graphs comparing the Winkler and Huglin index values per locality for V1 (2006/2007), V2 (2008/2009) and V3 (2009/2010) for two AWSs as examples. Fig. 4.2 (A) indicates the indices calculated for the data of the three dataloggers assigned to the Els AWS, whereas Fig. 4.2 (B) was calculated from the data of the four dataloggers assigned to the Bonfoi AWS. Middle points represent the mean values, while boxes indicate the SD from the mean and whiskers the min-max values observed per vintage.

In addition to the change in topography over a short distance, and the distances of the AWSs from the research sites, as noted in Table 3.3, the datalogger being located within the site, and therefore being more representative of the ambient air temperature of the site, is another reason for using the datalogger data in further analyses. It needs to be remembered, however, that the exposure of the leaves and the berries to abiotic factors (sunlight, wind, the availability of soil moisture) determines the actual temperature of the organs, and therefore the photosynthetic activity of the leaves (sugar accumulation) and the metabolic activity (berry composition) (Iland, 1989). When referring to temperature in this study, the ambient air temperature is described, as the temperature of the berries was not monitored.

Differences between data generated by the AWS and the datalogger networks cannot be attributed entirely to differences in the landscape, however, as the differences due to the sensors and screens used have to be considered when interpreting the data.

Due to the reasons mentioned above, for the purpose of this study, data from the datalogger network will be used in all analyses in relation to the sites.

The regional climate per vintage for the study area were determined using data obtained from the AWSs, due to the scale of monitoring.

4.3.3 Defining the regional climate per vintage

4.3.3.1 Regional climatic parameters per vintage

Stellenbosch is defined in the literature as a region with a Mediterranean climate, with cool, wet winters and warm, dry summers (Carey, 2005; Bonnardot & Cautenet, 2009). In addition, the occurrence of the sea breeze during the ripening period, and the impact thereof on the afternoon and evening temperatures and relative humidity of the region, cannot be ignored (Bonnardot *et al.*, 2001). Stellenbosch can furthermore be defined as having a maritime climate (near the coast), therefore an area with relatively small differences in temperature between seasons. It must be noted, however, that in addition to distance from the coast, elevation and latitude have significant effects (Smart & Dry, 1980), and the complex topography of this region therefore results in varied mesoclimatic conditions, as discussed in detail by Carey (2005).

Temperature and rainfall were decided upon as the descriptors to define the regional vintages, as this data was readily available from the AWS network datasets. As can be seen in Table 1 in the addendum, the vintages included in this study can generally be defined as:

Vintage 1: A dry vintage, with cool nights in February and warm daily temperatures.

Vintage 2: A very wet vintage, with temperate night temperatures in February and relatively warm daily temperatures.

Vintage 3: A wet vintage, with temperate nights in February and warmer day temperatures than those of the comparative vintages.

This, however, is a general climatic description of the vintages for the complete Stellenbosch Wine of Origin district using mean data. Table 1 in the addendum contains complete datasets of the climatic indices per vintage, calculated from both the AWS and the datalogger network, except for rainfall, which was calculated using data obtained from the AWS network. In addition, the number of hours per day that temperatures above 30°C were monitored for two AWSs, as examples within the area of interest, are indicated in Figure 6 in the addendum to show the occurrence of heat waves per vintage.

Fig. 4.3 (A) illustrates the extent of the SD and the minimum and maximum values in relation to the mean values per climatic index per vintage. The same trend is seen for the parameters represented in Fig. 4.5 (B), indicating the variability of the AWS values within a vintage due to topographical diversity.

The markedly high maximum values for winter rainfall in V2 and V3, shown in Fig. 4.3 (C), can be attributed to the high rainfall values measured by the Thel AWS, as can be seen in Table 1 in the addendum. It is clear from the raw data (Table 1 in the addendum) that the regional rainfall differs significantly across the study area, mainly during the winter rainfall period and again due to the topographical diversity.

The inclusion of both the MJT and the MFT for classification is justified in Fig. 4.3 (B), where it is clear that February was warmer than January in V1, with the opposite situation seen in V2. There was no notable difference between the MJT and the MFT in V3. These differences across vintages may be attributed to the timing of the heat waves during the vintage.

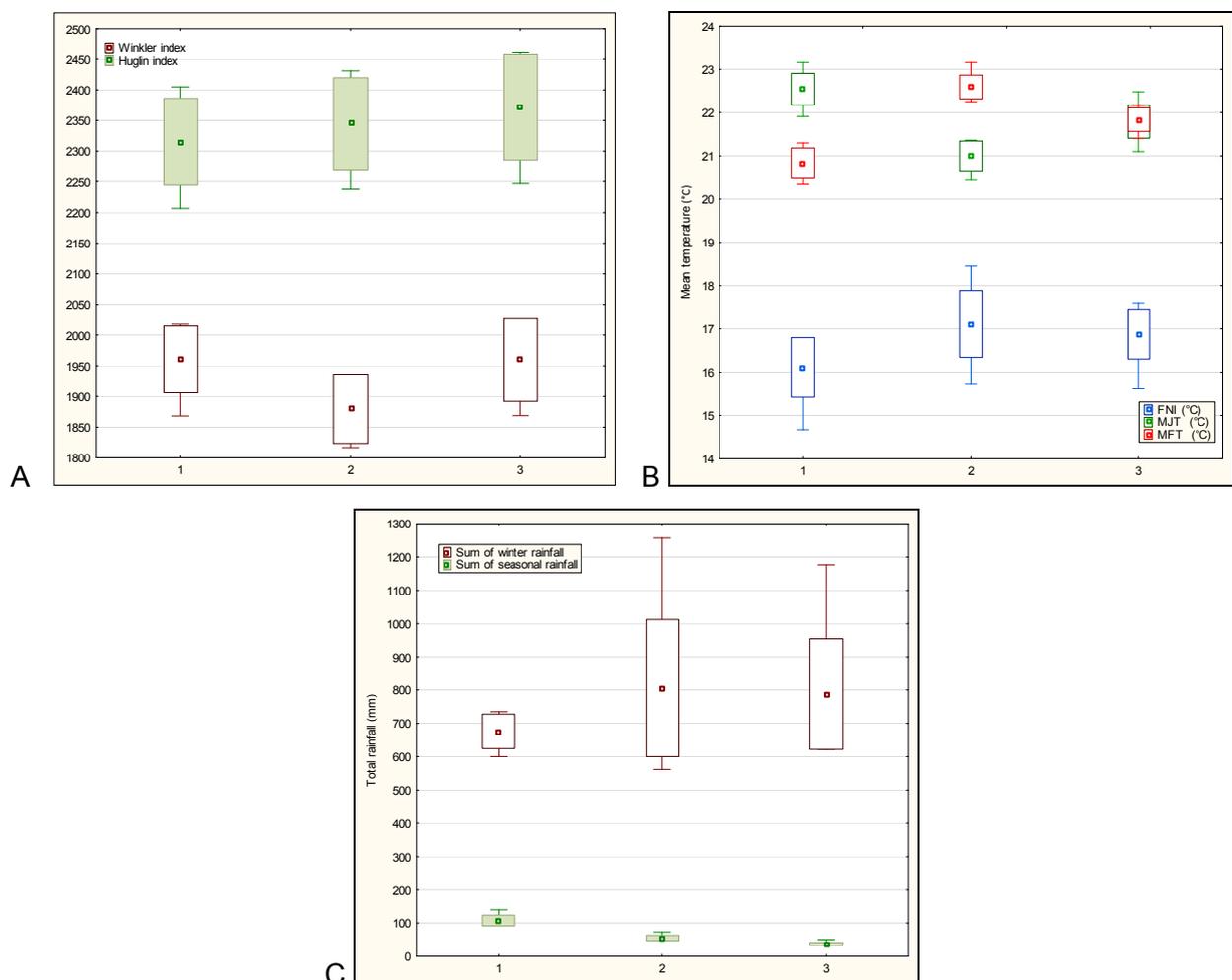


FIGURE 4.3

The mean climatic parameter values for the Stellenbosch Wine of Origin district calculated from the AWS network for vintage 1 (2006/2007), vintage 2 (2008/2009) and intage 3 (2009/2010), with A indicating the Winkler and Huglin indices per vintage, B the fresh night index (FNI), the mean January temperature (MJT) and the mean February temperature (MFT), and C the cumulative winter rainfall from April to November and the cumulative seasonal rainfall from December to March. The middle point represents the mean value, boxes the standard deviation from the mean value, and the whiskers the minimum and maximum values.

Jones and Davis (2000) noted that, in Bordeaux, heat summations, as used in the WI and HI, lacked a strong relation to grapevine physiology, production and quality, implying that heat summations may only be applicable in regional and global comparisons of grape growth and maturation potential in viticultural studies.

The use of the mean temperature of the warmest month, according to Kirk (1986), may lead to an underestimation of heat available for plant functioning during the season in maritime climates. This method requires minimal computation and is easy to use, however, and has been used in conjunction with other climatic indices and variables to classify regional climates (Smart & Dry, 1980). The timing of the occurrence of heat waves within the vintage may in addition explain differences in MFT notices between vintages.

A heat wave is defined by Hayman *et al.* (2012) as an extended period of excessive warm weather. Due to the occurrence of heat waves in the region of study during the growing and ripening period, and the subsequent influence of these on the ripening profile of the berries, a graph indicating the number of hours above 30°C per day is included in the addendum (Fig. 6), with two AWSs used as examples. From these graphs it is clear that not only did the duration and timing of the heat waves differ between vintages and the location of the AWS, but also the number of daily hours these temperatures were experienced. During V1, the heat waves occurred earlier in the vintage, after *véraison*, whereas low-intensity heat waves occurred at *véraison* in V3, with high-intensity heat waves experienced during the final ripening period for mainly CS. V2 experienced many, but low-intensity, heat waves during the final ripening period for both SB and CS, with the first heat wave occurring at the phenological stage of bunch closure. It is also clear that the Groen AWS experienced daily temperatures above 30°C more frequently than the Thel AWS. This may be due to the exposure of the Thel AWS to the sea breeze in the afternoon, with a subsequent drop in temperatures, in addition to the altitude of this AWS, whereas the Groen AWS was shielded from the sea breeze and therefore the increase in temperature in the afternoon was registered by the AWS.

4.3.3.2 Regional mean monthly temperatures per vintage

To better illustrate the fluctuation of temperatures across the vintages, the mean, maximum and minimum monthly temperatures were calculated from the AWS network data. This also provides an indication of the climate during the period from budburst to *véraison* (BV; September to December), which is also regarded as the growing period, and during the period from *véraison* to harvest (VH; January to March), which is seen as the ripening period. In the study area, budburst usually occurs in September and *véraison* in January, while SB is harvested in February and CS from late February to March. Please note that these periods refer solely to a general climatic timeframe and are not intended as an indication of the grapevine cycle per site.

From the mean regional monthly temperatures, as shown in Fig. 4.4, it is clear that V1 started out noticeably warmer than the remaining vintages, with a mean monthly temperature of 3.1°C higher than the vintage mean for September. In contrast, the temperature for September during V2 was 2.2°C lower than the vintage mean.

Although V1 was generally warmer than the other two vintages, key periods were cool, although still classified as temperate according to the system of Tonietto & Carbonneau (2004). December, the month prior to *véraison*, and the final two months of ripening in VI were the coolest of the three vintages.

Vintage 2 started out as the coolest of the three vintages, but had the warmest December and February. Interesting to note is the inverse of January and February being the warmest month for V1 and V2. No differences could be seen regionally between the mean monthly temperatures for December and January, although the differences are clear for the minimum and maximum values.

Regionally, V3 was quite stable, with constant mean temperature values in relation to the other vintages, ending with March being the warmest month in this vintage.

Interestingly, the main differences between vintages can be seen in September (budburst) and from January to March, the months of ripening. There was very little difference between temperatures per vintage for the months of October and November, the months of anthesis.

The following graphs depict the mean (Tmean), maximum (Tmax) and minimum (Tmin) monthly temperatures per vintage, normalised against the mean, minimum and maximum monthly temperature for the three vintages (also referred to as the vintage mean).

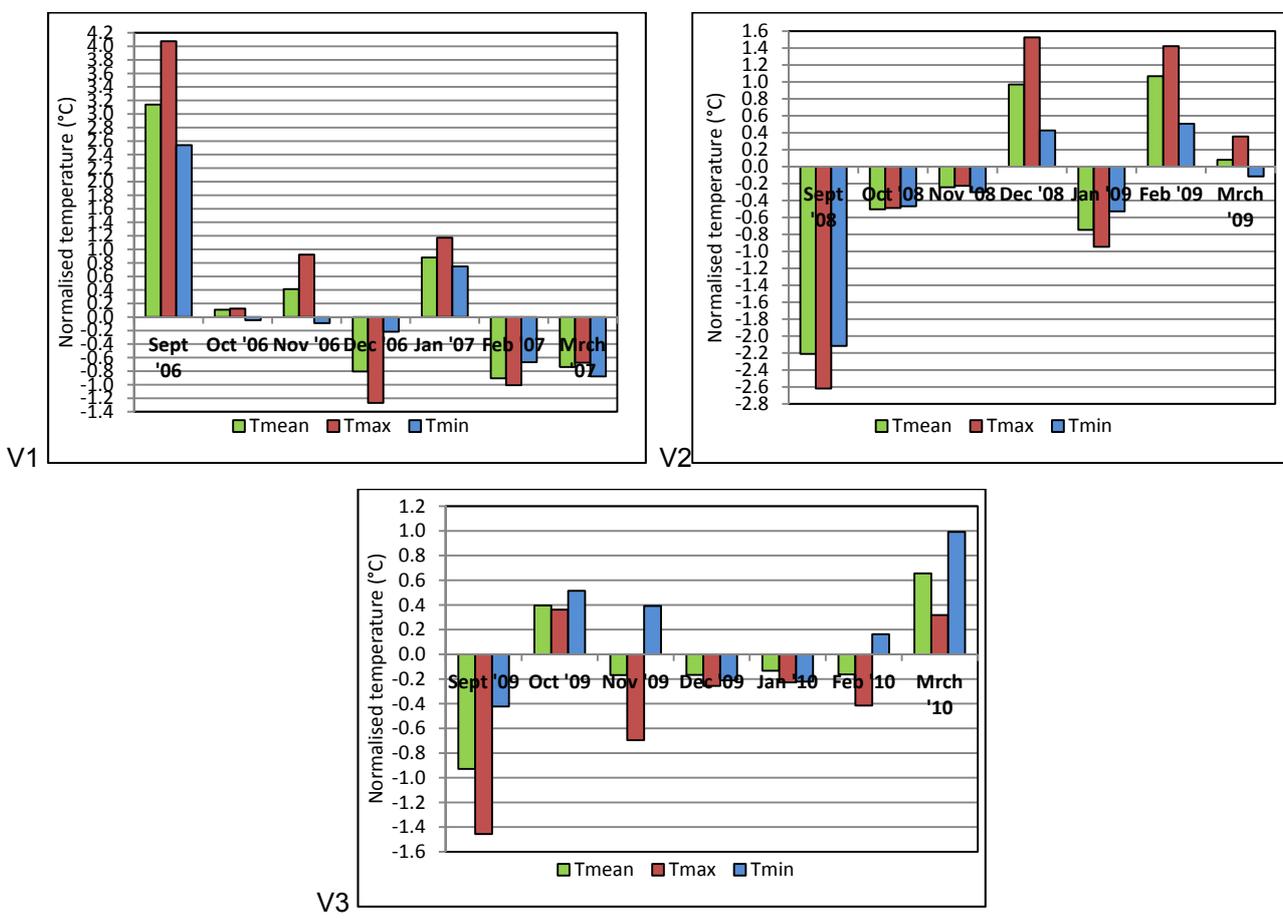


FIGURE 4.4

The mean (Tmean, shown in green), minimum (Tmin, shown in blue) and maximum (Tmax, shown in maroon) monthly temperatures normalised against the mean, minimum and maximum mean monthly temperatures calculated for the three vintages (vintage mean) for the Stellenbosch Wine of Origin district. The data was collected by the AWS network and separated for vintage 1 (V1) (2006/2007), vintage 2 (V2) (2008/2009) and vintage 3 (V3) (2009/2010).

These are regional representations and, as can see by the differences in the minimum and maximum values, the data of the AWSs need to be examined separately, as the climate changes over short distances due to topography, elevation, etc.

4.3.3.3 Climatic parameters per AWS per vintage

When the climatic parameter data from the individual AWSs is inspected (Table 1 in the addendum), the variability between the AWSs and between the vintages become apparent, as expected.

Across the three vintages, Thel was the wettest locality, with a mean annual rainfall of 1 117 mm measured over the three vintages, while Els, Groen and Goed were the driest, with a mean annual rainfall of less than 700 mm. This can be explained by the location of these AWSs within the study area. Thel is located on the slopes of Simonsberg, which may act as a “barrier” and create an area of compression of the passing wet cold front air. In comparison, Els is located on a plain. Groen and Goed are located to the north of the Bottelary Hills and the air of the cold front usually approaches from the north-west, but the wet air may continue inland because there is no area of compression.

Within the AWS network, Alto, Groen and Bonfoi have the highest thermal indices. The higher temperatures measured at Groen may be attributed to the diminished influence of the sea breeze due to the position of the AWS north of the Bottelary Hills. The Bottelary Hills have been shown to be the point beyond which the influence of the relative humidity coming from the ocean declines rapidly, therefore reducing the effect of the sea breeze on the afternoon temperature (Bonnardot *et al.*, 2001; Bonnardot *et al.*, 2005; Du Preez, 2007). The higher values measured at Alto can be attributed to warmer night-time temperatures, as seen in the FNI values. The lower thermal index values, as observed from the Thel AWS, can be attributed to the altitude of this AWS (423 m). In this regard, Saayman (1981) noted that, in South Africa, the temperature drops on average 0.3°C for every 100 m increase in altitude. In addition, Goed shows low thermal index values due to the altitude (235 m) of this weather station, even though it has a northern aspect. The coolest night temperatures were observed at Els due to the location of the AWS on a plain and the subsequent accumulation of cold air in this region at night. This also contributes to Els being a cooler location according to the thermal indices. In contrast, the warmest night temperatures were noted for the AWS located at Alto. This is due to the high altitude of the position of this logger on the Helderberg Mountain, in addition to a steep slope. At night, mountain breezes blow the cool air down the slope into the valley below, and Alto therefore “escapes” this cool layer as it is located above the layer of thermal inversion (Bonnardot *et al.*, 2012).

4.3.3.4 Climatic parameters per locality per vintage

As shown earlier, there was a distinct difference between the index values for data from the AWS and from the datalogger network per vintage. This is not only seen on a regional scale, but also per location. Fig. 4.5 shows the variation in the mesoclimatic datalogger network Huglin index values per location across vintages.

Again, from Fig. 4.5 it is clear that there is great variability per locality within the region. The use of macroclimatic data was therefore not useful in this study and it was necessary to use the mesoclimatic data per site in the analyses.

The necessity of using mesoclimatic data per site is therefore again emphasised during the locality analyses, as the AWS data might be either an under- or overestimation of the site temperature due to the topographical differences between the position of the AWS and the position of the vineyard.

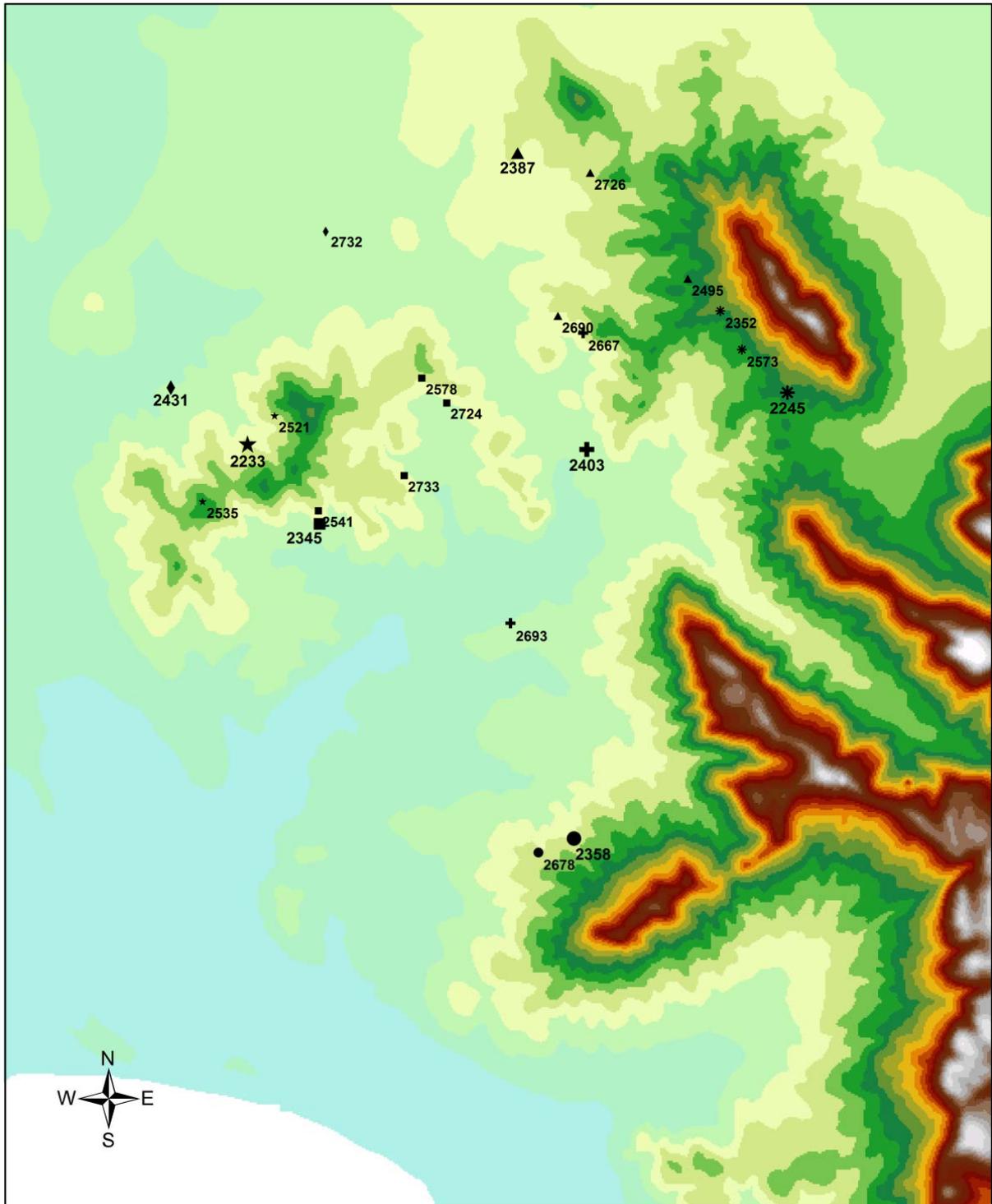


FIGURE 4.5

Figure indicating the variability of the mean Huglin index values of the datalogger and AWS networks per locality for three vintages (2006/2007, 2008/2009 and 2009/2010). Similar symbols indicate dataloggers and AWS assigned to a specific location, with the AWS value and symbol the larger of the two. Values are projected on a 50 m digital elevation model (DEM), indicating the variability of the elevation across the research area. The DEM was classified with ESRI® ArcMap™ 10.0 (digital data compiled from data supplied by National Geo-spatial Information (NGI), Mowbray South Africa).

4.3.3.5 The climatic indices

The mean values for both the HI and WI were calculated as the mean of the hourly temperatures (T_{mean}) per day ($n = 24$). This method of calculation ensures that extended periods of warm or cool temperatures in the day are included in the calculation, as the daily temperature curve for the study region is not concave (Roltsch *et al.*, 1999) and drops rapidly in the afternoon due to the presence of the sea breeze. In addition to the T_{mean} , the HI furthermore includes the T_{max} hour temperature measured for the day. Please refer to equation 3.1 and equation 3.2 for the calculation of these indices as used in this study.

The consistently higher values of the HI (Table 4.1 and Fig. 4.2) can be explained by the use of the maximum temperature values in the calculation of the Hugin index in addition to the mean value (as seen in equation 3.2).

The fact that the SD and min-max values for the HI vary more than those for the WI, as is evident in Fig. 4.2, provides an indication that the mean daily maximum temperatures during the season varied between the sites (further illustrated in Fig. 4.6). The mean maximum daily values per site vary greatly due to the influence of the location, the topography of the site (mainly aspect and altitude) and the distance from the ocean (extent of the influence of the sea breeze) on the daily temperature kinetics per site.

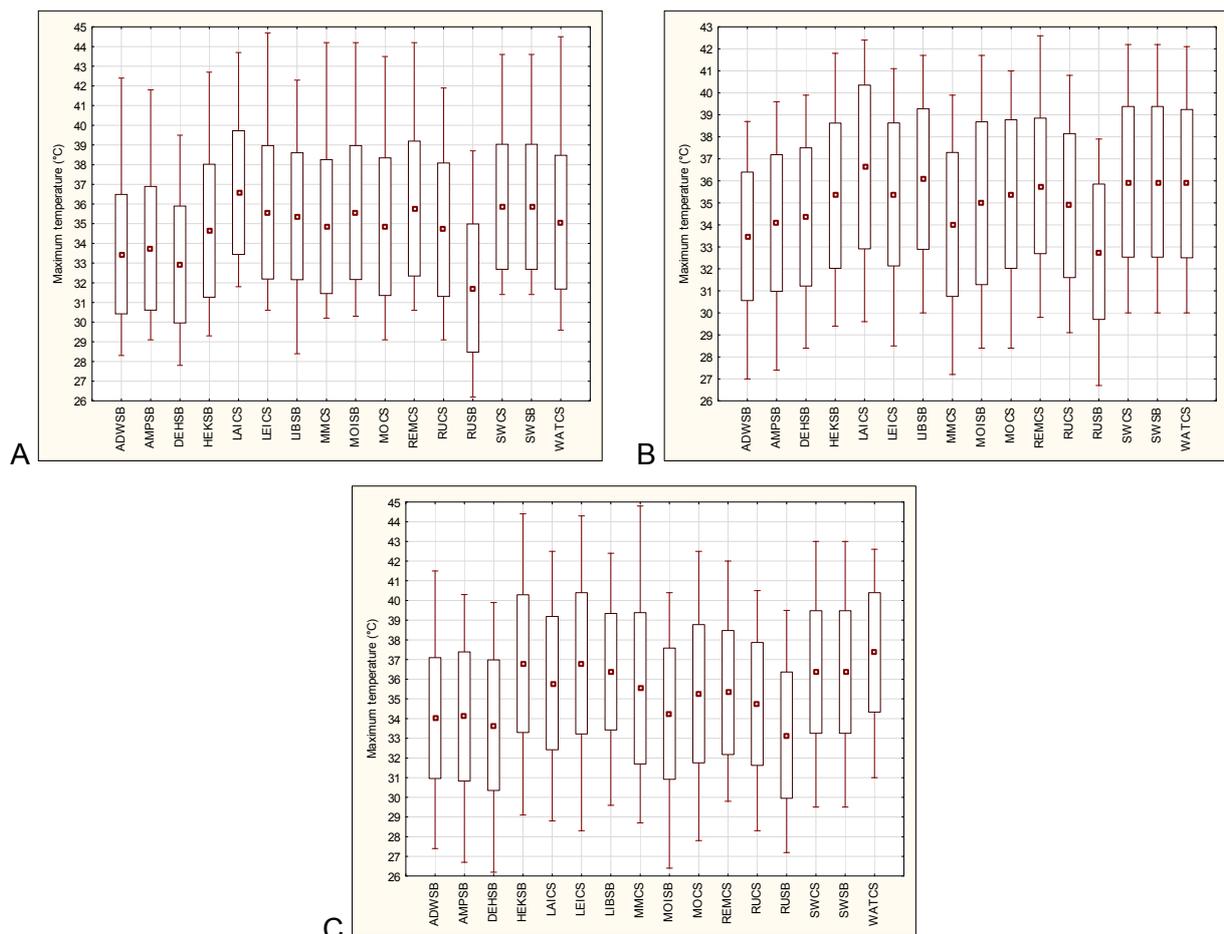


FIGURE 4.6

Graphs indicating the variability of the mean daily maximum temperatures per site for the period 1 September to 31 March in V1 (2006/2007) (graph A), V2 (2008/2009) (graph B) and V3 (2009/2010) (graph C). Middle points represent the mean of the daily maximum temperatures, while boxes indicate the SD from the mean and whiskers the min-max values of the maximum for the observed temperatures across the vintage.

The location of the site within the study area played a large role in the differences between the maximum temperatures, as the sea breeze markedly lowered the afternoon temperature of certain sites. In addition, the topography of the site (altitude and the aspect) played a large role in the air temperature, not only due to the effect of sunlight exposure in the case of aspect, but also the exposure to the sea and land breezes.

When comparing the WI and HI values per site ($n = 16$ per index, calculated from the datalogger network) and per vintage, a significant correlation ($p \leq 0.05$) was found between the indices for all three vintages, with mean r -values greater than 0.84 (Fig. 4.7).

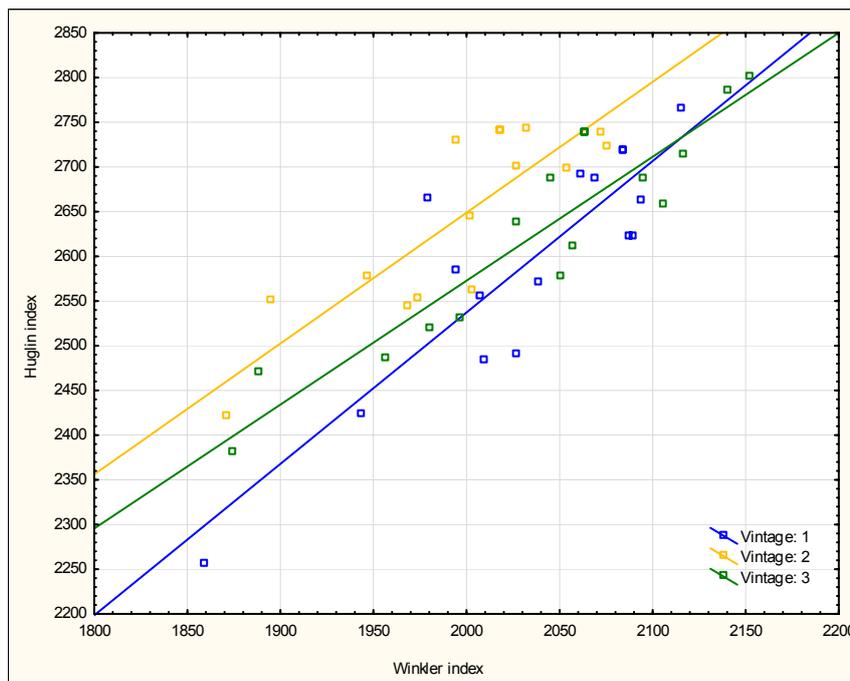


FIGURE 4.7

The relationship between the regional Huglin and Winkler index values from the datalogger network, classified per vintage, where V1 ($n = 16$) is indicated in blue ($y = -851.4716 + 1.6944*x$; $r = 0.8686$, $p = 0.00001$; $r^2 = 0.7545$), V2 ($n = 15$) in orange ($y = -279.1768 + 1.464*x$; $r = 0.8460$, $p = 0.00007$; $r^2 = 0.7157$) and V3 ($n = 16$) in green ($y = -199.6414 + 1.3862*x$; $r = 0.9259$, $p = 0.00000$; $r^2 = 0.8573$).

From Fig. 4.7 it is clear that there was a positive relationship between the WI and HI per vintage. As mentioned earlier, it has to be noted that neither of these indices might be suitable in this type of study, even if monitored spatially on a mesoclimatic level.

The WI may be less of a true representation of the daily kinetic temperature, however, especially in a region like Stellenbosch, where there is an irregular occurrence of heat waves in summer and a more regular occurrence of sea breezes, of which the intensity and extent of the influence change according to the location in the landscape. The HI may thus be a better representation of the prevailing climate, as the T_{max} emphasises the high daily temperature in the study region during the ripening period. The HI will also better distinguish between the cooler and warmer sites, as sites exposed to the sea breeze had a lower and shorter duration at T_{max} (Bonnardot, 1997).

As the study area has a highly diverse topography, and also experiences sea breezes, the use of the indices need to be adapted to incorporate these attributes and diversity.

According to Tonietto & Carbonneau (2004), the HI is more applicable in the classification of viticultural areas, as the thermal component is calculated over the period closest to the average

cycle of the grapevine. Regarding the time period of the calculations in this study, the HI is the better representation of the cycle of the grapevines, as budburst occurs from mid- to late September in the area of interest. In addition, Tonietto & Carbonneau (2004) report that the daily temperatures used in the calculation have a good relation to the potential sugar content of the grape, and therefore include qualitative information.

The use of the HI was preferred in this study as there was a noticeable fluctuation of temperatures between sites, not only between the mean daily temperatures, but also between the maximum daily temperature values. Using the WI may thus underestimate the thermal value. The topographical diversity of this region induces great climate diversity, as will be illustrated further on in this chapter and in Chapter V. In addition, as high daily temperatures and heat waves are regular occurrences in the area of interest, incorporating T_{\max} in the calculation, as for the HI, provided a truer representation of the thermal units.

Following the discussions in this section regarding the climate indices and climatic monitoring, the HI calculated with the data from the datalogger network was used in all further analyses of site observations. For regional and locality descriptions of the vintages, AWS data was used in addition to the datalogger data for interpretation.

4.3.4 Phenology

The date of budburst is influenced by the soil temperature, which may be influenced by the soil water content, which in turn is influenced by the winter rainfall (Conradie *et al.*, 2002). The date of pruning, including a human attribute in the determination of this date, therefore also influences the date of budburst. As is well known, the daily mean temperature also influences the date of budburst, as budburst occurs after the daily temperature exceeds 10°C for five consecutive days (Jones & Davis, 2000) if the chilling requirements to break dormancy are met (Chuine *et al.*, 2003). These requirements, however, vary greatly according to site and also to cultivar (García de Cortázar-Atauri *et al.*, 2009). As the remaining phenological responses are driven mainly by temperature, the use of growing degrees to predict phenological events is accurate (Oliveira, 1998; Chuine *et al.*, 2003).

An indication of the variability of the phenological dates per vintage and per cultivar is provided in Table 4.2. The differences are indicated, firstly, as number of days between the phenological stages from budburst to anthesis (B-A), anthesis to *véraison* (A-V) and *véraison* to harvest (V-H), and secondly as variability in the number of days from anthesis to *véraison* (V_DOA) and harvest (H_DOA).

From Table 4.2 it is clear that there is great variation in the data, not only between the cultivars, but also between the vintages and within the vintages. Greater variation between the days per stage can be noted for SB than for CS for all the indicators. For example, the number of days from anthesis to harvest for SB varies from a mean value per vintage of 92 days to 101 days, and a greater variability from 107 to 122 days for CS.

The length of one interval per vintage does not dictate the length of the following interval in that vintage. From Table 4.2 it furthermore is clear that there was no trend between the vintages and in the extent of the intervals for both CS and SB, e.g. SB in V1 had the longest period of ripening when calculated from anthesis, although the actual period of ripening from *véraison* was the shortest of the three vintages, therefore indicating a longer growth period followed by a shorter ripening period. Consequently, it is necessary to divide the data into phenological intervals.

TABLE 4.2

An indication of the variability of the days between the major phenological stages in the Stellenbosch Wine of Origin district per cultivar (CS: n = 8, n = 7 for V2; SB: n = 8, n = 7 harvest V2 and V3) for V1 (2006/2007), V2 (2008/2009) and V3 (2009/2010). B-A indicates the number of days between budburst and anthesis, A-V the number of days between anthesis and *véraison*, and V-H the number of days between *véraison* and harvest. A-H represents the number of days from anthesis to harvest. Values are indicated as mean values, followed by the standard deviation.

		B-A	A-V	V-H	A-H
SB	V1	48.00 ± 4.23 b	71.58 ± 4.62 a	29.29 ± 4.78 b	100.88 ± 5.33 a
	V2	55.81 ± 6.46 a	60.76 ± 6.90 b	31.52 ± 7.61 ab	92.29 ± 2.29 b
	V3	53.43 ± 4.65 ab	59.57 ± 5.74 b	36.71 ± 7.34 a	96.29 ± 3.45 b
CS	V1	50.17 ± 3.09 c	69.46 ± 3.86 a	53.13 ± 5.44 a	122.58 ± 8.02 a
	V2	61.75 ± 4.30 a	59.88 ± 6.92 b	47.25 ± 7.59 a	107.13 ± 10.08 b
	V3	53.83 ± 2.10 b	61.88 ± 4.05 b	51.13 ± 5.06 a	113.00 ± 6.53 b

*Values designated by different letters within each column per cultivar differ significantly ($p \leq 0.05$)

The influence of the temperature on the date of phenology is well known and described, and has been so for a long time (Due *et al.*, 1993; Oliveira, 1998; Jones & Davis, 2000; Chuine *et al.*, 2003; García de Cortázar-Atauri *et al.*, 2010; Parker *et al.*, 2011). In this study, a statistical analyses was firstly done to investigate the correlation of the mean cumulated thermal units with the phenological periods of *véraison* and harvest per vintage and per cultivar. The mean number of days was determined as the number of days from the dates of anthesis to *véraison* and from anthesis to harvest, and correlated with the mean accumulated daily thermal units (calculated as for the HI) from the dates of anthesis to *véraison* and anthesis to harvest respectively.

A correlation was drawn between the mean number of days per phenological stage and the mean accumulated daily thermal units per stage for the periods of anthesis to *véraison* and *véraison* to harvest per vintage and per cultivar. The results for these correlations are indicated in Table 4.3.

The correlations differed greatly between vintages, as well as between cultivars, as expected. Table 4.3 indicates a positive correlation between all the stages per vintage for CS and SB. From the table it further seems that a better correlation was found between the total number of days until harvest and the mean accumulated thermal units for CS in comparison to the number of days to *véraison*. This is not seen for SB, where a stronger correlation was found between the number of days to *véraison* and the accumulated thermal units than for the number of days to harvest. SB also clearly shows a weaker correlation between the total number of days to harvest and the accumulated thermal units.

The accumulated GDD are therefore a good indication of the phenological date, as has also been reported by Jones & Davis (2000). Jones & Davis (2000) furthermore noted that the actual date of the phenological events is less important than the interval between the phenological dates, as this indicates the overall climate during the interval.

TABLE 4.3

The correlation of the cumulative daily thermal units of the Hugin index from anthesis to *véraison*, anthesis to harvest and *véraison* to harvest per cultivar (CS: n = 8, n = 7 for V2; SB: n = 8, n = 7 harvest V2 and V3) for V1 (2006/2007), V2 (2008/2009) and V3 (2009/2010) with the corresponding number of days from anthesis to *véraison* and anthesis to harvest. Confidence intervals are indicated with * where $p < 0.05$, ** where $p < 0.01$, and *** where $p < 0.001$.

Cabernet Sauvignon			Sauvignon blanc		
V1	Anthesis to <i>véraison</i>	0.8928**	V1	Anthesis to <i>véraison</i>	0.8772**
V2	Anthesis to <i>véraison</i>	0.9771***	V2	Anthesis to <i>véraison</i>	0.9556***
V3	Anthesis to <i>véraison</i>	0.8259**	V3	Anthesis to <i>véraison</i>	0.9359***
V1	Anthesis to harvest	0.9571***	V1	Anthesis to harvest	0.5301
V2	Anthesis to harvest	0.9780***	V2	Anthesis to harvest	0.5326
V3	Anthesis to harvest	0.9025***	V3	Anthesis to harvest	0.5306
V1	<i>Véraison</i> to harvest	0.9828***	V1	<i>Véraison</i> to harvest	0.8342**
V2	<i>Véraison</i> to harvest	0.9634***	V2	<i>Véraison</i> to harvest	0.9843***
V3	<i>Véraison</i> to harvest	0.9710***	V3	<i>Véraison</i> to harvest	0.9673***

It has been mentioned by several authors that temperature is furthermore strongly correlated with the accumulation of sugar in the berry (Winkler, 1962; Kliewer, 1964; Buttrose *et al.*, 1971; Coombe, 1987), which might explain the high correlation found for the time period of *véraison* to harvest, as all sites were harvested at a similar level of total soluble solids (TSS) as far as was possible. This level of technological ripeness was chosen to ensure that a potential unripe character of the wine did not influence the sensory evaluation. The TSS is an indication of the concentration per berry, however, and the berry volume is not included in the rate of change for this value, which means that it may not be the better indicator to normalise the harvest date. Due to logistical reasons and sampling variability, the vineyards could not always be harvested at the desired TSS level. Table 4.4 provides an indication of the differences in the sugar content per berry between vintages and between sites.

From Table 4.4 it can be noted that, for both CS and SB, the TSS values at harvest for all the sites across vintages differed significantly, even though the differences between sites were not that great. However, no significant differences are seen for the sugar content per berry for either CS or SB. Due to the larger size of the berries, the sugar content per berry is much higher for SB than for CS, even though the TSS per cultivar is quite similar. There is variability in the SD of around 10% per vintage from the mean of the sugar content for both CS and SB, indicating variability between the sites per vintage.

SB harvest dates differed greatly between V1 and V2/V3, with V1 being harvested earlier in relation to V2 and V3, although no significant difference can be seen in the sugar content per berry (Table 4.4) between the vintages. As the SB sites were harvested early in February in V1, this occurrence could be explained by the higher temperatures experienced during January in V1 in comparison with V2 and V3 (as reported earlier in the chapter).

TABLE 4.4

The mean sugar content and total soluble solids values at harvest per vintage and per cultivar for V1 (2006/2007), V2 (2008/2009) and V3 (2009/2010). Values are indicated as mean values, followed by the standard deviation.

	Sauvignon Blanc			Cabernet Sauvignon		
	N	TSS	Sugar/berry	N	TSS	Sugar/berry
V1	23	21.17 ± 1.15 b	411.99 ± 48.8 a	23	20.29 ± 1.63 a	303.24 ± 34.96 a
V2	14	21.64 ± 1.19 ab	402.62 ± 26.88 a	21	22.54 ± 1.41 b	311.88 ± 32.17 a
V3	18	21.95 ± 0.88 a	413.18 ± 40.17 a	24	23.32 ± 1.85 b	311.88 ± 30.47 a
All groups	55	21.54 ± 1.11	409.99 ± 40.69	68	21.76 ± 3.19	306.72 ± 46.61

^{*}Values designated by different letters within each column differ significantly ($p \leq 0.05$) per sugar indicator

When examining the correlation data between TSS, the sugar per berry and the mass per berry and the accumulated GDD at *véraison* and harvest from flowering and per stage (Table 4.5), no clear and consistent correlations can be seen in the dataset for CS and SB. However, CS does show a significant correlation between TSS and sugar/berry at *véraison* in V2 and V3, although a significant correlation was found at harvest only in V3 when calculated per stage. The TSS, sugar per berry and mass per berry per stage show a high correlation with the HI in V1, with *r*-values greater than 0.85. There is great variability between the sites per vintage, due to differing ripening kinetics, and therefore a low correlation is expected because of the low *N*-values. In addition, the sampling was not done on the exact phenological dates, therefore a low correlation is again expected, as the HI units were calculated per stage according to the phenological dates. When the correlation was done according to the HI units calculated according to the sampling dates, the correlation still was low (data not shown), therefore indicating that the variability between sites plays a large role in the low correlations found. The large SD seen for the sugar per berry for SB in V1:V for the period from flowering is due to the large variation seen in the data, as the TSS content varied significantly for this sampling period (from 4 to 17 °Brix), as did the sugar content per berry (from 38 to 288 mg/berry).

It has to be borne in mind, however, that sugar accumulation is dependent on the rate of photosynthesis, which in turn is dependent not only on the ambient temperature, but also on the soil moisture and wind conditions (Iland, 1989). No clear and consistent correlations were seen for berry volume, as berry volume is not dependent only on the temperature, but is influenced mainly by the water available to the plant during ripening (Ojeda *et al.*, 2001).

In the further analyses, the content per berry per indicator was used in the analyses, as it excludes the influence of the berry volume as an additional factor contributing to variability in the data.

TABLE 4.5

The relationship between TSS, sugar/berry and mass/berry and the accumulated HI per cultivar per vintage and per stage (CS: n = 8, n = 7 for V2; SB: n = 8, n = 7 harvest V2 and V3). Data is furthermore calculated from flowering to véraison and harvest, and per stage from anthesis to véraison and véraison to harvest. Confidence intervals are indicated with * where $p < 0.1$.

		From flowering						Per stage					
		TSS	SD TSS	Sugar/berry	SD sugar	Mass/berry	SD mass	TSS	SD TSS	Sugar/berry	SD sugar	Mass/berry	SD mass
SB	V1:V	-0.28	3.47	-0.24*	71.78	-0.23	0.26	-0.28	3.47	-0.24	71.78	-0.23	0.26
	V2:V	0.01	2.38	0.10	38.56	0.16	0.12	0.01	2.38	0.10	38.56	0.16	0.12
	V3:V	0.62*	2.03	0.57	26.20	0.21	0.08	0.62*	2.03	0.57	26.20	0.21	0.08
	V1:H	-0.15	0.88	-0.35	45.43	-0.32	0.20	-0.12	0.88	-0.27	45.43	-0.24	0.20
	V2:H	-0.38	0.92	0.56	7.86	0.48	0.10	-0.57	0.92	0.82*	7.86	0.73	0.10
	V3:H	-0.30	0.70	0.25	31.16	0.29	0.18	0.01	0.70	0.53	31.16	0.41	0.18
CS	V1:V	-0.57	2.83	-0.60	36.29	-0.45	0.12	-0.57	2.83	-0.60	36.29	-0.45	0.12
	V2:V	-0.82*	3.42	-0.78*	36.37	-0.35	0.12	-0.82*	3.42	-0.78*	36.37	-0.35	0.12
	V3:V	-0.81*	1.54	-0.75*	11.26	0.08	0.07	-0.81*	1.54	-0.75*	11.26	0.08	0.07
	V1:H	-0.35	1.49	0.33	35.30	0.47	0.18	-0.18	1.49	0.55	35.30	0.59	0.18
	V2:H	-0.65	1.21	0.57	29.85	0.62	0.19	-0.49	1.21	0.71	29.85	0.66	0.19
	V3:H	0.30	1.50	0.70	28.83	0.61	0.10	0.39	1.50	0.90*	28.83	0.77*	0.10

* The large SD value and the subsequent negative correlation value for the sugar per berry in V1 can be explained by the extended sampling period during V1.

4.3.5 The content of malic and tartaric acid per vintage

The MA and TA contents are discussed at two key phenological stages, *véraison* and harvest, and separated per cultivar.

Regarding the analyses, as mentioned before, all values used for MA and TA are indicated as content per berry, as the berry volume is also influenced by the terroir and cultivation practices and therefore adds an additional variable to the concentration values for of TA and MA. For the remainder of the chapter, therefore, the content per berry is implied when referring to MA and TA.

It is well known, and has been indicated in the literature, that the MA and TA content is influenced by vintage as well as by cultivar (Terrier & Romieu, 2001). Table 4.6 shows the variability in the mean MA and TA content at *véraison* and harvest for both SB and CS.

As seen in Table 4.6, the TA does not vary significantly between vintages per cultivar, as also noted by Ruffner *et al.* (1976), although variability was found for SB at harvest due to a noticeably high mean TA content in V1, as high values of TA were measured for five sites at harvest. However, there is no climatic explanation for this occurrence in V1. No significant variability in the mean values of SB can also be seen between vintages.

However, variability is seen within vintages when considering the SD values per cultivar per stage. It seems that the SD of the TA per vintage at *véraison* and harvest did not differ as greatly as the MA for both CS and SB, indicating a more constant value between sites per vintage (also refer to Tables 4.6 and 4.7). This could be attributed to the differing temperature regimes per site, as the MA content is influenced more by temperature than TA (Kliwer, 1964; Buttrose *et al.*, 1971; Coombe, 1987; Terrier & Romieu, 2001).

It is also clear that the MA content at harvest was higher for SB than for CS. This can be attributed mainly to the earlier harvest date for SB than for CS, hence a shorter period for MA degradation.

TABLE 4.6

Indication of the variability in the mean site TTA, TA and MA content per berry (mg/L) per cultivar between vintages at *véraison* and at harvest. Values are indicated as mean values, followed by the standard deviation.

		<i>Véraison</i>			Harvest				
		N	TTA	TA per berry	MA per berry	N	TTA	TA per berry	MA per berry
CS	V1	8	28.53 ± 5.95 a	8.16 ± 4.86 a	15.39 ± 5.39 a	8	5.05 ± 0.42 b	12.10 ± 3.29 a	3.01 ± 1.31 a
	V2	8	28.767 ± 6.65 a	9.20 ± 0.68 a	15.09 ± 3.41 a	7	5.69 ± 0.67 a	10.88 ± 1.24 a	3.72 ± 1.89 a
	V3	8	32.59 ± 1.90 a	7.89 ± 0.43 a	16.86 ± 2.91 a	8	5.27 ± 0.29 ab	11.44 ± 0.72 a	4.16 ± 1.16 a
SB	V1	8	26.77 ± 6.31 a	8.19 ± 5.41 a	16.42 ± 5.27 a	8	7.59 ± 0.66 a	19.17 ± 5.75 a	6.82 ± 1.44 a
	V2	8	33.46 ± 3.94 b	10.38 ± 2.97 a	18.97 ± 2.95 a	6	6.91 ± 1.43 a	11.02 ± 1.48 b	8.27 ± 1.45 a
	V3	8	32.08 ± 2.87 b	9.92 ± 2.10 a	21.82 ± 6.90 a	7	7.09 ± 1.48 a	12.82 ± 1.20 b	8.10 ± 2.88 a

*Values designated by different letters within each column per cultivar differ significantly ($p \leq 0.05$) per organic acid

As the climate dynamic differs greatly between sites per vintage, as reported in this chapter and to be discussed further in Chapter V, the variability of TA and MA will be discussed further per cultivar, focusing on the variability of the contents of these organic acids per site between vintages.

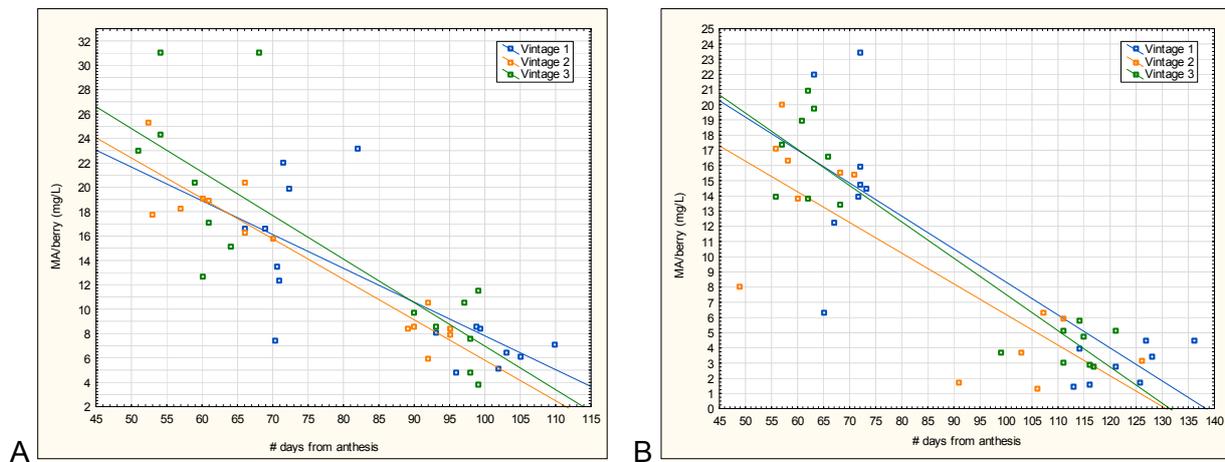


FIGURE 4.8

The dynamics of the change of malic acid per berry per site from *véraison* to harvest per vintage, with vintage 1 (2006/2007) indicated in blue, vintage 2 (2008/2009) indicated in orange and vintage 3 (2009/2010) indicated in green. A represents Sauvignon Blanc (vintage 1: $y = 35.4971 - 0.2769 \cdot x$, $r = -0.7085$, $p = 0.0021$, $r^2 = 0.50207$; vintage 2: $y = 38.998 - 0.3319 \cdot x$, $r = -0.9395$, $p = 0.00000$, $r^2 = 0.8827$; vintage 3: $y = 42.6649 - 0.357 \cdot x$, $r = -0.8052$, $p = 0.0003$, $r^2 = 0.6484$) and B represents Cabernet Sauvignon (vintage 1: $y = 30.0326 - 0.217 \cdot x$, $r = -0.8202$, $p = 0.00010$, $r^2 = 0.6727$; vintage 2: $y = 26.3837 - 0.2019 \cdot x$, $r = -0.7986$, $p = 0.0011$, $r^2 = 0.6378$; vintage 3: $y = 31.3868 - 0.2387 \cdot x$, $r = -0.9316$, $p = 0.00000$, $r^2 = 0.8680$).

The need for separation of the vintages is further emphasised by the data in Fig. 4.8, where it can be seen, firstly, that there are clear differences between the values of the malic acid per berry, not only per site, but also between vintages and between the dates of sampling, as indicated by the number of days after anthesis. This indicates the clear differences between sites per vintage for both SB and CS, even though no significant differences were found between vintages when the absolute values were considered. It is furthermore clear from Fig 4.5 that an earlier date of *véraison* results in an earlier date of harvest, as expected, with less variation seen at harvest than at *véraison* for the values of the malic acid per berry per site for both cultivars.

4.4 Conclusions

It is clear that care has to be taken when using climatic parameters to interpret data on a vineyard scale, as it is only useful to classify regions climatically. In a region like Stellenbosch, with extensive topographical diversity, it will be necessary not only to determine the most representative scale of monitoring, but also the climatic parameters most suitable for this region. The final decisions are of course determined according to the aim of the data. For research purposes, data per site will be optimal if available on the smallest scale and time interval possible. Alternative climatic indications will therefore be investigated in Chapter V.

The averaging of data, for instance to monthly data, is not optimal, as using one mean monthly value for classification during the ripening period may over- or underestimate the total seasonal temperature available. This also only provides an indication of one month in the growing and ripening cycle, therefore does not incorporate any conditions during the vegetative growth period or, in the case of the organic acids, during the period of synthesis.

The further averaging of data, i.e. hourly to daily data, will eliminate the trend of daily temperature evolution. An index incorporating another climatic component other than the average, like the HI containing a component of the maximum temperature, might therefore be more applicable. Heliothermal indices like the HI are simple to use, but one-dimensional and again regional.

When thermal indices are used to explain biochemical compounds, like MA and TA in this study, it definitely is advisable to divide the climatic data into the period of synthesis and breakdown, as the variations in heat summation during berry development can be related to the seasonal variations in the organic acid and sugar concentrations at harvest. As *véraison* is the point at which the synthesis of both acids ends in the grape berry, it is advisable to divide data into the periods of flowering to *véraison* and *véraison* to harvest to accommodate this turning point. This is not only applicable to the organic acids, but to a range of compounds found in the grape berry.

When considering topographical diversity and the occurrence of the sea breeze in the Stellenbosch region, and the climatic diversity these introduce, it is clear that an index has to be developed for use in this winegrowing region that incorporates these diversities as parameters.

In this study, even though clear climatic differences were seen between vintages, these differences could not explain the trends in the TA or MA content per site for either cultivar at the time of measurement.

It has to be noted that, due to the size of the original research project, berry samples were taken over a one-week period and as close to possible to the phenological stage being examined. The samples therefore were not taken on the true phenological date. Care hence should be taken when

interpreting data between vintages per site during ripening, as differences cannot be attributed only to the climatic variability between vintages, as the samples were taken at different phenological ripeness levels. When interpreting the variations between vintages per site at harvest, the sites were harvested at relatively the same level of ripeness, therefore excluding the date of harvest as an additional phenological difference.

When averaging data between sites, the differences in phenology have to be taken in account, as outlying sites (either early or late) will have an effect on the mean values obtained, as these sites were not at the same phenological ripeness as the remaining sites. For this reason, Chapter V will focus on the differences per site within vintages, thereby excluding the thermal and ripeness differences between vintages.

As temperature is not the only factor driving organic acid synthesis and breakdown in the case of MA, additional factors have to be taken into consideration when examining these two organic acids. Further research thus is needed on the influence of factors other than temperature, e.g. berry composition (mainly the K content), canopy manipulation (mainly changes in the sink to source relationship) and water availability (influence on the physiology of the grapevine), on the content of the organic acids per cultivar and per vintage. Some of these influences are still under investigation, and the interactions between these factors are intricate and some are not yet understood well (Jackson & Lombard, 1993).

4.5 Literature cited

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Chapter V: The effect of site on malic and tartaric acid contents of grapes in commercial vineyards of Sauvignon blanc and Cabernet Sauvignon in the Stellenbosch Wine of Origin district

5.1 Introduction

L-tartaric acid (TA) and L-malic acid (MA) are the main organic acids found in grapes, contributing to more than 90% of the total acids found in grape berries and wine (Winkler, 1962). According to Boulton (1980), the ratio in which these two acids are found is an important reaction among the many chemical and physical systems found in wine. These acids play an important role in wine, as they contribute mainly to the sensory perception (Boulton, 1980; Boulton *et al.*, 1998) and microbial stability of the wine by inducing a low pH (Jackson, 2008a).

Malic acid is synthesised primarily through CO₂ assimilation pathways (Ribéreau-Gayon, 1968), whereas TA originates mainly from ascorbic acid (Conde *et al.*, 2007). The acids are synthesised actively up to *véraison*, after which the MA content decreases, mainly due to respiration (Ruffner, 1982b), while the TA content stays fairly constant (Ruffner, 1982a).

Temperature plays an important role in the synthesis and respiration of MA, and to a lesser extent in the synthesis of TA (Iland, 1989). In addition, diurnal variations in temperature during the growth and ripening stages of the berries influence the rate of synthesis and the rate of degradation of MA (Kobayashi *et al.*, 1967; Kliewer & Lider, 1970; Hunter & Bonnardot, 2011).

Among the environmental factors contributing to viticulture, climate, and especially temperature, have an important effect on grapevine growth, wine quality and character (Coombe, 1987). In this chapter, climatic monitoring will be a focus and the effect of temperature differences between sites on the MA content of the berries will be discussed. Attention will also be given to the vegetative and productive variation between the sites. All these factors contribute to a certain degree to the final acid content of the berries (refer to Chapter II). As these interactions are intricate and not well defined as of yet, and many additional factors (not all monitored) contribute to these interactions, temperature will be the focus of this study.

5.2 Project aim

The aim of this project was to determine the range of malic and tartaric acid contents in grapes of Cabernet Sauvignon and Sauvignon Blanc from the Stellenbosch Wine of Origin district – at two key phenological stages and within three vintages – by investigating the relationship between the climatic characteristics of the vineyards and the tartaric and malic acid contents of the grapes.

5.3 Results and discussion

5.3.1 Data

As the Huglin index (HI), calculated from data collected from the dataloggers, will be the main thermal index used for the calculations from hereon as determined in Chapter IV, the three study periods per vintage relevant in this chapter therefore are:

Vintage 1 (V1):	1 October 2006 to 31 March 2007;
Vintage 2 (V2):	1 October 2008 to 31 March 2009;
Vintage 3 (V3):	1 October 2009 to 31 March 2010.

Each vintage includes data from 16 Tinytag[®] dataloggers, except for V2. All climatic parameters calculated from the datalogger data of the “LACS” site for vintage 2 were excluded in the statistics due to exceptionally low values in comparison to the complete dataset and in relation to the nearest automatic weather station. This can be attributed to a sensor that was faulty during the period of observation.

5.3.2 Climatic variability between sites

As discussed in Chapter IV, there are climatic differences between the three vintages included in the study period. When multivariate analyses (principal component analyses – PCA) were performed on the regional mesoclimatic thermal parameters to indicate these differences according to the vintage, the differences become clearer (Fig. 5.1).

In Fig. 5.1 it can be seen that sites are grouped according to the mesoclimatic parameters per vintage, therefore clear climatic differences between vintages are apparent on a mesoclimatic level. It is evident that 58% of the variance in the data can be described by the differences between vintages, and 37% by the variability of the sites within the vintages.

The sites furthermore can be split based on principal component (PC) 2, therefore indicating a consistent grouping of sites across the vintages, even though the distribution of the sites changed between vintages.

It is also noticeable that RUSB continuously fall outside the grouping per vintage. This is due to the consistently low values for the HI and mean February temperatures (MFT) per vintage as a result of the altitude, distance from the ocean and the southern aspect of this site (Carey *et al.*, 2008). The separation of DEHSB in V1 can be attributed to the low HI value in relation to the remaining sites in V1, which can be attributed to the low FNI for this site in V1 and a low MFT value (Fig. 5.1).

It is clear from Fig. 5.1 that the differences between vintages are driven mainly by the value of the MFT, while the differences between the sites are driven mainly by the FNI and the HI per vintage, although these last two also contribute to vintage differences. Multivariate analyses (Fig. 5.2), separating the regional mesoclimatic data per vintage, show a further separation between sites, emphasising the climatic differences between the sites, which are due mainly to topographical differences and the extent of the influence of the sea breeze, as will be discussed later. In addition, the distribution of the sites per vintage when compared between vintages is noticeably different, emphasising the climatic diversity of the sites, not only within, but also between, vintages.

Per vintage, small clusters of sites are noticeable that cannot be attributed solely to locality, hence indicating similar summated and mean climatic data. However, the distribution of the sites within these clusters is dissimilar per vintage.

As can be seen in Fig. 5.1, there appears to be a similar separation of sites along PC2 within all three vintages (also seen in Fig. 5.2 A-C), indicating a similar climatic trend regarding the FNI and the HI per site between vintages.

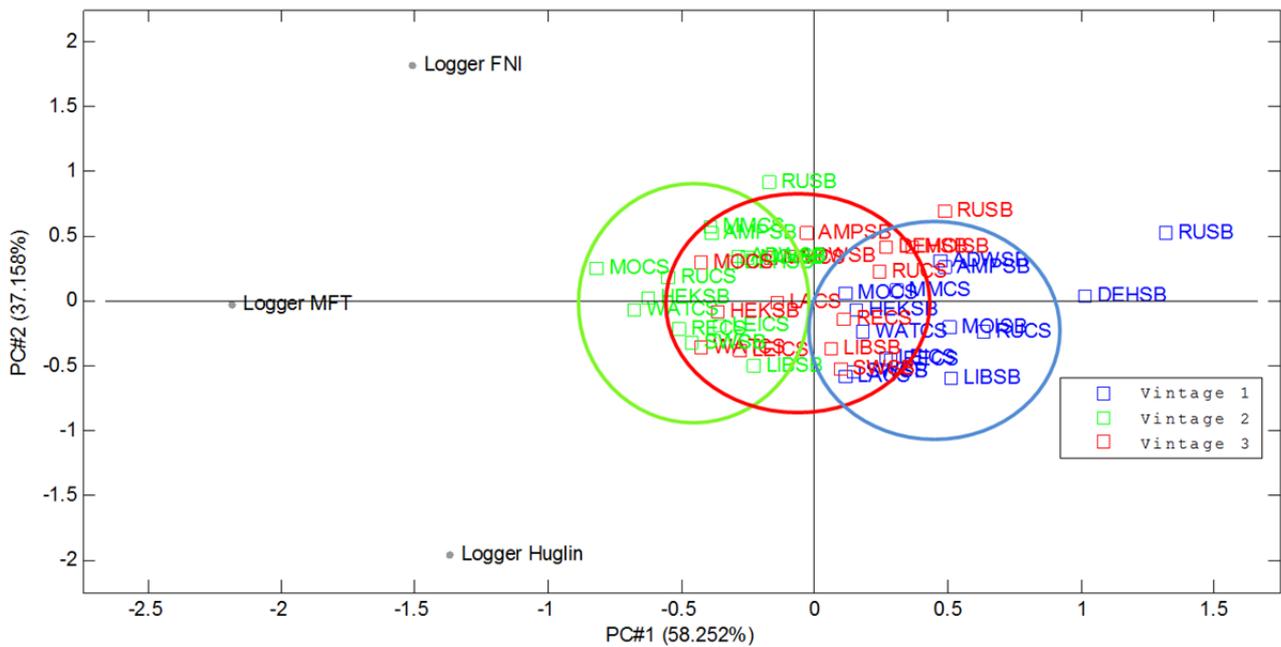


FIGURE 5.1

PCA score plot of sites modelled against the mesoclimatic parameters (Huglin index, fresh night index and the mean February temperature) and coloured according to vintage, with V1 (2006/2007) indicated in blue, V2 (2008/2009) in green and V3 (2009/2010) in red. PC1 describes 58.3% and PC2 37.2% of the variance in the dataset, therefore this plot explains 95% of the variance.

Considering the PCA plot in Fig. 5.1, each vintage was handled separately in further statistical analyses in which sites were compared to exclude the additional influence of the differences between vintages on the statistics.

The data points are not a true indication of the kinetics of the vintage, however, and the differences within the vintage are not represented. For instance, two sites may have a similar fresh night index (FNI) value, which is a mean minimum value for 28 days (1 February to 28 February) in a specific vintage, but the daily kinetic temperatures (both day and night) may be dissimilar, and therefore also the daily minimum values from which the FNI values were calculated. For instance, as seen in Table 1 in the addendum, in V1, RUSB and SWCS had a similar FNI value of 14.6°C, with corresponding MFT values of 19.7°C and 21.9°C respectively. The same is seen in V3 for LACS, RUSB and HEKSB, with a similar FNI of 15.9°C and corresponding MFT temperatures of 22.0°C, 21.2°C and 22.7°C respectively. During V2 it can be seen that DEHSB, ADWSB, WATCS and HEKSB had a similar FNI value of 16.1°C. The corresponding values for the MFT are 23°C for DEHSB and ADWSB and 23.6°C for WATCS and HEKSB, indicating similar mean daily temperature values, but this is not an indication of similar temperature kinetics for these sites. Care therefore has to be taken when interpreting summated and mean thermal data. This is one of the reasons the use of the summated thermal indices, e.g. the HI and the Winkler index (WI), is not suitable in ripening studies, as the differences in the kinetic daily temperatures, as well as the kinetics of the temperature data across the vintage, are not represented.

It can be noted from the PCA plot in Fig. 5.2 (A), however, that during V1, MOCS had the highest FNI value and LIBSB the lowest. The higher FNI of MOCS can be attributed to the radiation at night from the soil and from the large stones located on the surface of the soil. The same effect is seen in V2 and V3. LIBSB lies on the lower footslopes of Stellenbosch Mountain, therefore explaining the lower FNI for both V1 and V2, as cold air tends to accumulate in lower-lying areas.

RUSB consistently lies outside the main cluster in all three vintages due to a low MFT, in addition to low values for the HI, for the reasons explained previously. In V1 and V3, DEHSB (and MOISB in V3) also separates from the cluster due to the lower values for the HI and the MFT as a result of the altitude of these sites. Even though MOISB faces north, the effect of altitude on the temperature overcomes the effect of the aspect.

In all three vintages, ADWSB, AMPSB and MMCS cluster together due to higher FNI and lower HI values per vintage. This can be attributed mainly to the southerly aspect of these sites and therefore the moderating influence of the sea breeze on the temperatures, leading to a shorter duration at T_{\max} (Bonnardot, 1997) and ultimately lowering the HI values.

In Fig. 5.2 (B), MOCS is separated from the cluster due to the highest FNI for the vintage, in addition to higher HI and MFT values, as also seen in V1 and V3. This site faces west, therefore is situated on a warmer slope facing the afternoon sun. The cooling effect of the sea breeze on this site may not be able to overcome the topographical influence on the temperature due to the aspect of the slope and the distance from the ocean. LIBSB separates from the cluster during V1 and V2 because of the lowest value of the FNI calculated, although the HI and the MFT values were close to the mean value for the vintage (data not shown).

No clear outliers can be identified when looking at Fig. 5.2 (C), and the sites are more tightly arranged along PC1. However, there is a clear clustering of sites. Firstly, RUSB, RUCS, DESB and MOISB are clustered together due to the low values for the HI. These four sites have the highest altitude, thereby explaining the lower daily temperatures. In V3, SWSB and SWCS cluster together with LIBSB and RECS, as these sites have the lowest FNI values. SWCS and SWSB have a low FNI due to their location on a plain and the subsequent accumulation of cold air in this region at night. In contrast, AMPSB, MOCS, MMCS and ADWSB had the highest FNI values and therefore clustered in the opposite quadrant. Correlations performed on the topographical and temperature data did not produce any significant correlations, indicating that either more than one topographical factor drives the differences in temperature, or that topographical aspects in conjunction with other factors drive temperature differences between the sites.

Due to the proximity of SWCS and SWSB, with no change in topography or soil surface, data from one data logger, situated in the SWSB site, is assigned to both sites, which explains the overlapping of these sites on the PCA for all vintages.

As noted before, these indicators are daily mean and cumulative values per site per vintage, therefore can only be used for a broad interpretation and general comparison of the sites. The PCAs in Fig. 5.2 therefore represent the overall differences between sites per vintage. As these comparisons are not a true representation of the vintage, this data therefore cannot be used for comparison of the organic acids per site within a vintage, as the dynamics of the vintage have to be incorporated.

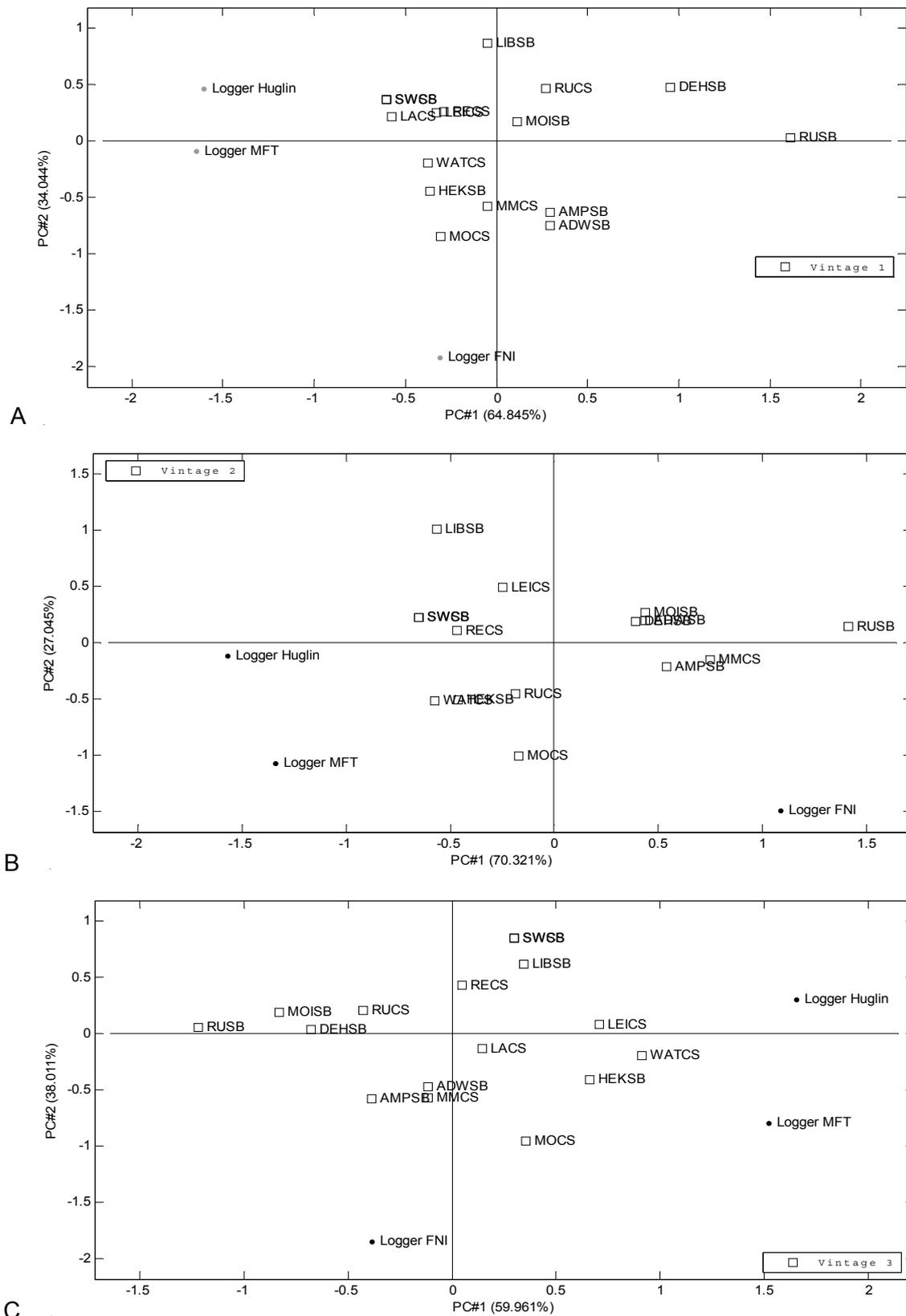


FIGURE 5.2

PCA score plots of the experimental plots distributed according to the HI and FNI per vintage. For vintage 1 (A), PC1 describes 64.8% and PC2 34.0% of the variance; for vintage 2 (B), PC1 describes 70.3% and PC2 27.1% of the variance in the data; and for vintage 3 (C), PC1 describes 59.9% and PC2 38.0% of the variance in the data. The scores are indicated in squares, while the loadings are indicated by circles.

Véraison is seen as the point at which the process of ripening starts (Hrazdina *et al.*, 1984). There are two distinct phases in the curve of organic acid synthesis and breakdown (in the case of MA) in the grape berry, with the turning point at *véraison*, (literature cited in Conde *et al.*, 2007). Separating data into a pre- and post-*véraison* period is crucial, as *véraison* is seen as the point when organic acid synthesis is terminated (Ruffner, 1982a, 1982b). Therefore, conditions pre-*véraison* can be attributed to the synthesis of MA and TA, and those post-*véraison* to the rate of degradation of MA and the degree of dilution of TA.

5.3.3 Climatic variability between sites according to growth and ripening periods

The Huglin index data was separated into two periods of three months each – a growth period (GP) from 1 October to 31 December, and a ripening period (RP) from 1 January to 31 March. As the Huglin index is calculated from October to March, the GP excludes the month of September for the calculation, as previously defined in Chapter IV. When a simple scatter plot is drawn, indicating the sum of the thermal units (TU) for the GP and the RP per vintage, the regional climatic differences per location and per vintage are again emphasised.

From Fig. 5.3 it is clear that, during the RP, more thermal units are cumulated than during the GP, although both periods are three months in length, indicating a warmer RP than GP for all sites across all vintages. In addition, between vintages per site, a warm GP can be followed by a cooler RP and vice versa. It seems that the GP was more stable between vintages, whereas the RP showed greater differences between vintages, except for the values measured at RECS, SWSB/SWCS. This can be attributed to heat waves occurring mostly in January and February in the Stellenbosch Wine of Origin district.

It is also clear that a lower number of TU were accumulated in the majority of the sites during the GP and RP in V1, therefore indicating a cooler vintage. The lower FNI, indicating lower minimum temperatures during February in this vintage, may contribute to the trend during the RP.

Even though the time periods for which the indices per site were calculated are similar, it is clear that the GP and RP values for the majority of the SB sites are less than those of the CS sites. This can be attributed mainly to the higher altitude of the SB sites in general. There also is less variability in the TU values for the CS sites than for the SB sites during the GP and the RP, mainly in V1 and V2. When comparing two sites in the same vintage with a similar TU accumulation during the GP, a value for the RP cannot be predicted, therefore indicating different temperature regimes during the ripening period and therefore different ripening sequences per site. It has to be borne in mind, however, that even though the same TU accumulation was seen during the GP, berry ripening will not be the same between the sites, as other factors, such as the soil, water status of the vines and canopy architecture, play a significant role in addition to the temperature.

When the cumulative TU for the GP and the RP is examined per cultivar (Fig. 5.3), it is apparent that the cooler sites are MMCS and RUCS, which consistently are the cooler CS sites across all vintages. This may be due to the south and south-westerly aspect (exposure to the sea breeze) of MMCS and RUCS respectively, and the higher altitude of both sites. In addition, MMCS may receive fewer sunlight hours in the afternoon due to the shading effect of the Bottelary Hills to the west and north-west. The same effect is not seen for LEICS in the same location and with the same aspect, as this site is located further to the southeast of MMCS and at a lower altitude (Fig. 3 in the addendum). In comparison to MMCS and RUCS, no sites seem to be consistently warmer in a specific vintage, with LAICS and SWCS being the warmest during V1 (location, aspect), no

specific sites showing distinctly warmer temperatures in V2, and LEICS and WATCS as the warmer sites in V3. What is very noticeable is the great variation in the data across vintages for the RP of WATCS, in relation to a GP with no variation across vintages.

For SB, the distinction between consistently warmer and cooler sites is more apparent, with HEKSB, LIBSB and SWSB (location and aspect) being the warmer sites for the three vintages and RUSB (altitude and aspect) the coolest.

It can further be seen in Fig 5.3 that, for example, ADWSB has a cooler RP in relation to GP in comparison with the remaining sites. This is a clear indication of the cooling effect of the sea breeze on this site during the RP (southern aspect, open landscape and distance from the ocean). The sea breeze is more pronounced during the RP in this region due to the increased temperature difference between the land and the ocean, therefore strengthening air circulation. SWCS and SWSB, which are situated on a flat plain with no exposure to the sea breeze (behind the Bottelary Hills, which act as a border to the penetration of the sea breeze, as mentioned before), show very little variation in temperature between vintages. The greatest variation can be seen for RUSB, as the topography of this site (altitude, landscape, aspect) is the great driver of the climatic difference in comparison to the other sites. The effect of the topography on climate at this site is not consistent during vintages, however, indicating the contribution of other, inconsistent factors, such as the occurrence of the sea breeze and heat waves, to the climatic variability of the site.

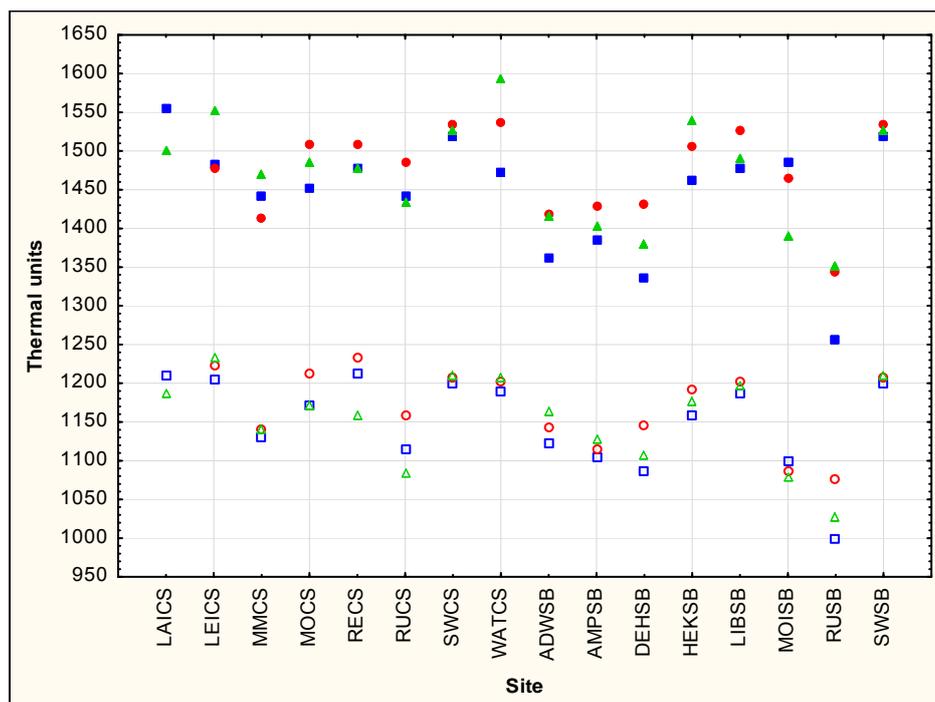


FIGURE 5.3

Scatterplot indicating the cumulative thermal units for the growth period from 1 October to 31 December (presented in symbol outlines) and the ripening period from 1 January to 31 March (presented in solid symbols) per site per vintage for the region, with V1 (2006/2007) indicated in blue squares, V2 (2008/2009) in red circles and V3 (2009/2010) in green triangles.

Fig. 5.3 provides an indication of the same time period as dictated by the calculation of indices per site, therefore it still gives a general overview per season, without incorporating differences in the real phenological periods per site in the representation. Jones & Davis (2000) mention that the

date of the phenological events is not as important as the interval between these dates, which provides an overview of the climate during these periods.

5.3.4 Climatic variability between sites according to phenological stages

When creating the scatter plots of the accumulated TU per vintage according to the phenology per site (Jones & Davis, 2000), a completely different pattern can be seen in the values. The “growth period”, as defined for Fig. 5.3, is represented by the period from anthesis to *véraison* (AV) in Fig. 5.4, therefore indicating the berry growth period, and the “ripening period” from *véraison* to harvest (VH) therefore is the true phenological ripening period.

It is clear from Fig. 5.4 that further analyses need to be separated according to SB and CS, as the length of the phenological stages differs considerably between vintages. This was not found in the study of Jones & Davis (2000), however, where a high correlation was found between phenological dates, where events were highly correlated with the preceding event and the growth interval being relatively constant regardless of the climatic conditions during the growing season. The study of Jones & Davis (2000) was conducted on data covering 45 years, whereas our study only represents three vintages, therefore emphasising the climatic differences between vintages.

The effect of the date of harvest on the calculation is an additional point that needs to be taken into consideration, as a lengthened period of time from *véraison* to harvest may negatively influence the end content of TA and MA when comparisons have to be drawn per site. This delay in the time of harvest may be as a result of a slower rate of sugar loading due to inhibiting factors particular to the site (irrigation availability and frequency, associated with soil water availability, canopy size, which is associated with transpiration rate, exposure to wind, which is associated with transpiration rate, and stomatal efficiency, which is related to the nutrient status of the soil and the plant, etc.). These factors will not be investigated in this study, as sugar loading was not investigated, but all viticultural and environmental factors need to be considered when conclusions are drawn. However, the interactions of these factors are intricate and cannot be simplified to the few factors mentioned here.

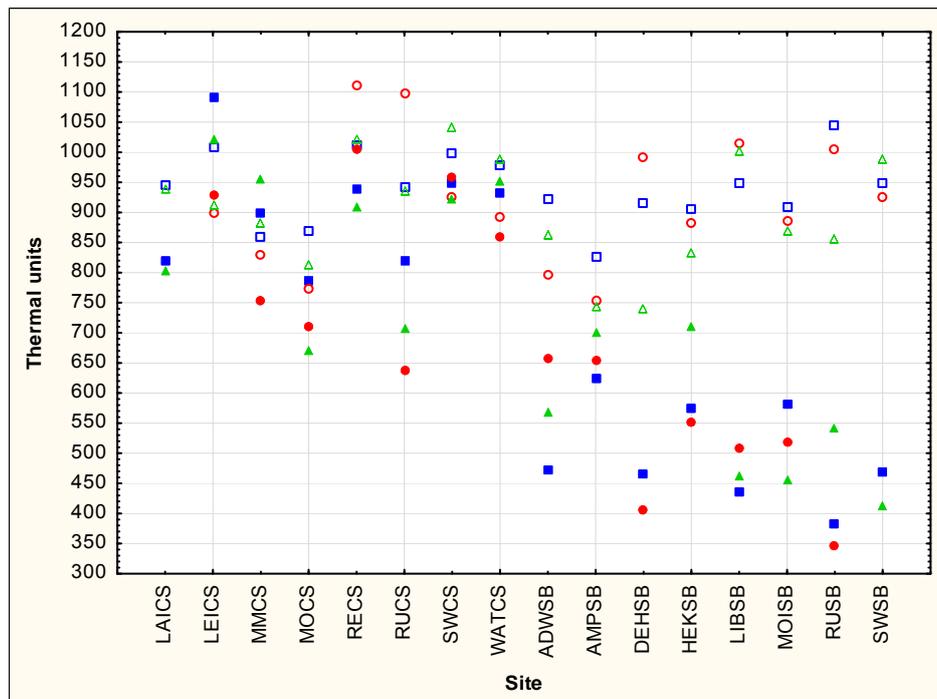


FIGURE 5.4

Scatterplot indicating the cumulative thermal units for the phenological periods of anthesis to *véraison* (presented in symbol outlines) and *véraison* to harvest (presented in solid symbols) per site per vintage, with V1 (2006/2007) indicated in blue squares, V2 (2008/2009) in red circles and V3 (2009/2010) in green triangles.

What is interesting is that, even though the length of the phenological periods (Table 4.2) and the cumulative thermal units (Fig. 5.4) differ greatly between sites per vintage, the thermal units per day remain relatively constant when calculating the mean daily HI thermal units per site per stage per vintage, with a small SD value at *véraison* and harvest (Table 5.1).

The SD is greater at harvest than at *véraison* (Table 5.1). However, the date of harvest was determined according to a ripeness indicator (sugar only), which means that sugar is loaded at different rates per cultivar and per site (data not shown). The period of time per phenological stage varies according to the cultivar, the climate and the geographical location (Jones & Davis, 2000), and a clear correlation was seen between the number of thermal units and the period of time per phenological stage (Table 4.4). It therefore can be concluded from Table 5.1 that phenology is driven by the accumulation of thermal units per vintage, although this number differs slightly between vintages and, as stated before, between cultivars. Jones & Davis (2000) also noted that the actual date of the phenological event is less important than the interval between the events, as this indicates the climate during this period of time.

TABLE 5.1

The daily rate of change of the Huglin index for all the sites per cultivar for V1 (2006/2007), V2 (2007/2008) and V3 (2009/2010). The data is further separated per stage, with *véraison* representing the period from anthesis to *véraison*, and harvest the period from *véraison* to harvest.

			N	# days per stage ¹	HI per stage ²	HI rate per day ³
CS	<i>Véraison</i>	V1	8	69.46 ± 3.86 a	951.83 ± 59.87 a	13.70 ± 0.38 a
		V2	8	59.86 ± 7.47 b	933.11 ± 126.97 a	15.58 ± 0.46 b
		V3	8	61.88 ± 4.05 b	932.86 ± 68.20 a	15.08 ± 0.63 b
	Harvest	V1	8	53.13 ± 5.44 a	904.58 ± 97.30 a	17.02 ± 0.36 a
		V2	7	47.25 ± 7.59 a	832.21 ± 150.96 a	17.35 ± 0.87 a
		V3	7	51.13 ± 5.06 a	855.30 ± 130.65 a	16.89 ± 1.05 a
SB	<i>Véraison</i>	V1	8	71.58 ± 4.62 a	927.59 ± 60.83 a	12.96 ± 0.43 a
		V2	7	60.67 ± 6.39 b	922.67 ± 92.56 a	14.95 ± 0.47 b
		V3	8	58.88 ± 5.67 b	861.67 ± 96.41 a	14.63 ± 0.60 b
	Harvest	V1	8	29.29 ± 4.78 b	500.56 ± 82.80 a	17.14 ± 1.63 b
		V2	6	31.52 ± 7.61 ab	497.31 ± 108.31 a	16.55 ± 0.72 b
		V3	7	36.71 ± 7.34 a	550.02 ± 118.47 a	14.97 ± 0.83 a

Values designated by different letters within each column per cultivar per stage differ significantly ($p \leq 0.05$)

¹ The number of days per stage, calculated from the date of anthesis to the date of *véraison* for *véraison*, and from the date of *véraison* to the date of harvest for harvest.

² The summated Huglin index thermal units per day, calculated from the date of anthesis to the date of *véraison* for *véraison*, and from the date of *véraison* to the date of harvest for harvest.

³ The mean daily Huglin thermal units (HI per stage divided by the # days per stage), calculated from the date of anthesis to the date of *véraison* for *véraison*, and from the date of *véraison* to the date of harvest for harvest.

5.3.5 Hourly temperature variation between sites according to thresholds

As MA varies more than TA across vintages (Ruffner *et al.*, 1976), and little or no effect on the tartaric acid content was seen in previous studies regarding temperature (Kliwer, 1964; Buttrose *et al.*, 1971; Coombe, 1987; Terrier & Romieu, 2001), the temperature thresholds for MA synthesis and degradation will be focused on in this section.

5.3.5.1 Mean hourly temperature data per site

The hourly minimum, mean and maximum temperatures per site were determined for the pre- (1 November to 31 December) and post-*véraison* (1 January to 28 February) period per vintage, as shown in Figs 5.5 and 5.6. Two sites will be shown as case studies. The sites selected are a warmer site (Fig. 5.5), represented by RECS, and a cooler site (Fig. 5.6), represented by RUSB (data not shown). These specific cultivars were chosen according to the sites, as SB generally is planted on cooler sites and CS on warmer sites. These examples therefore indicate the differences in temperature experienced by the two cultivars.

The ambient temperature thresholds for optimal malic acid synthesis and degradation, as noted in Chapter III, were superimposed on the graphs to indicate the periods of the diurnal optimum temperature thresholds for MA synthesis in the pre-*véraison* and MA degradation in the post-*véraison* period.

Although some authors (Kliwer & Lider, 1970; Buttrose *et al.*, 1971; Lakso & Kliwer, 1978) have noted temperatures of around 20°C as optimal, a temperature threshold of 20 to 25°C between the hours of 06:00 and 18:00 (Kliwer, 1964; Kliwer & Lider, 1970; Ruffner *et al.*, 1976; Lakso & Kliwer, 1978) and night temperatures of around 15°C between 18:00 and 06:00 (Kliwer & Lider,

1970; Hunter & Bonnardot, 2011) were used for the purpose of this study. Ruffner (1982b) also noted that 10°C is the temperature limit below which half of the maximum activity is observed and therefore can be taken as the lower threshold value for the night-time temperatures. Note that high temperatures during the pre-*véraison* period were not considered, as previous studies show that higher temperatures have little effect on MA accumulation, but rather affect MA degradation (Buttrose *et al.*, 1971; Ruffner *et al.*, 1976).

The temperature threshold for MA respiration during the post-*véraison* period was noted as 30°C due to an increase in enzyme activity and the subsequent metabolism of MA (Winkler, 1962). The temperature for MA respiration is lower than that for TA (Winkler, 1962), however, and was indicated as 35°C by Gerber in 1897, as reported by Kliewer (1964). No new research reports on firstly, the possible respiration of TA, and secondly the temperature at which the respiration may occur, have been published since then, which, according to me, calls the validity of this statement into question. A constant relationship between cooler and warmer vintages was not found by Kliewer (1964), while Peynaud & Maurié (1958) have stated that respiration is not in direct relationship to the temperature during ripening.

The daily temperature thresholds are also the thresholds for optimum photosynthesis (Iland, 1989; Ferrini *et al.*, 1995; Hunter & Bonnardot, 2011), therefore ensuring a constant supply of the substrate for MA synthesis. Furthermore, a threshold of 30°C is the upper limit for plant photosynthetic activity, above which photosynthesis slows down notably (Spayd *et al.*, 2002). At temperatures above this threshold, MA is used as carbon source due to the low supply of photosynthetic substrate (Kanellis & Roubelakis-Angelakis, 1993). Kobayashi *et al.* (1967) also noted 30°C as the upper temperature limit for favourable berry maturation.

For RECS (Fig. 5.5), it is clear that the mean hourly temperatures during the pre-*véraison* period mostly fell outside the boundaries of the thresholds in the three vintages, while the mean night-time temperatures fell within the threshold boundary of 15°C from 00:00 to 06:00. In contrast, for RUSB (Fig. 5.6), a longer period of the mean daytime hours fell within the threshold and the mean night temperatures fell within the threshold boundary of 15°C from 21:00 to 06:00 during all three vintages. Due to the altitude of RUSB and the exposure of this site to the sea breeze, the optimum night temperature was reached earlier than at RECS, which, even though it is an almost similar distance from the ocean and also exposed to the sea breeze, reaches the optimum temperature four hours later than RUSB. This may be attributed to the starting day temperature being higher than that of RUSB, mainly due to the difference in altitude. In addition, RECS has a closed landscape to the south (as seen in Fig. 3 in the addendum), therefore it is not as directly exposed to the sea breeze as RUSB. This therefore provides a good indication of how topography can influence the daily kinetics of temperature.

When considering the threshold temperature for MA respiration, neither of the two sites indicate a mean hourly temperature above 30°C for the time period, although the maximum hourly temperature for both sites indicates values of around 40 to 45°C for RECS and 35 to 40°C for RUSB. This shows that the mean can mask extremes; in fact, RECS and RUSB were exposed to a mean of 186 and 95 hours at temperatures above 30°C respectively, across all three vintages.

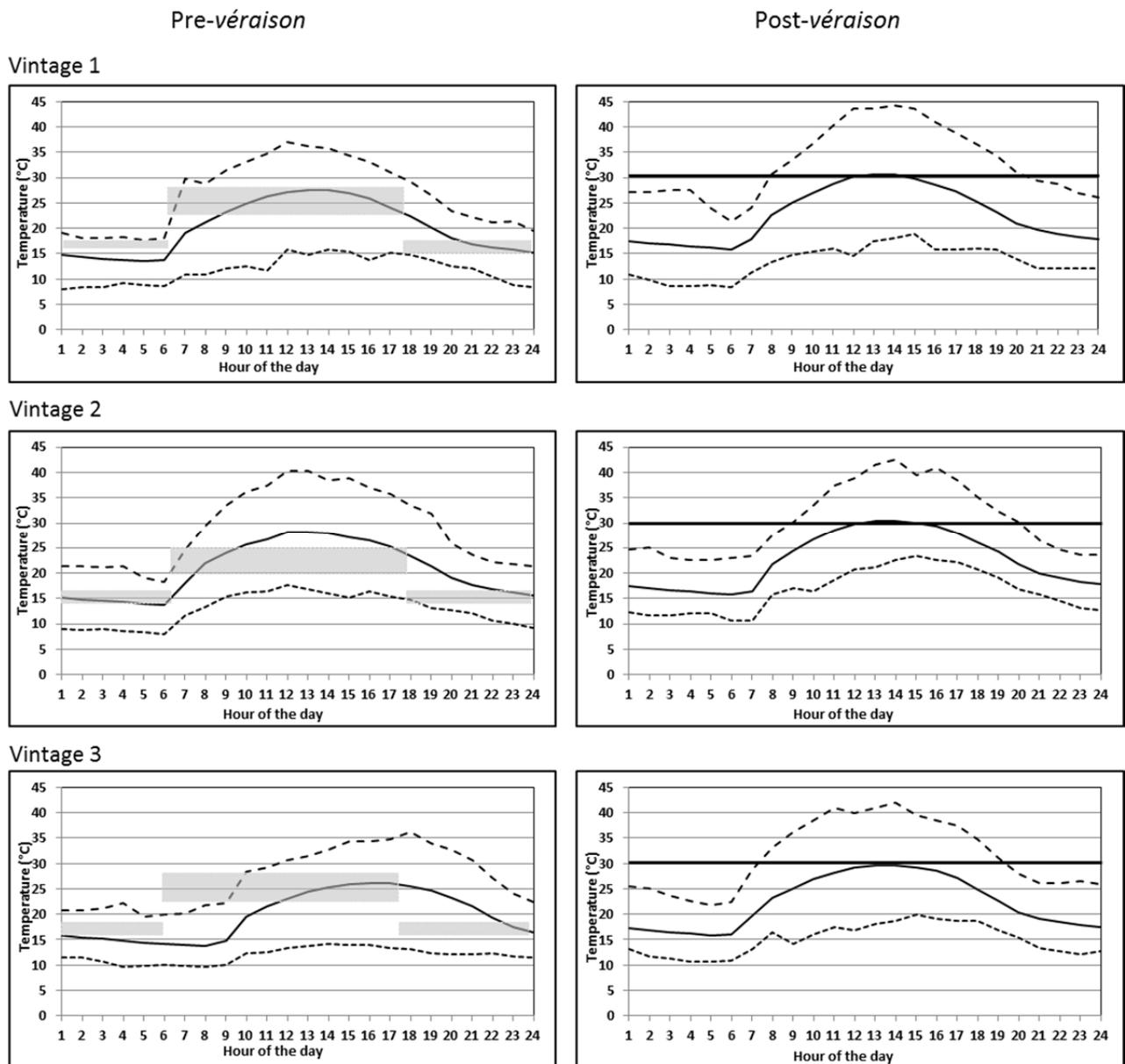


FIGURE 5.5

The hourly mean (solid line), minimum (short dashed line) and maximum (long dashed line) temperatures recorded for the pre- (1 November to 31 December) and post-*véraison* periods (1 January to 28 February) for RECS for vintage 1 (2006/2007), vintage 2 (2008/2009) and vintage 3 (2009/2010). Superimposed with grey blocks are the optimal temperature thresholds for MA synthesis in the pre-*véraison* period (20 to 25°C during the daytime; 10 to 15°C during the night), and the solid line indicates the temperature threshold for MA respiration (above 30°C) in the post-*véraison* period. Values were calculated as the mean, minimum and maximum values of the daily hourly values as measured by the datalogger during the period specified.

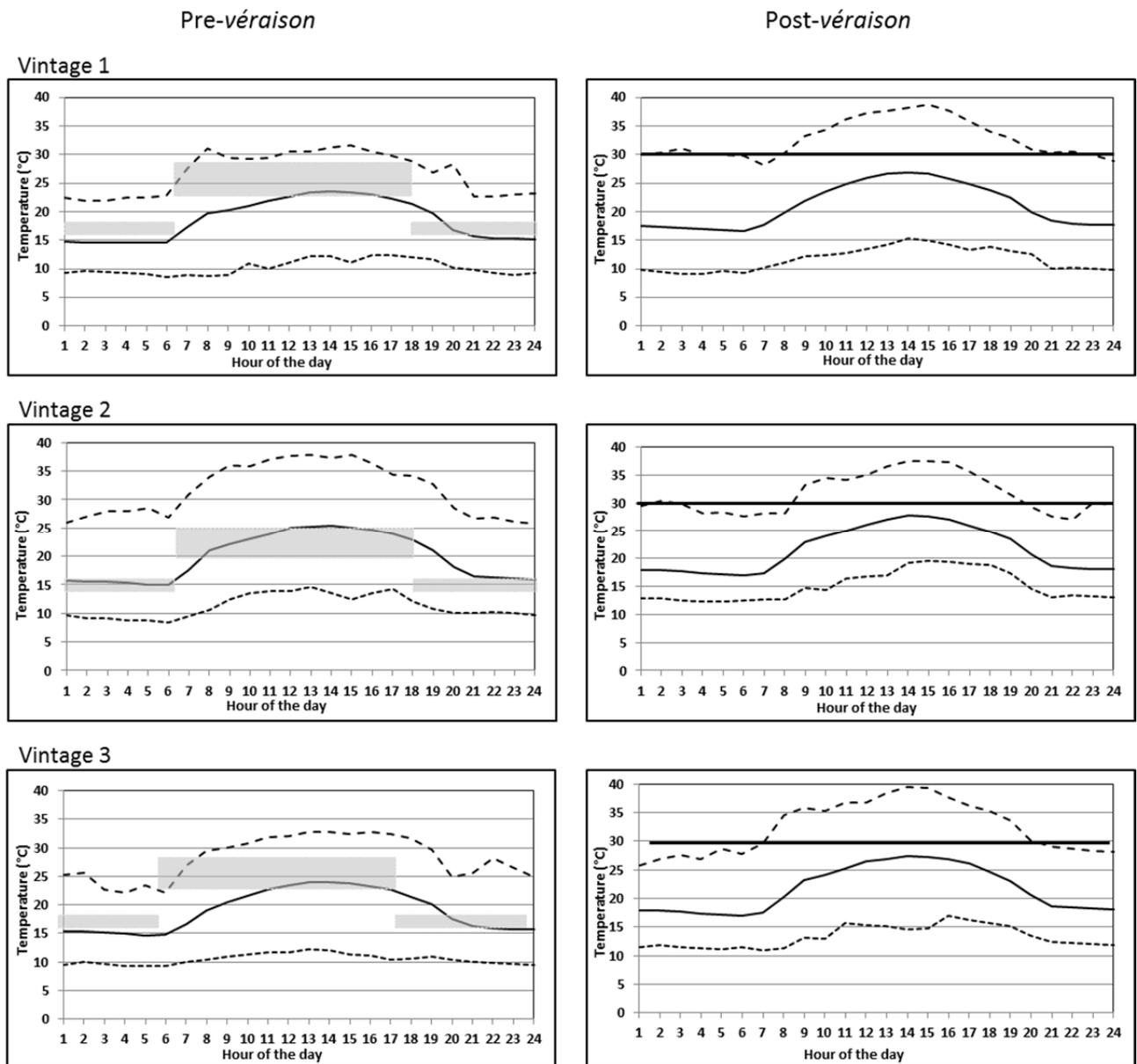


FIGURE 5.6

The hourly mean (solid line), minimum (short dashed line) and maximum (long dashed line) temperatures for the pre- (1 November to 31 December) and post-*véraison* periods (1 January to 28 February) for RUSB for vintage 1 (2006/2007), vintage 2 (2008/2009) and vintage 3 (2009/2010). Superimposed with grey blocks are the optimal temperature thresholds for MA synthesis in the pre-*véraison* period (20 to 25°C during the daytime; 10 to 15°C during the night), and the solid line indicates the temperature threshold for MA respiration (above 30°C) in the post-*véraison* period. Values were calculated as the mean, minimum and maximum values of the daily hourly values as measured by the datalogger during the period specified.

5.3.5.2 Optimum temperature hour summations according to temperature thresholds

Winkler (1962) stated that heat summation during the ripening period is important, therefore the method of Hunter & Bonnardot (2011), which incorporates temperature thresholds, was used in the calculations and adapted to the thresholds for MA synthesis and respiration (see Chapter III).

The total number of hours within or above the threshold was calculated for the pre-*véraison* (1 November to 31 December) and post-*véraison* period (1 January to 28 February) for the thresholds noted in section 5.3.3.1 and indicated in Fig. 5.7 as a percentage of the total number of hours monitored.

Firstly, from Fig. 5.7 it is clear that there are great differences between vintages per site for all indicators that were calculated, with none of the sites showing a constant trend across the three vintages. It is also evident that, on average, V3 had the higher percentage of optimal hours for the pre-*véraison* period, but the smaller percentage of hours above 30°C, and we therefore would expect the highest MA for this vintage. Small differences were noticed between V1 and V2 for these indicators. In addition, V1 and V2 showed the same trend between vintages for the majority of the sites, mainly for the indicators of the pre-*véraison* period, while V3 cannot be predicted according to this trend. It is also evident that the majority of the plots were more likely to be exposed to more hours per night at temperatures between 10 and 15°C than between the daily threshold hours of 20 and 25°C.

When considering the pre-*véraison* period in V1, V2 and V3, MOISB consistently fell in the group with the higher percentage of optimal day and night hours per vintage (62.18%, 68.96% and 70.32% respectively). The remainder of this group consistently comprised DEHSB and RUSB across all three vintages. During V3, the Simonsberg ward sites (DESB, RECS, RUCS, and RUSB) were indicated as having the greater percentage of optimal hours (68.75%, 65.37%, 73.11% and 70.67% respectively), together with AMPSB (67.14%). MOCS showed the minimum percentage of optimal hours for MA synthesis in V1 (43.51%) and V2 (47.02%), and fell into the median percentage group during vintage 3 (57.81%). It seems that the differences in the percentage of hours at the optimal night temperature vary more between the sites than the percentage of hours at the optimal day temperatures. This is due to the topographical differences and to the extent of the cooling effect of the sea breeze per site. Sites that are more exposed to this cooling effect will reach the optimum temperatures earlier in the late afternoon, therefore increasing the number of hours within the optimum temperature threshold at night (see also Figs 5.5 and 5.6). It also was interesting to note that, although MOCS consistently show the lowest percentage of hours per day within the optimal temperature thresholds, it also consistently had the lowest percentage of hours at the optimal night-time temperature threshold during the pre-*véraison* period (24.73%, 25.41% and 32.24% across vintages).

It furthermore is clear that the sites exposed to the sea breeze and with a higher altitude had a smaller percentage of hours at temperatures above 30°C during the post-*véraison* period, for the same reason as stated in the previous paragraph regarding night temperatures. RUSB, for instance, consistently showed the minimum percentage of hours below 30°C post-*véraison* (12.29%, 12.15% and 15.96% across vintages) due to altitude and aspect of this site. On average, during the ripening period sites in the Stellenbosch area experienced temperatures of above 30°C for about a fifth of the daily hours.

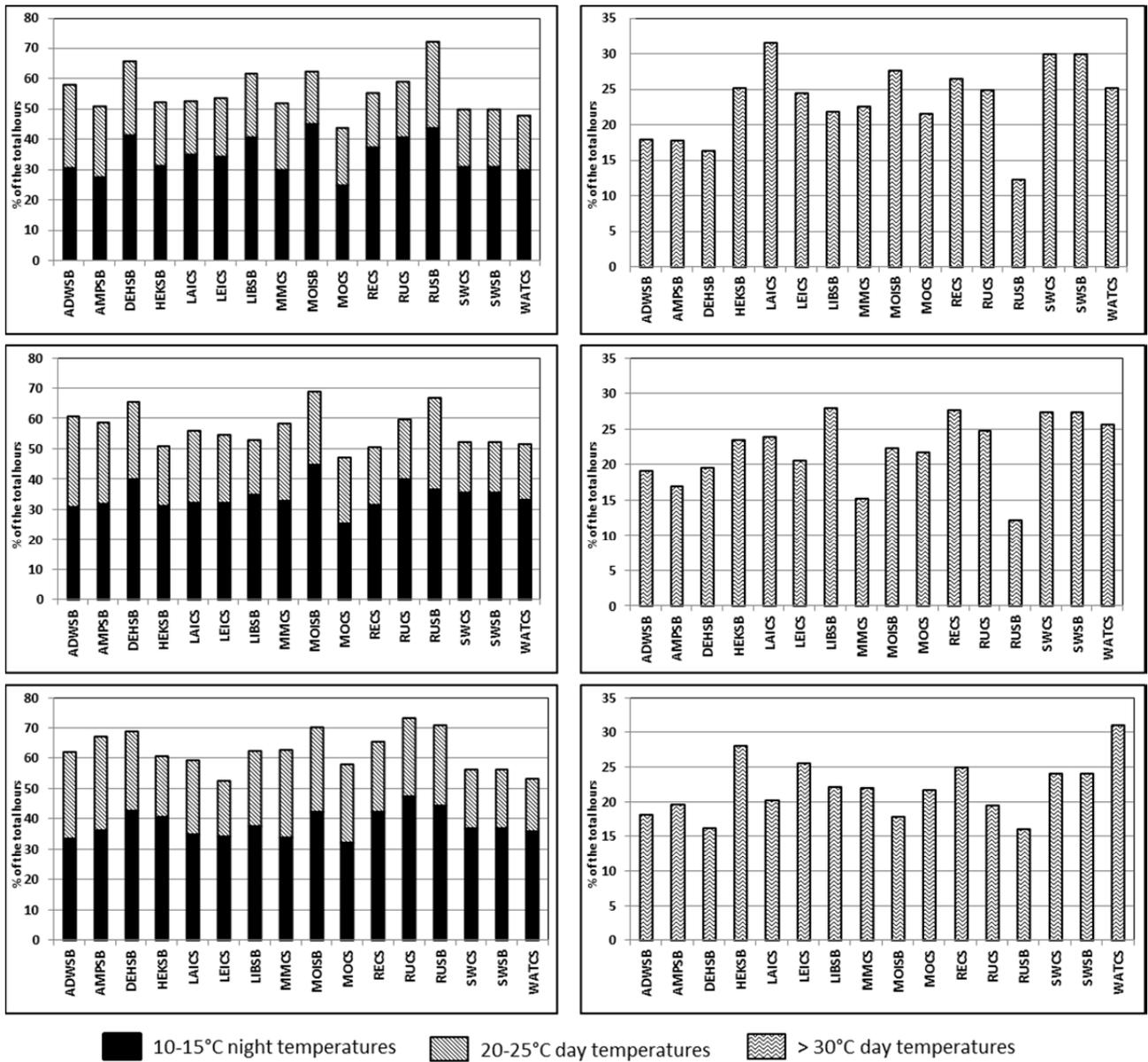


FIGURE 5.7

Summation per site for the pre- (1 November to 31 December) and post-*véraison* (1 January to 28 February) period for the thresholds of MA synthesis and respiration, represented as a % of the total number of hours monitored ($n = 732$ for pre-*véraison*; $n = 708$ for post-*véraison*) from top to bottom for vintage 1 (2006/2007), vintage 2 (2008/2009) and vintage 3 (2009/2010).

5.3.6 Vegetative and reproductive variation between sites

Table 2 in the addendum provides the complete dataset of the vegetative and reproductive measurements per site per vintage.

As vegetative and reproductive indicators, the yield per vine and the pruning mass per vine were selected to calculate the yield:pruning mass index, as this index is a good indicator of the balance of the vines. It should be noted that the sites are commercial vineyards, therefore certain sites were either pruned or harvested by the producer in some vintages. The yield:pruning mass index was consequently not calculated in these cases.

By using the score card of Smart & Robinson (1993) as adapted for South Africa (Archer, 2002) (see Fig. 7 in the addendum), a percentage value per site was obtained, indicating a canopy qualitative score.

Finally, the point quadrat (PQ) (Smart & Robinson, 1993) values provide a good indication of the canopy architecture. The values presented for the PQ measurements are the percentage gaps present, the leaf layer number (LLN) and the percentage shaded bunches and leaves present in the canopy.

The canopy structure firstly differs greatly according to cultivar (e.g. shoot and internode length, tendency of lateral shoot growth, and leaf structure and size). The vintage does have an influence on the canopy architecture, which in turn has an effect on photosynthesis and also on the microclimate of the berries. As the soil type stays constant per site, the canopy architecture is mainly changed by the amount of water available in the soil due to rainfall (and irrigation where available) per vintage, and by the soil water-holding capacity. This change in the architecture induces a change in the microclimate of the canopy and the bunches, thereby influencing photosynthesis and berry ripening and, ultimately, berry composition. In this study, the temperature was not monitored on a microclimatic level, and therefore is representative only of the ambient temperature of the site and not of the canopy or the bunches.

The differing canopy structure per vintage furthermore includes the different canopy management practices applied by the producers per site. Standard management practices applied in these commercial vineyards are pruning in winter to two-bud spurs, and mainly shoot positioning, suckering, tipping and topping as summer canopy maintenance.

These attributes are presented in this chapter to accentuate the fact that all the sites differed greatly according to vegetative and reproductive attributes, therefore contributing to the meso- and microclimatic and physiological differences and, ultimately, to the TA and MA content per site. The influence of the vegetative and reproductive attributes on the TA and MA content will not be included in any further discussion or analyses in this study, however, due to the high level of interaction between these attributes and the physiology and ripening of the vines.

5.3.7 Phenological variability between sites

To exclude the date of phenology per vintage as an additional variable in the data, an index, developed by Barbeau *et al.* in 1996 (as cited in Barbeau *et al.* (1998b)), known as the *indices de précocité* (precocity indices) for flowering (iP_f), *véraison* (iP_v) and the seasonal cycle (iP_{cy}), was included in the study. The index is based on a baseline of 100 (representing the mean phenological date of the vintage for all sites per cultivar), with the indices per site indicated above

or below this value. This serves to indicate whether the site is phenologically early or late in comparison with the mean phenological date, and the extent of the advance or delay.

As can be seen in Table 5.2, the phenological dates per phenological stage per site differ significantly, not only between sites per index, but also between vintages, as indicated by the SD values. For the majority of the sites, if a site is delayed or advanced in terms of one of the indices, it will remain delayed or advanced for the other indices. Furthermore, the variability between sites stays fairly constant between the indices. The variability of iP_f for CS, however, does not respond to the variability seen for the remainder of the indices. The least variability for both cultivars is noticed at anthesis, while the greatest variability is noticed for iP_{cy} . The iP_{cy} serves as a prediction of the date of harvest according to the dates of flowering and *véraison* to determine if full ripeness will be reached in a vintage according to this date, specifically in the Northern hemisphere, where later vintages are associated with low sugar levels and difficulty to reach optimal ripeness.

It is notable that the iP_f is not that variable for CS and SB with an increase in the variability of iP_v . The greatest variability in values is seen between and within the iP_{cy} , which indicates the variability of the cycle between the sites and between vintages.

From Table 5.2 it can be concluded that, for SB, RUSB is constantly late between vintages for all stages, while AMPSB is constantly the earliest of the sites. For CS, RUCS is the latest site for all indices. Please note that RUCS has a high infection incidence of leafroll virus, which may contribute to the differences seen for this site for phenology and berry composition in relation to the remainder of the CS sites, in addition to it being situated in a cooler location.

TABLE 5.2

The variability in phenology between the sites across the three vintages ($n = 3$) per cultivar through the indices of precocity for flowering (iP_f), *véraison* (iP_v) and the vintage (iP_{cy}) of Barbeau *et al.* (1998a).

		iP_f	iP_v	iP_{cy}
SB	ADWSB	100.50 ± 0.40 b	101.00 ± 0.88 bd	104.33 ± 7.19 bd
	AMPSB	100.40 ± 0.90 b	102.25 ± 0.48 b	112.11 ± 2.94 b
	DEHSB	100.05 ± 1.05 ab	99.87 ± 2.04 cd	99.09 ± 9.33 cd
	HEKSB	100.10 ± 1.71 ab	101.02 ± 1.09 bd	105.96 ± 0.84 bd
	LIBSB	99.93 ± 1.54 ab	98.90 ± 0.35 c	92.92 ± 8.02 ac
	MOISB	99.744 ± 0.26 ab	99.69 ± 0.34 cd	99.08 ± 1.32 cd
	RUSB	98.35 ± 0.73 a	96.68 ± 1.37 a	87.03 ± 6.62 a
	SWSB	100.93 ± 0.83 b	100.58 ± 1.25 bcd	99.49 ± 6.54 cd
CS	LACS	100.00 ± 0.62 a	100.10 ± 0.11 c	100.35 ± 2.30 bc
	LEICS	99.79 ± 0.72 a	100.18 ± 1.18 c	102.14 ± 5.83 c
	MMCS	99.12 ± 1.80 a	100.18 ± 0.72 c	104.20 ± 2.35 c
	MOCS	99.64 ± 2.39 a	102.31 ± 0.22 a	116.12 ± 12.41 a
	RECS	100.55 ± 1.05 a	99.40 ± 1.04 bc	93.96 ± 7.85 bc
	RUCS	99.79 ± 0.36 a	98.16 ± 1.21 b	89.33 ± 9.13 b
	SWCS	100.34 ± 0.32 a	99.39 ± 0.42 bc	95.05 ± 3.77 bc
	WATCS	100.77 ± 1.02 a	100.27 ± 0.77 c	98.84 ± 2.79 bc

* Values designated by different letters within each column per cultivar differ significantly ($p \leq 0.05$) within indices.

5.3.8 Variability in chemical composition at *véraison* and harvest according to temperature and phenological parameters

Tables 5.3, 5.4 and 5.5 provide the correlations of temperature parameters and phenological indices, as discussed previously in the chapter, with some grape chemical composition components. The correlations have been separated per cultivar and determined from the mean values of the sites per cultivar per stage (*véraison* and harvest) for all vintages.

Significant correlations, where $p \leq 0.1$, will be discussed further in this section and separated into thermal index parameters (Table 5.3), phenological indices (Table 5.4) and the hourly thresholds (Table 5.5). The *véraison* data was calculated according to the dates from flowering to *véraison*, and for harvest from the dates of *véraison* to harvest per site.

As temperature parameters, the heliothermal index of Huglin (HI) and variations thereof (HI and the HI thermal units per stage) (Tonietto & Carbonneau, 2004) were used for the correlations. As phenological indices, the *indices de précocité* of flowering (iPf), *véraison* (iPv) and the seasonal cycle (iPcy) of Barbeau *et al.* (1998b) were used in the analyses, along with the thermal hourly thresholds (the number of hours and the percentage of hours per site) as used by Hunter & Bonnardot (2011). These parameters were chosen because they were discussed previously in this chapter and in Chapter IV as temperature indicators in this study.

These indicators were correlated with some general ripeness indicators (the TSS, pH, TTA, mass per berry and sugar per berry), TA indicators (TA concentration and content) and MA indicators (MA concentration and content, the MA content per stage and the daily rate of change of MA).

The rate of change per day of MA per site at *véraison* was calculated by dividing the MA content value at *véraison* by the number of days from flowering to *véraison*. The rate of change value per site at harvest was determined by calculating the difference in the MA content between the value at *véraison* and harvest and dividing it by the number of days between *véraison* and harvest.

The correlations at *véraison* are in accordance with what was expected, with indicators that increased during pre-*véraison* showing negative correlations, while indicators that decreased during the pre-*véraison* period showed positive correlations. However, this trend was not seen at harvest, which could be attributed to the climatic conditions post-*véraison*. For instance, heat waves are commonly experienced in this region during the ripening period (the number, duration and intensity varying between vintages, as can be seen in Fig. 6 in the addendum). These pronounced fluctuations in temperature have a big influence not only on the functioning of the grapevine, but also on the ripening sequence of the grape berry. During these periods of high temperatures, the metabolic functioning of the grapevine is driven by the availability of water to the vine, which is regulated by soil water availability, and by the water requirements of the vine, which is influenced mainly by the canopy size and density. Soil properties will not be discussed in this project, but the differences in canopy structure per site are discussed briefly in section 5.3.4. Finally, the extended period of time over which the sites were harvested could play a role, as this would influence the thermal units included in the summation.

Even though significant correlations were seen between the TSS and the mass per berry at *véraison*, and certain parameters, this significance was not necessarily seen in the resulting sugar per berry value. As sugar is only actively loaded into the berry after *véraison*, and temperature has no effect on the values at this stage, these correlations are not of importance. At harvest, a significant correlation seen for the mass per berry with the thermal indices resulted in a significant correlation for the sugar per berry, although no significant correlations were seen for the berry

volume and the corresponding TSS values, as expected. Temperature therefore directly influenced the loading of the sugar into the berry, but other factors, e.g. the water availability to the plant, also influence the berry volume and therefore the TSS.

From the data it is clear that, in this study, no single indicator was a significant predictor of the MA content or of the rate of change in MA or of any of the ripeness indicators at either *véraison* or harvest. The N values in this study were very low, however, and in a study with so much variation between sites a low correlation between a small number of sites is expected.

5.3.8.1 Thermal index parameters and berry chemical composition

Many previous studies have confirmed the influence of different climatic parameters on berry composition (Kliewer, 1964; Kliewer & Lider, 1970; Buttrose *et al.*, 1971; Lakso & Kliewer, 1975b; Ruffner, 1982a, 1982b; Iland, 1989; Jackson & Lombard, 1993; Jones & Davis, 2000; Spayd *et al.*, 2002; Van Leeuwen *et al.*, 2004; Van Leeuwen & Seguin, 2006), with thermal indices used most broadly as the climatic indicator (Winkler, 1962; Spayd *et al.*, 2002; Tonietto & Carbonneau, 2004; Happ, 2007). As shown earlier in this study, the HI was determined to be the more representative index, and datasets need to be separated between cultivars and into a pre-*véraison* and post-*véraison* period. The HI and the HI thermal units were thus calculated per cultivar from anthesis to *véraison* for *véraison*, and from *véraison* to harvest for harvest, and used in the correlations per cultivar with the berry composition components, as seen in Table 5.3.

From Table 5.3 it is clear that the technological ripeness indicators firstly correlate more significantly with both thermal indicators at *véraison* than at harvest, and secondly correlate better for CS than for SB. The low incidence of significant correlations of SB at *véraison* and harvest for all indicators may indicate the higher climatic sensitivity of SB compared to CS (Marais *et al.*, 1999; Carey *et al.*, 2008), in addition to other factors influencing the ripening parameters (water status, canopy architecture, and berry temperature due to different canopy architecture). Fig. 8 in the addendum provides an indication of the variability of the malic acid content in the Huglin thermal units per phenological stage per cultivar at *véraison* (A) and at harvest (B).

Although many authors have found that temperature has little or no effect on the TA content in grape berries (Kliewer, 1964; Buttrose *et al.*, 1971; Coombe, 1987; Terrier & Romieu, 2001), the [TA] did show a significant positive correlation with the HI for SB at *véraison*, and a significant negative correlation at harvest. Therefore, in this study, warmer periods before *véraison* ensured higher [TA] at *véraison*, while warmer vintages post-*véraison* were related to lower values for the [TA].

This significance was not seen in the [TA] for CS and the TA content for either CS or SB in this study. The fact that this trend was not seen in the TA content may be related to the influence of temperature on the berry volume. There was a clear negative correlation of berry volume with temperature up to *véraison* for CS, which switched to a positive correlation up to harvest. SB, on the other hand, showed no clear correlations either at *véraison* or at harvest. As noted before, the water availability to the plant, in addition to temperature, influences berry volume. Pre-*véraison*, the availability of the soil water was determined mainly by the winter rainfall and the level of the resulting water table in the soil. The irrigated plots were irrigated mainly from *véraison* on, and the irrigation schedule may therefore have contributed to the differences in berry volume.

The MA content per stage correlated positively with HI for CS, but not for SB at either *véraison* or harvest. From Table 5.3 it appears that the HI was the best indicator when considering the MA

rate per stage per day, as significant positive correlations were seen for both CS and SB at *véraison*, and significant negative correlations were seen at harvest. These results appear to support the findings of other authors that lower temperatures between anthesis and *véraison* favour the synthesis of MA, and that higher temperatures from *véraison* to harvest favour respiration, resulting in a lower MA content.

TABLE 5.3

The correlation between certain berry composition components and thermal index parameters for the Cabernet Sauvignon (CS) and Sauvignon Blanc (SB) sites within the study area at *véraison* and harvest across all three vintages. Values indicated in bold italics correlate significantly, with $p < 0.1$.

			TSS ¹	pH	TTA ²	Mass/ berry	Sugar/ berry ³	[MA] ⁴	[TA] ⁴	MA/ berry ⁵	TA/ berry ⁵	MA per stage ⁶	MA rate per stage per day ⁷
<i>Véraison</i>	CS n = 23	HI ⁸	-0.67	-0.47	0.44	-0.63	-0.39	0.22	0.17	0.22	0.23	0.40	0.40
		HI stage ⁹	-0.56	-0.49	0.47	-0.47	-0.15	0.18	0.03	0.18	-0.11	0.23	0.09
	SB n = 23	HI ⁸	-0.26	-0.12	0.35	-0.24	-0.15	0.09	0.38	0.09	0.04	0.12	0.42
		HI stage ⁹	0.24	0.26	-0.10	0.21	0.16	-0.29	0.19	-0.29	-0.56	-0.34	0.10
Harvest	CS n = 22	HI ⁸	0.08	0.27	0.25	0.61	0.42	0.45	-0.13	-0.34	-0.11	0.37	-0.42
		HI stage ⁹	-0.16	0.52	-0.24	0.57	0.58	0.39	-0.01	-0.33	0.13	0.26	-0.38
	SB n = 20	HI ⁸	0.23	0.15	-0.50	-0.06	-0.16	0.16	-0.62	-0.37	-0.17	0.22	-0.59
		HI stage ⁹	-0.24	0.30	-0.54	0.00	0.10	-0.19	-0.04	-0.35	-0.07	-0.21	-0.07

¹ Total soluble solids (^oBrix)

² Total titratable acidity (g/L)

³ Sugar per berry, as calculated from the mass per berry and the TTS (mg/berry)

⁴ Malic and tartaric acid concentration (g/L)

⁵ Malic and tartaric acid content per berry, calculated from the [MA] and [TA] and the mass per berry (mg/berry)

⁶ Change in malic acid content from anthesis to *véraison* for *véraison*, and from *véraison* to harvest for harvest

⁷ Daily rate of change in the malic acid content from anthesis to *véraison* for *véraison*, and from *véraison* to harvest for harvest

⁸ The Huglin index (Tonietto & Carbonneau, 2004)

⁹ Sum of the Huglin index thermal units from anthesis to *véraison* for *véraison*, and from *véraison* to harvest for harvest

Thermal index parameters and berry chemical composition per cultivar at véraison

Cabernet Sauvignon: As can be seen in Table 5.3, the thermal indicators showed a significant negative correlation with the TSS, pH, the mass per berry and the sugar per berry ripeness indicators (no significance seen for the HI per stage with the sugar per berry). Therefore, in this study, the lower the temperatures during the berry growth period, the higher the values for these indicators. The TTA showed a significant positive correlation with both thermal indicators, therefore indicating that the higher the temperature, the higher the value of the TTA. This is not in accordance with what is seen in literature, where lower temperatures favour organic acid accumulation. These thermal indicators are summated values, however, and not an indication of optimal conditions for organic acid accumulation, as will be discussed further in section 5.3.5.6.

Both thermal indicators correlated positively with all the organic acid indicators (except for the TA content with the HI thermal units per stage), but none were significant and the correlations were weak. This is in accordance with what was also found for the TTA.

The only significant positive correlations were seen for the HI with the MA content and MA rate per stage per day, indicating that higher temperatures favour higher synthesis of MA pre-*véraison*.

Again, this finding is opposite to what is found in the literature, but it may be an indication of the rate of photosynthesis, as noted before.

Sauvignon blanc: As seen for CS, the TSS, pH, mass per berry and sugar per berry correlated negatively with the HI, but the opposite correlation was seen with the HI per stage in comparison to CS. The same trend is seen for the organic acid indicators (including the TTA), where a positive, and mostly weak, correlation was seen for the HI and a weak negative correlation for the HI per stage.

Significant positive correlations were seen between the [TA] and the MA rate change per day and the HI, and a significant negative correlation was seen between the HI per stage and the TA content. Therefore, the higher the daily temperatures, the higher the [TA], whereas higher temperatures resulted in a lower TA content, thus indicating that higher temperatures might result in smaller berries. In addition, higher temperatures resulted in a higher rate of change in the MA per day. As this is a cumulative value, and not an indication of the hours at optimal temperatures, it cannot be seen as a definite indicator.

Thermal index parameters and berry chemical composition per cultivar at harvest

Cabernet Sauvignon: Positive correlations were found for all the ripeness indicators with the HI, and a higher TTA was also noted, which is not in accordance with previous findings. The correlation of the HI is only significant for the mass per berry and the sugar per berry, however, with the remainder of the indicators showing weak correlations.

Significant positive correlations are seen for the pH and the mass and sugar per berry with the HI per stage, indicating that the higher the temperature between *véraison* and harvest, the larger the berry and the higher the content of sugar per berry. However, the berry size is not only related to temperature, as the irrigation regime and the subsequent availability of soil water during this period play a large role.

Both thermal indicators showed a significant positive correlation with the [MA] and the MA content per stage, which again is not in accordance with the literature, as a higher temperature in general resulted in a lower MA content. Again, these temperature indicators are summated values and not an indication according to temperature thresholds. The MA rate of change per day showed a significant negative correlation, which indicates that, the higher the temperature, the more negative the rate of change in the MA content, thereby showing that high temperatures, especially above 30°C, increase the rate of MA metabolism.

Weak, non-significant correlations were found for the TA indicators, emphasising the previous findings that temperature has little or no influence on the TA content at harvest.

Sauvignon blanc: The correlations for SB at harvest were generally very weak or non-existent. As can be seen in Table 5.3A, a significant negative correlation was found between both thermal indicators and the TTA value. Therefore higher temperatures post-*véraison* resulted in a lower total organic acid content, and therefore a lower TTA, in the berry at harvest, as found in previous literature.

As for CS, the correlations between the HI per stage and the [TA] and TA content were very weak, again indicating that temperature has little or no effect on the TA content at harvest. The HI did show a strong negative significant correlation with the [TA] at harvest, however, which may indicate

that the higher the temperature, the lower the synthesis of TA in the berry, or it may be attributed to the influence of the temperature on the berry volume which influences the [TA].

5.3.8.2 Phenological indices and chemical composition

Phenology plays a role in berry composition, as it indicates the differences in physical ripeness between the sites at the sampling point. Many studies have been conducted on the relationship between temperature indicators and phenology (literature cited in Coombe, 1987; Jones & Davis, 2000; Jones, 2003; Parker *et al.*, 2011) and between phenology and berry composition (Coombe & McCarthy, 2000). The *indices de précocité* (Barbeau *et al.*, 1998a; Barbeau *et al.*, 1998b; Tešić, 2001), which is used to normalise phenological dates, was found to correlate well with certain ripeness indicators.

Even though Barbeau *et al.* (1998b) and Tešić (2001) found significant correlations for similar datasets generated in the Loire Valley (France) on Cabernet franc and Hawke's Bay (New Zealand) on Cabernet Sauvignon, some indices of SB or CS values correlated well at *véraison*, but very few significant correlations were found at harvest in our study region, as seen in Table 5.4. The sites in this study were harvested at a similar technological ripeness of around 23 °Brix, whereas in the studies by Barbeau *et al.* the plots were harvested on the same date and in those by Tešić they were harvested according to the date determined by each producer of the vineyards used in the study.

Care has to be taken when interpreting the iP_{cy} data, as it is mainly used to indicate whether the length of the season will accommodate the full ripening of the grapes. As the climate in Stellenbosch is sufficient to ripen the grapes, it was not a concern in this study. The grapes were therefore harvested at a specific point of technological ripeness to ensure that an unripe character of the grapes would not influence the sensorial analyses.

Table 5.4 provides further discussion regarding the correlations of the phenological indicators with the chemical composition.

Phenological indices and chemical composition per cultivar at véraison

Cabernet Sauvignon: From Table 5.4 it is clear that no significant and weak correlations were seen for the iP_f with the ripeness indicators as well as with the organic acid indicators. The time of flowering therefore does not noticeably influence the berry composition, which can be expected, as the date of flowering does not differ that greatly between the sites, as seen earlier in this chapter.

A significant positive correlation was seen of the iP_v with the TSS, the pH and the mass per berry and the sugar per berry (not significant), while a significant negative correlation was seen with the TTA. A later date of *véraison* therefore results in a higher sugar content in the berry, in addition to a larger berry volume, while it results in a lower TTA content. This may be due to the larger volume of the berry, which results in a lower concentration of TTA. No correlation between berry volume and phenology has been seen in previous studies, however, as the climate and the irrigation regime are mostly responsible for berry volume.

When considering the organic acid indicators, the iP_v correlated significantly with the MA indicators. The timing of *véraison* therefore appears to have an influence on the MA content of CS at *véraison*, with a later date of *véraison* resulting in lower values. As the sampling was not performed on the true date of *véraison*, these correlations might not be a true indication of the effect of phenology on the organic acid content, as the sampling date might have been prior to or

after *véraison* and may include a time of synthesis or metabolism of MA. This is quite likely, as phenology and temperature are highly correlated and a later date of *véraison* indicates cooler temperatures prior to *véraison*, which, according to the literature, should result in higher MA values.

Sauvignon blanc: As seen for CS in Table 5.4, no significant correlations were seen between iP_f and the ripeness indicators. Unlike for CS, significant correlations also were not seen with the iP_v .

Again, a very weak correlation was seen between the TA content and both phenological indicators, therefore indicating that the date of phenology had little or no effect on the TA content per berry. A significant negative correlation was seen with the [TA], indicating that the earlier the date of phenology, the higher the concentration of TA. This may be explained by the positive, although not significant, correlation seen for the mass per berry, therefore indicating a trend that the earlier the date, the smaller the berry size and therefore the higher the [TA]. This is the most probable explanation, as the TA content per berry shows no correlation with either indicator. A negative significant correlation was seen between both phenological indicators and the rate of change of MA up to *véraison*, therefore indicating a lower rate of MA accumulation with a later date of *véraison* sampling. The metabolism of malic acid may already have started by the later sampling dates.

Phenological indices and chemical composition per cultivar at harvest

Cabernet Sauvignon: The iP_f , iP_v and iP_{cy} consistently correlated negatively with the TTA, the mass per berry and the sugar per berry. This correlation was only significant for the correlation of iP_v and iP_{cy} with the TTA, therefore indicating that the shorter the phenological time to *véraison* and for the complete cycle, the higher the acid content at harvest. It was found in previous studies that a shorter ripening period indicates a shorter period of time of MA degradation, resulting in a higher TTA. The climate during this time period obviously plays an important role in the rate of MA degradation, and therefore needs to be taken into account when interpreting this data.

Unlike other indicators, iP_v and iP_{cy} correlated positively and significantly with the TA content in the berries, therefore indicating that a later date of *véraison* and harvest ensured a higher TA content in the berry. This can be attributed to a longer period of synthesis in the berry prior to *véraison*, but does not explain the higher content at harvest. The TA content remains stable in the berry after *véraison*, therefore the positive correlation of a later harvest date with the TA content indicates a negative correlation with the berry volume. These correlations can be seen in Table 5.4, although the correlations are not strong or significant.

Sauvignon blanc: Regarding the ripening indicators of SB at harvest, inconsistent and low correlations were seen for all the indicators with all of the phenological indicators, except for TA content with iP_v and iP_{cy} (see Table 5.4). Therefore, the later the dates of flowering, *véraison* and the harvest, the higher the organic content per berry. Again, as seen for CS, the TA content per berry showed a significant positive correlation with the iP_v and the iP_{cy} . The same reason for this occurrence as for CS is applicable.

A positive correlation was seen for the rate of change of MA per day with all the indicators, indicating that a longer phenological time period implies a higher rate of change. This may be due to the fact that a longer phenological time period means more days at optimal hours for synthesis pre-*véraison*, or more hours above 30°C increasing the MA respiration rate post-*véraison*. As stated before, the climate during these periods therefore cannot be excluded from discussions.

TABLE 5.4

The correlation between certain berry composition components and phenological indices for the Cabernet Sauvignon (CS) and Sauvignon Blanc (SB) sites within the study area at *véraison* and harvest across all three vintages. Values indicated in bold italics correlate significantly, with $p < 0.1$.

			TSS ¹	pH	TTA ²	Mass/ berry	Sugar/ berry ³	[MA] ⁴	[TA] ⁴	MA/ berry ⁵	TA/ berry ⁵	MA per stage ⁶	MA rate per stage per day ⁷
<i>Véraison</i>	CS n = 23	iP _f ⁸	-0.12	0.05	0.07	-0.08	-0.05	-0.11	-0.26	-0.11	-0.20	-0.02	-0.21
		iP _v ⁹	0.63	0.74	-0.67	0.54	0.23	-0.39	-0.34	-0.39	-0.14	-0.46	-0.45
	SB n = 23	iP _f ⁸	0.35	0.29	-0.29	0.25	0.17	-0.05	-0.36	-0.05	0.00	-0.13	-0.41
		iP _v ⁹	0.27	0.19	-0.34	0.22	0.20	-0.01	-0.37	-0.01	0.23	-0.08	-0.44
<i>Harvest</i>	CS n = 22	iP _f ⁸	-0.25	0.06	-0.03	-0.21	-0.02	0.32	-0.32	0.25	0.28	0.32	-0.22
		iP _v ⁹	0.15	0.05	-0.48	-0.22	-0.28	0.12	-0.09	0.49	0.57	0.16	0.13
		iP _{cy} ¹⁰	0.25	0.02	-0.44	-0.11	-0.25	-0.04	0.01	0.31	0.37	0.00	0.17
	SB n = 20	iP _f ⁸	-0.29	0.26	0.05	-0.29	-0.14	0.32	0.26	0.36	0.37	0.38	0.29
		iP _v ⁹	-0.37	0.05	-0.24	-0.13	0.03	-0.03	0.39	0.30	0.58	-0.04	0.38
		iP _{cy} ¹⁰	-0.30	-0.12	-0.34	0.05	0.16	-0.23	0.35	0.15	0.52	-0.31	0.31

¹ Total soluble solids (^oBrix)

² Total titratable acidity (g/L)

³ The sugar per berry as calculated from the mass per berry and the TTS (mg/berry)

⁴ Malic and tartaric acid concentration (g/L)

⁵ Malic and tartaric acid content per berry, calculated from the [MA] and [TA] and the mass per berry (mg/berry)

⁶ Change in malic acid content from anthesis to *véraison* for *véraison*, and from *véraison* to harvest for harvest

⁷ Daily rate of change in the malic acid content from anthesis to *véraison* for *véraison*, and from *véraison* to harvest for harvest

⁸ *Indices de précocité* for flowering (Barbeau *et al.*, 1998b)

⁹ *Indices de précocité* for *véraison* (Barbeau *et al.*, 1998b)

¹⁰ *Indices de précocité* for the cycle (Barbeau *et al.*, 1998b)

5.3.8.3 Hourly thresholds and chemical composition

Although the optimal thresholds were determined according to the optimal temperatures for the synthesis of the organic acids up to *véraison*, it should be borne in mind that the optimal thresholds for synthesis also represent the optimal thresholds of photosynthesis up to *véraison* (Hunter & Bonnardot, 2011). The number of hours post-*véraison* represents the number of hours above 30°C, which is seen as the lower limit above which MA is actively respired.

Please refer to Table 5.5 for further discussion regarding the correlations of the hourly threshold indicators with the chemical composition.

Hourly thresholds and the berry chemical composition per cultivar at véraison

Cabernet Sauvignon: As for the climatic indicators, the number of hours within the optimal thresholds (or above in the case of post-*véraison*) (# hours) and the percentage of the number of hours in relation to the total number of hours (% hours) in relation to the thresholds, correlate negatively, and mostly significantly, with the TSS, pH, mass and sugar per berry, and positively with the TTA, as seen in Table 5.5. The positive significant correlation of both hourly indicators with the TTA is in accordance with the literature, as a higher number of hours within the optimal thresholds will ensure a higher content of organic acids, and mainly of MA, in the berry at *véraison*. Positive correlations were seen for the majority of the organic acid indicators with both hourly

indicators, as expected, except for the TA content per berry, which showed almost no, although a slight and negative, correlation (Table 5.5). As stated before, the TA content was influenced slightly or not at all by temperature, which was also seen in the correlation with the [TA].

A significant positive correlation was found for the number of hours with the MA content per stage and the daily rate of change of MA, which was not seen as strongly in the percentage of hours. This may indicate that the total number of hours spent at optimal temperatures was more important than the percentage of time within the season spent at these temperatures.

Sauvignon blanc: As can be seen in Table 5.5, the correlations of the hourly indicators with the ripening indicators generally are weak for SB. The same trend of correlation as in CS was seen for the correlation found with the number of hours in SB. When considering the organic acid indicators, the [TA] unexpectedly showed a significant positive correlation with the number of hours, and a significant negative correlation with the TA content per berry. There was a significant positive correlation between number of hours per stage and the MA rate per stage per day. Therefore, the higher the number of hours within the optimal threshold of synthesis, the higher the [TA], although it seems that the number of hours also had a positive influence on berry size, as the effect was negative on the TA content per berry.

Hourly thresholds and the berry chemical composition per cultivar at harvest

Cabernet Sauvignon: Significant positive correlations were seen for both hourly indicators and the pH, mass and sugar per berry. This is not in correlation with previous findings, as the rate of photosynthesis is not optimal at temperatures above 30°C (Hunter & Bonnardot, 2011) and therefore slows down the loading of sugar into the berry.

As discussed before, the hourly indicators showed little or no correlation with either the [TA] or the TA content at harvest, as temperature has little or no effect on the TA content of the berry.

A negative correlation, and significant for the percentage hours, was seen for the rate of change in MA per day, as expected. An increase in the number of hours above 30°C resulted in a more negative value for the rate of change, leading to a higher rate of degradation of MA. The same negative correlations were seen for the MA content per berry, therefore supporting this finding.

In comparison, a significant positive correlation was seen for the [MA] and number of hours per stage. This means that being exposed to more hours at $T > 30^{\circ}\text{C}$ was associated with a higher [MA]. As this was opposite to the MA content per berry, it can be assumed that it was due to a loss of berry volume under warmer conditions.

Sauvignon blanc: As seen for the correlations at *véraison*, the ripening indicators did not correlate significantly with either of the two hourly indicators. The hours above 30°C cannot be used as an indicator of photosynthesis or the size of the berry, as optimal photosynthesis is represented by differing thresholds, as noted for MA degradation, and the berry size was dictated by overall climate and the irrigation regime.

The TTA did, however, show a significant negative correlation with both hourly indicators, as seen in Table 5.5. Therefore, the greater the number of hours above 30°C, the lower the TTA, which is in accordance with previous studies, as temperatures above 30°C increase the rate of respiration of MA and therefore lower the TTA. The same trend was seen for the remainder of the MA indicators, although the correlations were not always strong. The rate of MA respiration would therefore appear to increase with the number of hours above 30°C.

Although the [TA] correlated significantly negatively with the number of hours, it has been shown before that temperature does not influence the TA content between *véraison* and harvest. This may be due to fluctuations in the berry volume and not the TA content, which in turn is related to temperature and irrigation scheduling. This negative correlation is seen for the TA content, but the correlations, as seen for CS, are very weak with the percentage hours.

TABLE 5.5

The correlation between certain berry composition components and hourly thresholds for the Cabernet Sauvignon (CS) and Sauvignon Blanc (SB) sites within the study area at *véraison* and harvest across all three vintages. Values indicated in bold italics correlate significantly, with $p < 0.1$.

			TSS ¹	pH	TTA ²	Mass/ berry	Sugar/ berry ³	[MA] ⁴	[TA] ⁴	MA/ berry ⁵	TA/ berry ⁵	MA per stage ⁶	MA rate per stage per day ⁷
<i>Véraison</i>	CS n = 23	# hours/stage ⁸	-0.76	-0.60	0.55	-0.70	-0.42	0.33	0.21	0.33	0.29	0.54	0.44
		% hours/stage ⁹	-0.62	-0.54	0.51	-0.53	-0.21	0.24	0.09	0.24	-0.02	0.33	0.19
	SB n = 23	# hours/stage ⁸	-0.21	-0.09	0.32	-0.20	-0.13	0.14	0.40	0.14	-0.01	0.15	0.44
		% hours/stage ⁹	0.12	0.10	0.04	0.10	0.08	-0.20	0.28	-0.20	-0.47	-0.23	0.24
Harvest	CS n = 22	# hours/stage ⁸	0.06	0.36	0.14	0.64	0.46	0.43	-0.04	-0.43	-0.15	0.34	-0.36
		% hours/stage ⁹	-0.13	0.53	-0.27	0.59	0.57	0.36	0.00	-0.36	0.10	0.23	-0.37
	SB n = 20	# hours/stage ⁸	0.22	0.04	-0.41	0.21	0.08	-0.05	-0.45	-0.65	-0.35	-0.07	-0.48
		% hours/stage ⁹	-0.11	-0.11	-0.44	0.17	0.19	-0.32	0.04	-0.29	0.20	-0.41	-0.02

¹ Total soluble solids (^oBrix)

² Total titratable acidity (g/L)

³ Sugar per berry as calculated from the mass per berry and the TTS (mg/berry)

⁴ Malic and tartaric acid concentration (g/L)

⁵ Malic and tartaric acid content per berry, calculated from the [MA] and [TA] and the mass per berry (mg/berry)

⁶ Change in malic acid content from anthesis to *véraison* for *véraison*, and from *véraison* to harvest for harvest

⁷ Daily rate of change of the malic acid content from anthesis to *véraison* for *véraison*, and from *véraison* to harvest for harvest

⁸ Number of hours per threshold per stage (*véraison* (1 November to 31 December): 20-25°C between 06:00 and 18:00 and 10-15°C between 18:00 and 06:00; harvest (1 January to 29 February): above 30°C)

⁹ Percentage of the number of hours per threshold of the total number of hours per stage

5.3.9 Variation in organic acid between sites within vintages

Following the discussions regarding the differences between vintages in the preceding chapter, the data was subsequently analysed between sites per vintage to exclude the effect of the variation between vintages from the analyses. Due to the added effect of cultivar on the organic acid content, as discussed previously, the data was again separated per cultivar.

As a reminder, all data connected to the TA content for AMP SB, DEHSB, HEKSB, LACS, MOISB, MOCS, RECS and SWCS in V1 at *véraison* clearly showed unrepresentative values, as discussed in Chapter IV, and subsequently was removed from all analyses in Tables 5.6 and 5.7.

As TA does not change significantly according to changes in temperature (Kliwer, 1964; Buttrose *et al.*, 1971; Coombe, 1987; Terrier & Romieu, 2001), the values for TA at *véraison* and harvest were not discussed according to temperature differences between sites, but mainly according to phenological differences. The main factors that greatly influenced the TA content at *véraison* and harvest were, firstly, the freezing of the samples of all the vintages at *véraison*, which can explain the difference of up 25% in the TA content as a result of precipitation due to salinification (García *et al.*, 2011). In addition, the K⁺ content can influence the values at both *véraison* and harvest.

Although the salt of TA with K^+ is not readily found in the berry due to the compartmentalisation of these two elements in the berry (DeBolt *et al.*, 2004), the salt will be able to form with the breaking of the berry during the preparation of the juice for analyses (Keller, 2010). The influence of K^+ is greater for MA than for TA, however, as it acts as an inhibitor against the degradation of MA during ripening (Keller, 2010). As the K^+ content was not included as an indicator in the study, only suppositions can be made.

5.3.9.1 Cabernet Sauvignon

From Table 5.6 it is clear that the TA content at *véraison* did not differ significantly between the sites in all three vintages. This is in accordance with the findings of Ruffner *et al.* (1976). The TA content did differ between sites at harvest, even though the TA content was supposed to remain relatively stable from *véraison* to harvest (Ruffner, 1982a; Iland & Coombe, 1988; Kanellis & Roubelakis-Angelakis, 1993). As noted before, this could be because the date of sampling was prior to the date of *véraison*, at which point TA synthesis is arrested, therefore explaining the lower TA content at *véraison* than at harvest. This occurrence could also be attributed to other physiological factors that were not discussed in this study, however, and needs further investigation.

Furthermore, the content at *véraison*, or the number of days between *véraison* and harvest, does not dictate either the MA content at harvest or the rate of change in MA content. Neither can any significant trend be detected for SB, as seen in Table 5.7.

MA content at véraison

In V1, the variability in the MA at *véraison* was lower than the variation found in the other vintages. MOCS separated from the other CS sites, largely because of the earlier date of *véraison* of this site, as seen in Table 5.2. The lower value of MA for this site in V1 might be due to the date of sampling being later than the date of *véraison*, therefore indicating a period of time in which degradation of MA had already occurred (*véraison* noted as 2006/12/28 and sampling performed on 2007/01/03, with 10 hours of temperatures above 30°C noted for this period). In addition, it could also be due to a lower rate of MA biosynthesis during the pre-*véraison* period. This site, in comparison with the other sites, showed high levels of hydric constraint early in the season (data not shown), which may contribute to the lower MA content at *véraison*.

It is interesting to note the significantly higher content of MA on the date of the *véraison* sampling for LEICS and MMCS, as they are spatially and topographically grouped together and this could have influencing the temperature during the growth period. This is not the case, however, as there are great differences between the cumulative GDD for the GP between MMCS and LEICS, as seen in Fig. 5.4, indicating that other factors, e.g. the high incidence of leafroll virus in these two plots (not investigated in this study), could have contributed to the high values of MA in these sites. Other factors contributing to a higher content of MA at *véraison* may be that similar high-vigour canopies and similar high soil water-holding capacities indirectly result in bigger canopies, which induce shaded conditions and change the bunch microclimate.

The same trend was noticed in V2, for similar reasons as those presented for V1, except that MMCS and LEICS group with the majority of the sites. It was also evident that the SD for the MA and TA content at *véraison* in V2 showed greater variability within the sites. This may be an indication that variability between the plots within the site was more pronounced in this vintage due to factors such as vigour differences, which might have been more pronounced due to water

availability per site during this vintage. Topography (water flow or logging) and soil properties, relating to soil water-holding capacity and soil drainage, therefore also could have contributed to these differences between the plots in this vintage. This is most probably the reason for these differences, as V2 was described in Chapter IV as being very wet, therefore indicating higher levels of available ground water. In addition, the grouping of MMCS and LEICS may indicate that the symptoms of leafroll virus were less pronounced earlier in this season, due to less hydric constraint experienced by the sites in this vintage.

V3, however, showed great variability between sites for the MA content at *véraison*. The same trend as in V1 and V2 was noticed for MOCS, with the same explanation, although RECS and SWCS also had low values for MA at *véraison*. This could be attributed to the longer time periods between anthesis and *véraison*, but also to the highest values for the cumulative GDD for this period, as shown earlier in the chapter. Furthermore, the later date of *véraison* may indicate, as for RUCS, that the samples were taken before the cessation of MA synthesis. These sites (RECS, SWCS and RUCS) had a great number of optimal threshold hours, especially night temperatures, indicating optimal synthesis of MA and hence supporting the argument that these samples might have been taken prior to the cessation of MA. These are not the only indicators for MA synthesis, however, and additional factors not discussed in this study, such as virus infection in the case of RUCS, might have contributed to these low values. Other factors could include waterlogging of the sites, specifically RECS and SWCS, due to the soil characteristics (data not shown), therefore decreasing the soil temperature during the growth period.

MA content at harvest

When the final content of MA at harvest is considered, the variability of *véraison* does not follow through and the grouping of the sites changes. The MA content per site furthermore does not correlate (data not shown) with either the number of days or the temperature during this period, as the initial MA content at *véraison* was not included in the correlation. The change in MA content between *véraison* and harvest must therefore be studied to further interpret the differences between the sites.

Of interesting is that MMCS was consistently at the lower end of the MA content at harvest, and consistently at the upper end of the rate of change of the MA content. This site was classified as one of the cooler CS sites according to the HI and the number of hours above 30°C (showing especially low values in V2), therefore indicating another factor contributing to the high rate of change during the ripening period. MMCS is a south-facing, dryland plot with a small canopy but a high percentage of shaded bunches (Table 2 in the addendum). The higher rate of change could therefore be attributed to higher berry temperatures in relation to the ambient temperature, as the bunches were not exposed directly to the cooling effect of the sea breeze as recorded by the logger. In addition, leafroll virus was found in this site.

V3 showed clearly higher values for all the sites in relation to the remainder of the sites for the MA content at harvest, even though the rate of change did not differ greatly from V2.

Change in MA content at harvest

Again, as for the values of MA in MOCS at *véraison*, the change in MA in V1 (six days) and V2 (five days) was small in comparison to the remainder of the sites. As previously explained, this was due to the sampling date being after the actual date of *véraison*. This fact is also confirmed by the early date of harvest of this site in relation to the remaining CS sites. As for *véraison*, LEICS and

MMCS showed a grouping in the change of MA in V1, and this can only be attributed to factors pertaining to locality, as discussed previously for *véraison*, as temperature and phenology did not show the same trend. As discussed before, the occurrence of leafroll virus in these sites could contribute to the rate of change in MA, as there were few other similarities (canopy, soil, yield, temperature) between these two sites.

The changes in MA in V2 seemed stable, except for MOCS as mentioned earlier, even though the values were marked as significantly different. The sites, except for RECS, were harvested during a short period of time (six days) after relatively the same number of days since *véraison* (around 56 days). The homogeneity of the data was not seen in the cumulative GDD for this period, with values ranging from mean values of 763 for MMCS to 925 for LEICS. RECS was harvested two to three weeks after the harvest dates of the remaining sites.

Vintage 3 was noted as a very warm harvest period for CS according to the GDD accumulation during the ripening period in relation to the remaining vintages. The value for MOCS was in relation to the values for the remaining CS sites, as the date of *véraison* and the date of sampling were similar in this vintage (0 days, data not shown), therefore excluding a period of MA degradation. In this vintage, SWCS had the lowest value of change for MA, which could be attributed to this plot having a relatively low MA content at *véraison* in relation to the remainder of the CS sites, most probably due to unfavourable conditions for MA synthesis (i.e. high GDD accumulation). In addition, the low rate of MA change, even though the final MA content at harvest did not vary from that of the remainder of the sites, cannot be attributed to either the number of days or the temperature during the ripening period, therefore indicating a possible buffering of MA degradation or more optimal canopy conditions during this vintage.

TABLE 5.6

Variability in the TA and MA per berry between sites per vintage (n = 8) for CS at *véraison* and harvest, and the rate of change in MA content between *véraison* and harvest, for V1 (2006/2007), V2 (2008/2009) and V3 (2009/2010). In addition, the number of days between *véraison* and harvest (# days) is noted.

		# days	TA content		MA content		
			<i>Véraison</i>	Harvest	<i>Véraison</i>	Harvest	Rate of change
V1	LACS	47	-	13.70 ± 1.77 b	12.12 ± 3.61 c	4.00 ± 0.30 ab	8.13 ± 3.53 bc
	LEICS	64	13.30 ± 1.52 a	10.69 ± 0.84 bcd	23.56 ± 4.19 b	4.52 ± 0.20 b	19.37 ± 6.07 a
	MMCS	53	12.62 ± 0.60 a	18.55 ± 3.49 a	21.97 ± 0.42 b	1.55 ± 0.68 c	20.42 ± 0.44 a
	MOCS	48	-	14.00 ± 1.60 b	6.28 ± 1.07 a	1.46 ± 0.12 c	4.82 ± 1.14 b
	RECS	55	-	9.44 ± 2.86 cd	15.86 ± 0.44 c	4.53 ± 0.45 b	11.33 ± 0.38 c
	RUCS	49	11.89 ± 0.87 a	12.08 ± 1.33 bc	14.67 ± 2.28 c	2.74 ± 1.31 ac	11.94 ± 1.43 c
	SWCS	54	-	7.95 ± 0.93 d	14.02 ± 2.56 c	1.81 ± 0.62 c	12.21 ± 3.12 c
	WATCS	55	12.84 ± 3.38 a	10.20 ± 0.61 cd	14.44 ± 1.69 c	3.56 ± 1.63 ab	10.88 ± 2.81 c
V2	LACS	41	9.51 ± 1.10 a	10.36 ± 0.51 ac	14.47 ± 1.78 ab	3.86 ± 0.27 c	10.62 ± 2.04 abc
	LEICS	54	9.97 ± 1.70 a	9.98 ± 0.75 ab	20.07 ± 4.42 b	5.96 ± 0.67 a	14.11 ± 5.08 c
	MMCS	47	9.46 ± 1.27 a	11.59 ± 0.10 c	17.05 ± 4.48 b	3.66 ± 1.11 c	13.39 ± 3.39 ac
	MOCS	42	8.20 ± 2.88 a	8.67 ± 0.19 b	8.05 ± 0.39 a	1.72 ± 0.24 b	6.32 ± 0.17 b
	RECS	58	9.88 ± 3.42 a	11.82 ± 0.93 c	15.65 ± 5.14 b	3.17 ± 0.66 c	12.48 ± 5.01 abc
	RUCS	35	9.50 ± 0.50 a	11.82 ± 0.62 c	15.34 ± 1.97 b	1.41 ± 1.13 b	13.94 ± 2.88 c
	SWCS	52	8.63 ± 0.93 a	11.88 ± 1.94 c	13.82 ± 1.64 ab	6.32 ± 0.57 a	7.50 ± 2.18 ab
	WATCS	49	8.57 ± 1.54 a	11.00 ± 0.79 ac	16.65 ± 6.27 b	5.12 ± 2.83 ab	11.53 ± 4.70 abc

TABLE 5.6 (cont.)

			TA content		MA content		
		# days	Véraison	Harvest	Véraison	Harvest	Rate of change
V3	LACS	49	8.34 ± 0.64 a	12.56 ± 0.33 b	20.92 ± 1.23 a	5.70 ± 0.75 a	15.22 ± 0.48 b
	LEICS	57	7.75 ± 2.02 a	11.67 ± 0.50 bc	17.39 ± 2.81 abc	2.84 ± 0.84 b	14.55 ± 3.12 b
	MMCS	56	7.71 ± 0.05 a	11.45 ± 0.58 bc	18.99 ± 1.38 ab	3.70 ± 0.29 ab	15.29 ± 1.10 b
	MOCS	43	7.38 ± 0.69 a	11.30 ± 0.35 abc	14.02 ± 1.76 cd	2.96 ± 1.18 b	11.06 ± 1.49 ab
	RECS	54	8.11 ± 0.78 a	10.07 ± 0.78 a	13.38 ± 5.02 cd	3.05 ± 1.63 b	10.34 ± 6.60 ab
	RUCS	45	8.58 ± 0.96 a	11.59 ± 0.66 bc	16.65 ± 0.65 bcd	5.11 ± 0.42 ab	11.54 ± 0.94 ab
	SWCS	53	7.47 ± 0.44 a	11.85 ± 1.54 bc	13.37 ± 0.35 d	4.84 ± 1.93 ab	8.53 ± 1.92 a
	WATCS	52	7.73 ± 0.14 a	13.70 ± 1.77 b	19.82 ± 1.64 ab	4.00 ± 0.30 ab	

¹Values designated by different letters within each column per vintage differ significantly ($p \leq 0.05$) per organic acid

5.3.9.2 Sauvignon blanc

When considering the values of MA and TA, there is greater variability between the values per site for SB than was noticed for CS, as shown in Table 5.7. SB shows great variability with changes in temperature due to location, which could contribute to this occurrence (as seen earlier in Chapter V and in Chapter IV) The values of MA at *véraison* and harvest per site are clearly higher than those of CS. The higher values at *véraison* could be attributed to the cultivar (Terrier & Romieu, 2001) and the fact that SB is usually planted in cooler sites at higher altitudes, therefore having an increased number of hours within the thresholds for optimal synthesis of MA. The higher values at harvest were expected, as the period of time between *véraison* and harvest was shorter than that for CS, therefore indicating a shorter period of time for MA degradation. The general selection of cooler sites for the planting of SB as discussed for *véraison* could also contribute to these higher values.

Of interest are the high values for TA at harvest in V1, which was noted for CS, but to a lesser extent than for SB.

MA content at véraison

At *véraison* in V1 there was a separation between ADWSB and RUSB and the remainder of the SB sites, and to a lesser degree for DEHSB. Interestingly, these three sites also group in relation to the FNI, the MFT and the HI in Fig. 5.2 (A). It therefore seems that the differences in the MA content between sites in V1 were mainly driven by temperature differences induced by the topographical attributes (aspect, altitude). SWSB, on the contrary, showed the lowest value during V1, which can be attributed to the fact that the date of sampling preceded the actual date of *véraison* (five days), therefore indicating the possible degradation of MA prior to sampling. In addition, a low number of optimal hours during pre-*véraison*, combined with a high number of hours of temperatures above 30°C, could have contributed to the low rate of synthesis, in combination with a high rate of metabolism.

During V2, the MA content was relatively constant in all the sites, except for AMP SB, which had a high value in relation to the remainder of the SB sites (could be due to viticultural practices or increased vigour; see Table 2 in the addendum), and RUSB, which had a low value in relation to the other sites. This can be attributed to the date of sampling being prior to the date of *véraison* (16

days), as RUSB was phenologically classified as a late site (Table 5.2), therefore indicating that the grapevine was still in an active period of MA synthesis at the time of sampling.

The MA content per site at *véraison* in V3 differed greatly, as also seen for CS. The high value for LIBSB and DEHSB in relation to the remaining SB plots can also not be explained according to the climate or location, except that LIBSB and DEHSB were highly vigorous sites (data not shown), which could have contributed, firstly, to a change in the microclimate in the canopy (Smart, 1992) and secondly, as noted by Smart *et al.* (1985) and Terrier & Romieu (2001), high-vigour vines with high yields lead to high MA concentrations at harvest.

MA content at harvest

When the values of the MA content at harvest are considered, the variability of *véraison* does not follow through and the grouping of the plots changes. As noted for CS, the change in the content of MA at harvest needs to be investigated to be able to interpret the differences between the sites, as the MA content at *véraison* differs greatly and the initial value before the start of breakdown of MA after *véraison* is not included in the correlation of the dataset at harvest.

Change in MA content at harvest

In V1, the low mean value for SWSB was repeated, as seen at *véraison*, reconfirming the onset of MA degradation at *véraison* prior to the date of sampling. The effect of the locality of RUSB was also reconfirmed with a high value of MA change due to the long period of time between *véraison* and harvest. Despite the big lapse in time, the final MA content at harvest was still high in relation to the remainder of the SB sites, explained by the topography of the site (high altitude, southern aspect) inducing low temperatures during the ripening period.

V2 showed no significant differences between the four sites included in the ANOVA when considering the MA content at harvest. The MA change at harvest was in accordance with the values measured at *véraison*, so little variability was seen at harvest. Due to the small number of data points, however, no clear interpretations can be made for this vintage.

For the MA content and change of MA at harvest in V3, a clear separation between the sites was again noticed, as for *véraison*. Unlike the remainder of the vintages, V3 showed significant variation in the MA content at harvest between the SB sites, which cannot be explained only by the rate of change of MA. The slow rate of change, with the resulting high value for MA content at harvest for ADWSB, can be explained by the locality and topography of this site and the influence of this on the climate (southern aspect, high altitude and distance from the ocean). LIBSB and HEKSB, in comparison, had high rates of MA change, resulting in a higher MA content at harvest in relation to the MA content at *véraison*. This can be attributed to the high HI in the ripening period, in addition the large number of hours above 30°C for these two sites in relation to the remainder of the sites in V3 (Fig. 5.6). Of interest is the short ripening period of only 26 days for SWSB in contrast to the remainder of the sites.

The variability in SB can be explained according to locality, as ADWSB and RUSB are considered cool sites due to their aspect and exposure to the sea breeze. The low value detected for SWSB can be attributed to the date of sampling being five days after the date of *véraison*, indicating the starting point of MA degradation. As for MOCS, if the starting value of the MA content was already low in relation to the remainder of the sites, this value will be even more noticeable.

TABLE 5.7

Variability in TA and MA per berry between sites per vintage (n = 8) for SB at *véraison* and harvest, and the rate of change in MA content between *véraison* and harvest for V1 (2006/2007), V2 (2008/2009) and V3 (2009/2010). In addition, the number of days between *véraison* and harvest (# days) is noted.

		# days	TA content		MA content		
			<i>Véraison</i>	Harvest	<i>Véraison</i>	Harvest	Rate of change
V1	ADWSB	28	11.39 ± 2.57 ab	23.38 ± 2.21 cd	21.90 ± 2.13 d	8.32 ± 0.54 b	13.57 ± 2.54 ac
	AMPSB	39	-	22.65 ± 3.18 cd	16.47 ± 4.65 bcd	6.17 ± 0.36 ab	10.30 ± 4.45 cd
	DEHSB	26	-	12.07 ± 0.44 b	19.98 ± 4.58 bd	8.51 ± 1.03 b	8.94 ± 0.79 cd
	HEKSB	32	-	19.55 ± 6.58 ad	13.73 ± 6.78 abc	6.60 ± 2.87 ab	7.13 ± 4.35 bd
	LIBSB	24	15.18 ± 3.57 b	25.65 ± 1.14 c	16.56 ± 2.42 bcd	8.11 ± 0.38 b	8.46 ± 2.75 cd
	MOISB	31	-	10.59 ± 0.94 b	11.87 ± 5.10 ac	5.12 ± 1.98 a	7.01 ± 3.53 bd
	RUSB	28	15.86 ± 3.12 b	15.71 ± 3.60 ab	23.17 ± 2.00 d	7.11 ± 1.23 ab	17.02 ± 4.50 a
	SWSB	26	8.66 ± 2.46 a	23.51 ± 1.60 cd	7.4 ± 2.26 a	4.86 ± 1.50 a	1.38 ± 1.40 b
V2	ADWSB	42	11.48 ± 0.26 d	11.04 ± 0.82 b	17.72 ± 0.55 bc	7.88 ± 0.79 a	9.84 ± 0.35 ac
	AMPSB	41	9.41 ± 2.29 a		25.30 ± 1.93 a		
	DEHSB	24	12.88 ± 0.71 cd	10.19 ± 0.57 bc	20.33 ± 1.83 b	8.51 ± 0.99 a	11.82 ± 1.51 a
	HEKSB	32	13.64 ± 0.79 c	13.67 ± 0.38 a	18.25 ± 3.92 bc	8.58 ± 3.98 a	9.67 ± 0.18 ac
	LIBSB	29	11.23 ± 0.60 ad	10.81 ± 0.57 bc	16.34 ± 1.26 bc	8.35 ± 0.75 a	7.99 ± 1.92 bc
	MOISB	31	6.24 ± 0.11 b	9.27 c	18.94 ± 0.82 bc	10.47 a	8.22 bc
	RUSB	22	12.30 ± 0.71 cd	11.12 bc	15.85 ± 3.77 c	5.94 a	5.56 b
	SWSB		5.78 ± 1.60 b		18.95 ± 3.67 bc		
V3	ADWSB	38	8.32 ± 1.46 ab	14.44 ± 1.67 a	20.36 ± 1.71 cde	10.55 ± 1.11 bc	9.80 ± 2.78 b
	AMPSB	47	10.31 ± 1.04 bc	12.43 ± 2.18 a	22.86 ± 2.99 cd	7.56 ± 0.43 d	15.30 ± 3.39 ab
	DEHSB		12.61 ± 0.42 c		31.02 ± 1.38 ab		25.80 ± 3.96 a
	HEKSB	44	11.41 ± 2.39 c	12.58 ± 3.36 a	24.64 ± 7.58 bc	4.90 ± 1.91 a	14.97 ± 12.44 ab
	LIBSB	31	12.04 ± 0.31 c	14.23 ± 0.71 a	31.06 ± 2.09 a	11.48 ± 2.10 b	22.11 ± 2.44 a
	MOISB	33	8.17 ± 2.00 ab	11.14 ± 0.36 a	12.65 ± 3.10 f	8.58 ± 0.23 cd	8.82 ± 3.08 b
	RUSB	38	9.99 ± 0.37 bc	13.10 ± 0.48 a	16.90 ± 2.65 def	3.83 ± 0.17 a	4.10 b
	SWSB	26	6.64 ± 2.23 a	11.84 a	15.04 ± 1.60 ef	9.75 bcd	

^a Values designated by different letters within each column per vintage differ significantly ($p \leq 0.05$) per organic acid

5.4 Conclusions

It is known from previous studies reported in the literature and the analyses done in this study that there is clear climatic variation not only between vintages, but also within vintages, according to the location of the site. These climatic differences can be attributed mainly to the diverse topography of the study area, which changes rapidly over short distances and thereby influences the mesoclimate per site. In addition, the occurrence of the sea breeze and the influence thereof on the site according to the topography and the distance from the ocean, largely contributes to these differences. It therefore is important when characterising the climate of a vineyard to assess data on a mesoclimatic scale.

Mesoclimate is a measurement at one point in the vineyard as representation of the ambient temperature. The degree of variability in temperature of the parts of the vine within the site, however, is determined by certain conditions, which include the ambient temperature, the soil moisture status and wind conditions. In addition, the canopy architecture influences the

microclimate, which in turn influences the organ temperature. Light intensity and exposure should not be excluded, however, as exposed berries and other organs are warmer than shaded organs. Therefore it must be borne in mind that this study was done on ambient temperature and that the temperature of the berries, depending on the position of the berry within the bunch and the exposure of the bunch, may be higher.

In the study area, the main driver for the differences between vintages was noted as the mean February temperature. This may be due to the occurrence of heat waves during the ripening period, of which the time of occurrence, the intensity and the duration differ greatly between vintages. The differences between sites within the vintages were driven mainly by the fresh night index and the heliothermal index, confirming that the differences in temperature can be attributed mainly to the topography of the site and the influence of the sea breeze.

However, these indices and parameters are summated values or mean temperatures for a time period, and not a true representation of the kinetics of temperature per site per vintage, and therefore need to be considered when interpreting data, especially regarding the content of compounds that had clear synthesis and breakdown periods within the period of monitoring. This is true for the organic acids discussed in this study, as both tartaric and malic acid are synthesised up to *véraison*, after which the content of tartaric acid remains constant and the content of malic acid declines due to respiration. When examining the temperature data for the pre- and post-*véraison* periods, the temperature variability within vintages per site become even more apparent, and it therefore is more appropriate to separate all data into periods of synthesis and breakdown. This is not only applicable to the organic acids, but also to other metabolic compounds found in the grape berry of which the synthesis and/or breakdown are influenced by temperature.

All biological systems have temperature thresholds according to which the system operates optimally and metabolic compounds are produced in optimum quantities. The same is applicable to TA and MA synthesis, as discussed in this chapter. These reactions are mainly enzyme driven and the temperature thresholds are according to the optimal temperatures at which the applicable enzymes will be active per reaction. As the enzymes for TA synthesis are as yet unknown, the optimal temperature thresholds are also not known. For this reason, and from reports in the literature that TA shows little or no effect in relation to temperature, this chapter focused on MA synthesis and breakdown, according to the optimal thresholds for MA synthesis during the pre-*véraison* period and MA breakdown post-*véraison*.

From the results of the threshold data, it seems that the summation of the hours according to the threshold is the best indicator for MA synthesis and breakdown, as has also been found by other authors. In this study, the use as optimal hours as a definite indicator of the organic acid content has not been proven, however, due to the choices made regarding the sampling dates and the date of harvest. Future studies should focus on sampling dates being closer to the true date of *véraison* to ensure that the content of MA is at the optimum level after synthesis, and prior to the onset of ripening and the subsequent possible respiration of MA. The date of harvest is generally determined according to the sugar content of the berry. The cycles of loading sugar into the berry and the respiration of MA are not connected, however, as the temperature thresholds of these cycles differ greatly, and determining an optimal date of sampling for harvest therefore is difficult, as many factors in addition to the temperature influence both these cycles in the berry.

When considering the phenological differences between the sites, as these differences have an influence on the content of the organic acid due to the sampling dates, clear phenological differences exist between the sites per vintage. The same trends are however seen per vintage

where certain sites are consistently late or early. Phenology therefore has to be considered in relation to sampling dates. Of interest is that a clear, significant correlation existed between the temperature and the number of days per phenological time period, as seen in Chapter IV. This correlation was seen for the summated thermal units per day as calculated for the Huglin index. Also, even though the days per phenological stage may differ between sites, and therefore also the accumulated thermal units for that period, the mean thermal unit value per site was similar per vintage.

There was no clear correlation between the MA and TA content per site and per vintage and temperature, indicating that temperature alone cannot be used as an indicator of the organic acid content of berries, as other climatic indicators, soil attributes (contributing to the water status of the vine) and vegetative and reproductive indicators would also appear to play a role in the organic acid content of berries. These interactions are intricate and not yet understood completely. Biological systems contain complex processes, with many enzyme systems regulated by different temperature thresholds and other environmental factors within the system. It therefore is not possible to predict the metabolic product using only one aspect. In addition, the role of the phenology of the vine in biological systems must not be underestimated, as the length of the ripening period contributes greatly to the length of time that berries are exposed to specific temperature thresholds. There also was no clear trend between vintages for the determination of MA and TA, due to all the factors involved in the synthesis of MA and TA, and the degradation of MA and salinification of TA in the berries and the juice respectively.

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Chapter VI: General discussion and conclusions

6.1 Discussion and conclusions

The study was undertaken to determine if temperature was the main factor determining the content of malic and tartaric acid in the berries of Cabernet Sauvignon and Sauvignon Blanc at *véraison* and harvest. Due to the complex topography of the research area, resulting in changes of the climate over short distances, the topoclimatic variability of the research sites were firstly determined and in addition the extent of the climatic differences per vintage.

Great temperature variability was seen in this study, firstly between vintages in Chapter IV, and secondly between sites within vintages in Chapter V. The differences in temperature between sites were mostly driven by topographical diversity, e.g. aspect, altitude and the distance from the ocean, as was seen also seen in previous studies done within the study area. The topoclimatic diversity was furthermore driven by the exposure of the sites within the study area to the sea breeze. No correlation between one specific topographical aspect and the temperature of the site existed though, indicating that a combination of factors influence the temperature per site.

Temperature is however not the only environmental aspect defining the climate, and all climatic factors need to be taken into account when determining the content of a metabolic product in grapes. The climate, together with other external environmental aspects, is a complex system of interactions influencing the functioning and performance of the grapevine.

Temperature furthermore has an effect on the timing of the phenology of the grapevine, which in turn modifies the ripening kinetic of the plant. This, together with other vineyard aspects (canopy architecture, soil water availability, etc.) influences the final berry composition. When working with compounds that have a synthesis and metabolism period, the monitoring period needs to be determined according to these respective periods. Therefore when sampling, if logistically possible, the samples need to be taken as close as possible to true phenological dates or within the same time period, noting the true phenological dates. These phenological differences could be included in the correlation via a phenological index like the index of Barbeau (Barbeau *et al.*, 1998a; Barbeau *et al.*, 1998b) as used in this study.

The content of compounds within berries needs to be indicated on a per berry basis, as the concentration values are not a true representation of these values due to differences in berry volume which are mainly due to differences in the climate and the hydric status of the vines.

From the study it was clear though that due to the diversity of the landscape, defining the climate on a macroclimatic scale is not representative on a vineyard scale, and is, as was discussed by other authors (Smart & Dry, 1980; Jones & Davis, 2000), only useful to define the climate on a regional scale.

This study was however not undertaken to create a new index for the determination of correlations, but to verify the current available indices in the prediction of organic acids at *véraison* and harvest. It is however clear that the indices need to be adapted according to the outcome and research purposes.

Future studies should focus on better defining the temperature thresholds of the main berry composition components, as the use of optimal hours at certain temperatures seems to be the most applicable in the predictions of the content of the compounds. Where these compounds have metabolic periods according to the phenology of the grapevine, phenology needs to be included in the determination of the sampling dates.

Therefore, temperature can be used as an indicator of the malic and tartaric acid content of berries at *véraison* and harvest, specifically when using optimal hour thresholds for synthesis up to *véraison*, and for degradation of malic acid up to harvest. It must always be kept in mind though that other factors, i.e. the water status of the plant, the canopy architecture and the microclimate of the berry, brought on due to the high variability between the sites, in addition influence the final content of these organic acids at the key phenological stages.

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List of abbreviations

Abbreviation	Description
[MA]	Malic acid concentration
[TA]	Tartaric acid concentration
°C	Degree Celsius (temperature)
A-H	The period of anthesis to harvest
A-V	The period of anthesis to <i>véraison</i>
Acetyl-CoA	Acetyl coenzyme A
ANOVA	Analysis of variance
ARC	Agricultural Research Council
AWS	Automatic weather station
B-A	The period of budburst to anthesis
BV	The period of budburst to <i>véraison</i> – 1 September to 31 December
Ca ²⁺	Calcium
cm	Centimeter
CS	Cabernet Sauvignon
DEM	Digital elevation model
dH ₂ O	Distilled water
E-L	Eichhorn-Lorenz phenological classification system
FNI	Fresh night index
g	Gram
g/L	Gram per liter
GDD	Growing degree day
GDP	Guanosine diphosphate
GP	Growth period (1 October to 31 December)
GPS	Global positioning system
HI	Huglin index
iP _{cy}	Precocity index of the cycle for a site
iP _f	Precocity index of flowering for a site
iP _v	Precocity index of <i>véraison</i> for a site
ISCW	Institute for soil, climate and water
K ⁺	Potassium
KHT	Potassium hydrogen tartrate
km	Kilometer
L	Liter
LAB	Lactic acid bacteria
LLN	Leaf layer number
m	Meter
MA	L-malic acid
MDH	Malate dehydrogenase
ME	Malic acid enzyme
MFT	Mean February temperature (°C)
mg	Milligram
MJT	Mean January temperature (°C)
mL	Milliliter
MLF	Malolactic fermentation
mm	Millimeter
N	Mole
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
nm	Nanometer (spectroscopy wavelength)
OAA	Oxaloacetate
OIV	L'Organisation Internationale de la Vigne et du Vin
PC	Principal component

PCA	Principal Component Analyses
PEP	Phosphoenolpyruvate
PEPC	Phosphoenolpyruvate carboxylase
PEPCK	Phosphoenolpyruvate carboxykinase
PVPP	Polyvinylpyrrolidone
PQ	Point quadrat
R ²	Coefficient of determination
RP	Ripening period (1 January to 31 March)
SAWIS	South African Wine Industry Information and Systems
SB	Sauvignon Blanc
SD	Standard deviation
TA	L-tartaric acid
TCA	Tricarboxylic acid
T _{max}	Maximum temperature
T _{mean}	Mean temperature
T _{min}	Minimum temperature
TSS	Total soluble solids (% Brix)
TTA	Total titratable acidity (g/L)
TU	Thermal unit
V1	Vintage 1
V2	Vintage 2
V3	Vintage 3
V-H	The period of <i>véraison</i> to harvest – 1 January to 31 March
WI	Winkler index
WMO	World Meteorological Organisation

Addendum

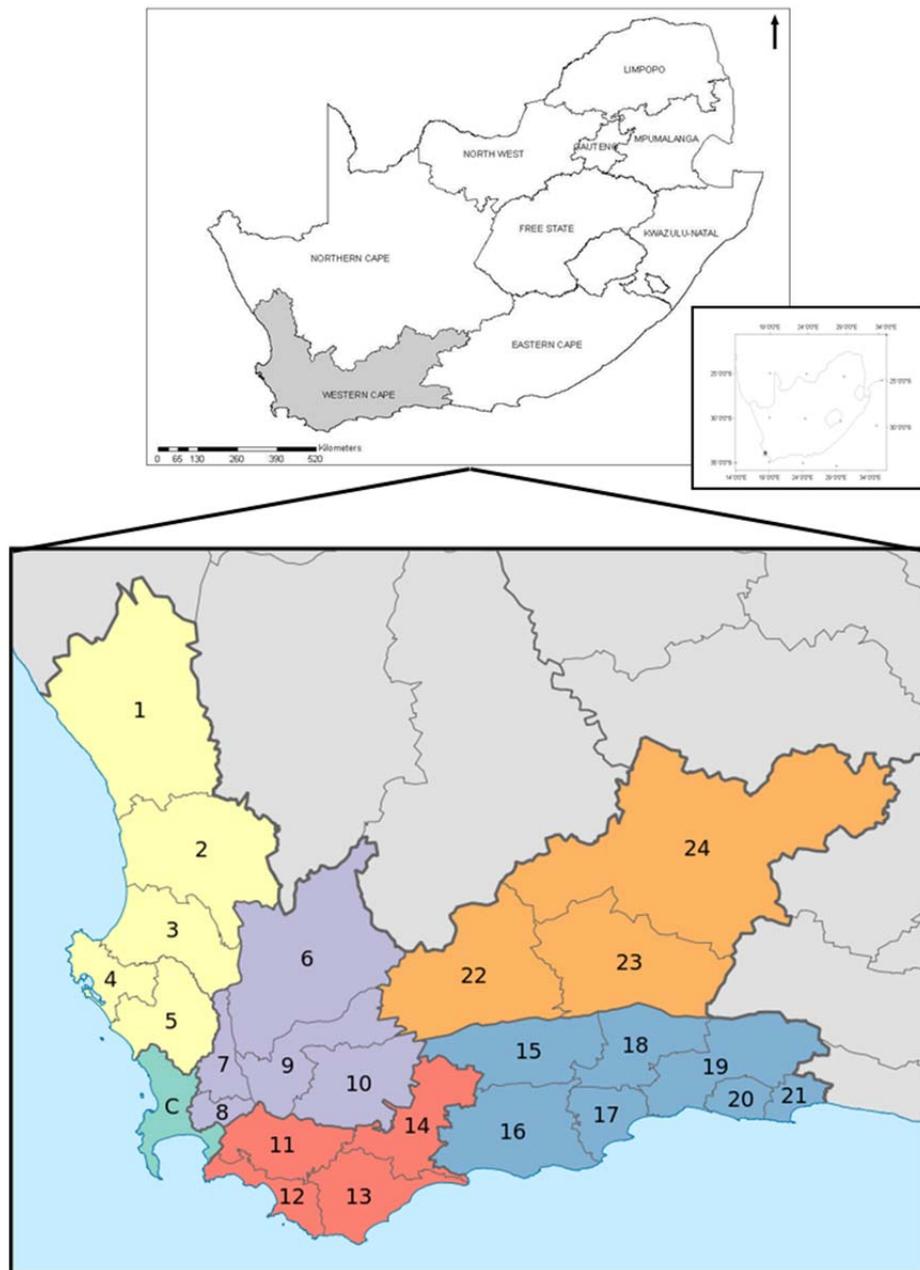


FIGURE 1

A map indicating the geographical location of the Western Cape within South Africa (top) and the municipalities within the Western Cape (bottom). The Stellenbosch Local Municipality is indicated as number 8 inside the area of the Cape Winelands Municipalities (numbered 6-10). Websites (<http://www.statssa.gov.za/>; <http://en.wikipedia.org/>) accessed 31 August 2012.

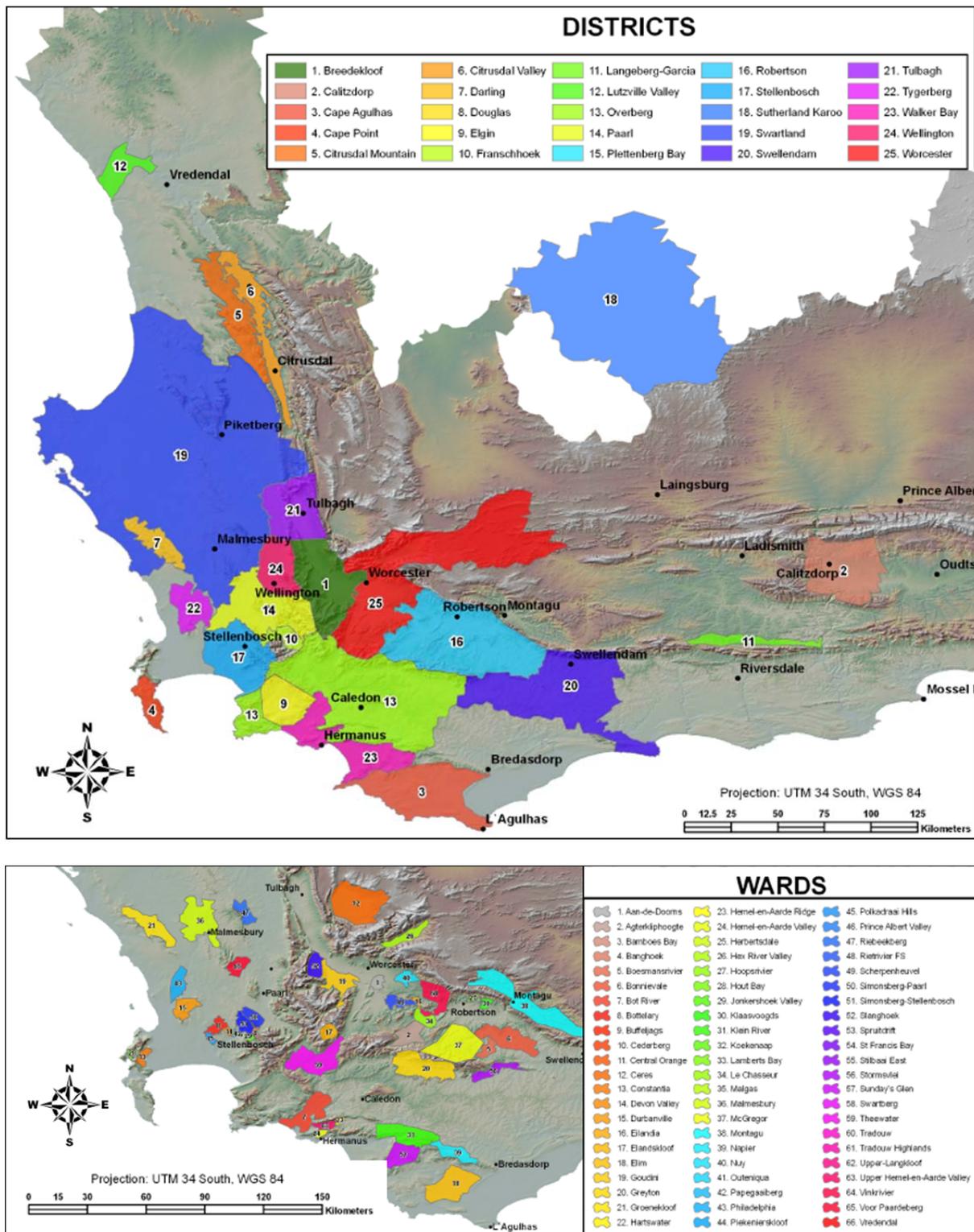


FIGURE 2

Maps indicating the wine production areas of South Africa according to districts (top) and the wards per district (bottom). On this map, Stellenbosch is indicated as district number 17 and includes the wards of Banghoek, Bottelary, Devon Valley, Jonkershoek Valley, Papegaaiberg, Polkadraai Hills and Simonsberg-Stellenbosch (www.wosa.co.za; accessed 29 August 2012).

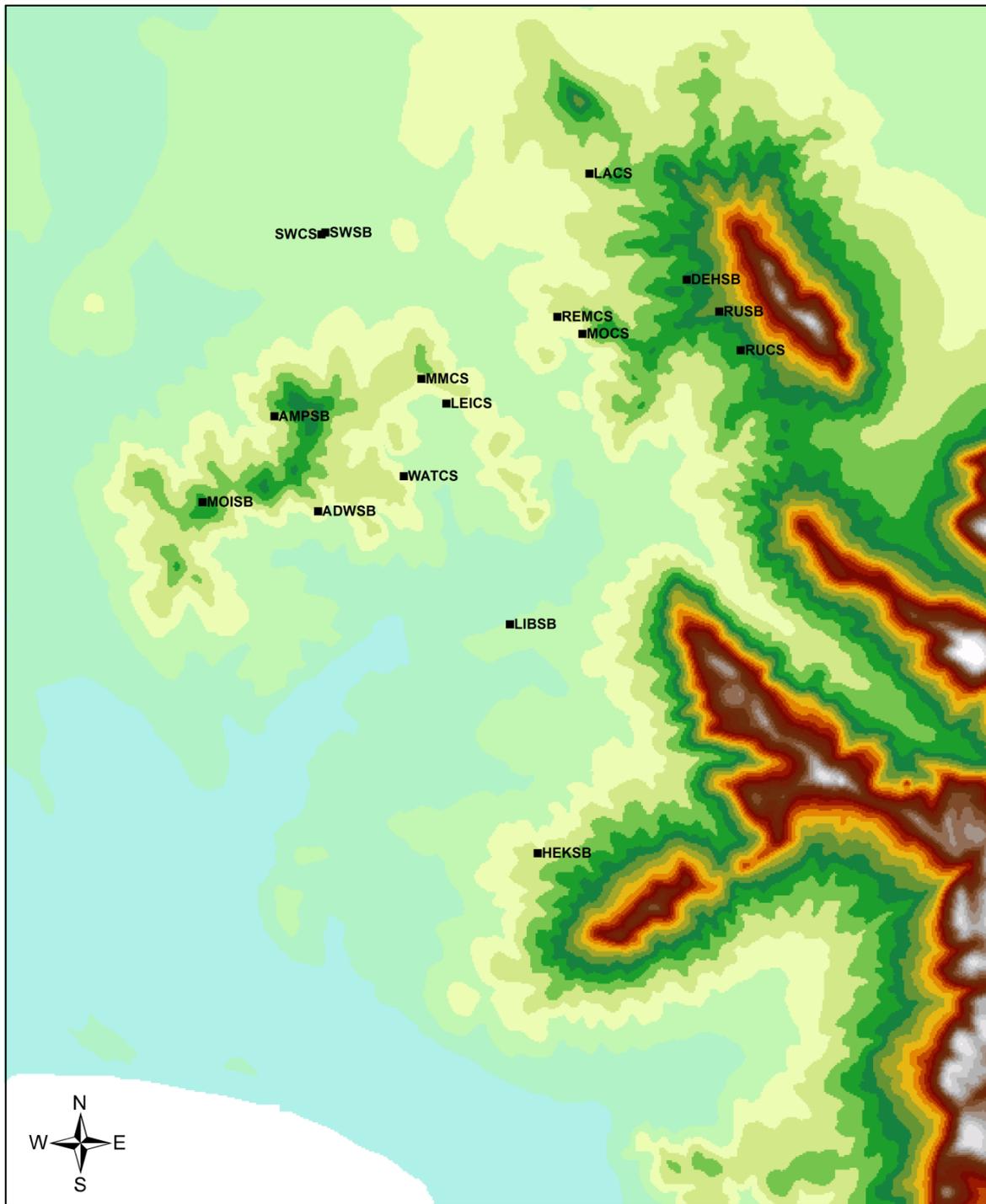


FIGURE 3

A digital data layer indicating the positioning of the 16 selected experimental sites within the area of interest, in addition indicating the topographical diversity through a 50 m digital elevation model as classified with ESRI® ArcMap™ 10.0 (digital data compiled from data obtained from National Geo-spatial information (NGI), Mowbray South Africa).

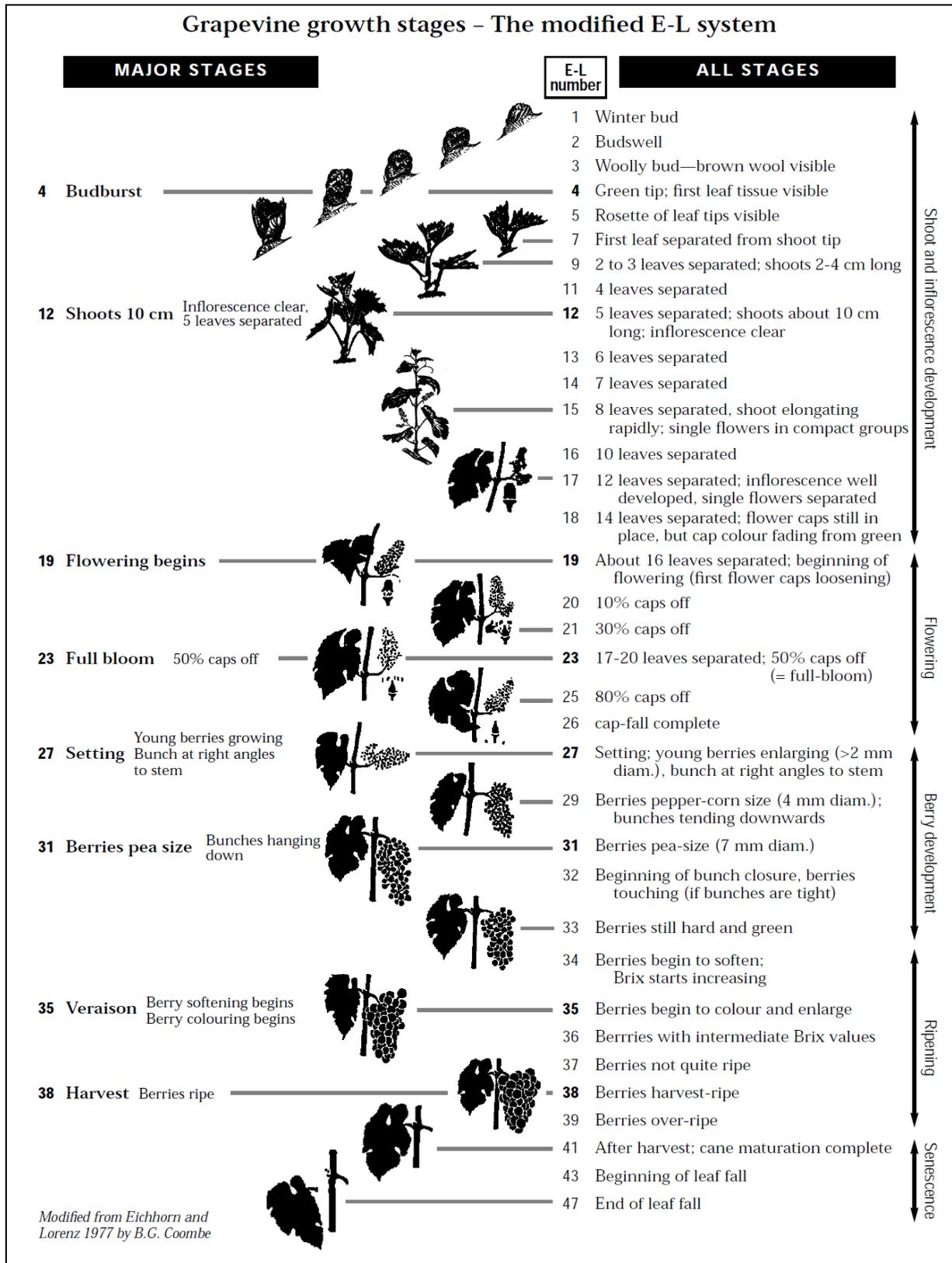


FIGURE 4

The E-L classification system of the phenological growth stages used in this study as modified by Coombe (1995) from the original system developed by Eichhorn and Lorenz (1977).

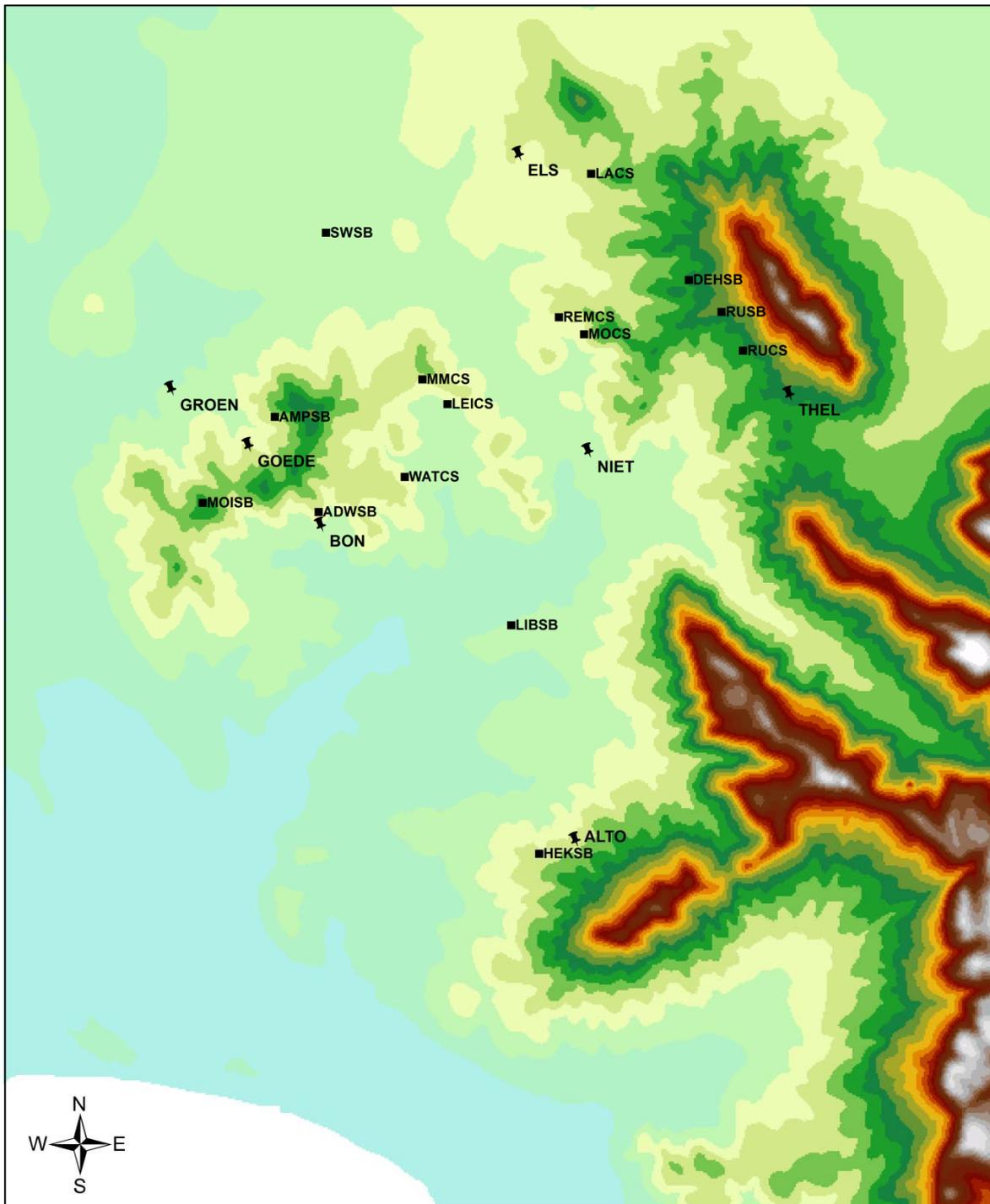


FIGURE 5

A digital data layer indicating the positioning of the datalogger network (indicated in black squares) and the automatic weather station network (indicated with a black pushpins) within the area of interest, in addition indicating the topographical diversity through a 50m digital elevation model as classified with ESRI® ArcMap™ 10.0 (digital data compiled from data obtained from National Geo-spatial information (NGI), Mowbray South Africa).

TABLE 1

The climatic parameters per experimental site (datalogger network data), including the climatic indices for the assigned weather station (AWS network) for vintage 1 (2006/2007), vintage 2 (2008/2009) and vintage 3 (2009/2010) and for Cabernet Sauvignon (Table A) and Sauvignon Blanc (Table B).

A	Site	Vintage	AWS ¹	Logger WI ²	Logger HI ³	AWS WI ⁴	AWS HI ⁵	Logger FNI ⁶	Logger MJT ⁷	Logger MFT ⁸	AWS FNI ⁹	AWS MJT ¹⁰	AWS MFT ¹¹	Winter Rainfall ¹²	Season rainfall ¹³
	LACS	1	Els	2116	2765	1868	2348	14.8	23.6	21.6	14.7	22.1	20.5	619	95
	LACS	2	Els	-	-	1817	2382	16.4	22.0	23.2	15.7	20.8	22.4	562	48
	LACS	3	Els	2096	2687	1913	2432	15.9	22.2	22.0	15.6	21.6	21.6	711	34
	LEICS	1	Bon	2069	2688	1991	2313	14.7	23.6	21.5	16.7	22.6	21.0	735	112
	LEICS	2	Bon	2027	2701	1925	2348	15.5	21.7	23.1	17.1	21.2	22.5	845	50
	LEICS	3	Bon	2140	2785	2004	2376	15.5	22.8	22.4	17.3	21.7	22.0	741	36
	MMCS	1	Bon	2039	2571	1991	2313	15.4	23.5	21.3	16.7	22.6	21.0	735	112
	MMCS	2	Bon	1974	2554	1925	2348	16.7	21.6	22.9	17.1	21.2	22.5	845	50
	MMCS	3	Bon	2058	2611	2004	2376	16.4	22.4	22.0	17.3	21.7	22.0	741	36
	MOCS	1	Niet	2088	2623	1984	2379	15.7	23.7	21.5	15.6	22.9	21.0	681	97
	MOCS	2	Niet	2076	2723	1919	2406	16.9	22.2	23.5	16.9	21.3	22.6	827	65
	MOCS	3	Niet	2106	2657	2015	2426	16.6	22.7	22.6	16.7	22.0	22.0	838	36
	RECS	1	Els	2061	2692	1868	2348	14.7	23.7	21.4	14.7	22.1	20.5	619	95
	RECS	2	Els	2032	2742	1817	2382	15.7	22.0	23.3	15.7	20.8	22.4	562	48
	RECS	3	Els	2027	2637	1913	2432	15.2	22.5	21.9	15.6	21.6	21.6	711	34
	RUCS	1	Thel	2008	2556	1948	2243	14.4	23.1	21.0	15.6	22.5	20.3	-	140
	RUCS	2	Thel	2003	2645	1817	2248	16.2	21.5	23.5	17.2	20.5	22.4	1257	62
	RUCS	3	Thel	1980	2519	1869	2247	15.5	22.0	21.7	16.7	21.6	21.5	1177	40
	SWCS	1	Groen	2085	2719	2003	2405	14.6	23.6	21.9	16.4	22.6	21.2	-	96
	SWCS	2	Groen	2019	2741	1922	2431	15.4	22.0	23.4	16.4	21.3	22.6	680	49
	SWCS	3	Groen	2064	2737	2011	2460	14.8	22.4	21.8	16.8	21.9	22.0	632	-
	WATCS	1	Bon	2094	2662	1991	2313	15.1	23.6	21.6	16.7	22.6	21.0	735	112
	WATCS	2	Bon	2073	2739	1925	2348	16.1	22.0	23.6	17.1	21.2	22.5	845	50
	WATCS	3	Bon	2152	2801	2004	2376	15.7	23.0	22.7	17.3	21.7	22.0	741	36

B

Site	Vintage	AWS ¹	Logger WI ²	Logger HI ³	AWS WI ⁴	AWS HI ⁵	Logger FNI ⁶	Logger MJT ⁷	Logger MFT ⁸	AWS FNI ⁹	AWS MJT ¹⁰	AWS MFT ¹¹	Winter Rainfall ¹²	Season rainfall ¹³
ADWSB	1	Bon	2010	2484	1991	2313	15.5	23.0	21.0	16.7	22.6	21.0	735	112
ADWSB	2	Bon	2003	2561	1925	2348	16.1	21.7	23.0	17.1	21.2	22.5	845	50
ADWSB	3	Bon	2050	2578	2004	2376	16.2	22.2	22.1	17.3	21.7	22.0	741	35
AMPSB	1	Goede	2027	2491	1910	2207	15.4	22.9	21.0	16.4	21.9	20.4	600	96
AMPSB	2	Goede	1969	2543	1826	2238	16.5	21.3	23.1	17.2	20.4	22.3	694	50
AMPSB	3	Goede	1997	2531	1885	2257	16.4	21.9	21.9	17.1	21.1	21.4	622	34
DEHSB	1	Els	1944	2423	1868	2348	14.3	22.5	20.3	14.7	22.1	20.5	619	95
DEHSB	2	Els	1947	2578	1817	2382	16.1	21.1	23.0	15.7	20.8	22.4	562	48
DEHSB	3	Els	1957	2487	1913	2432	15.8	21.7	21.5	15.6	21.6	21.6	711	34
HEKSB	1	Alto	2090	2622	2017	2313	15.3	23.3	21.7	16.7	22.7	21.0	674	130
HEKSB	2	Alto	2054	2699	1934	2362	16.1	21.7	23.6	17.9	21.1	22.9	783	73
HEKSB	3	Alto	2117	2714	2020	2402	15.9	22.6	22.7	17.4	22.0	22.1	762	50
LIBSB	1	Niet	1980	2665	1984	2379	14.1	22.7	21.2	15.6	22.9	21.0	681	97
LIBSB	2	Niet	1995	2729	1919	2406	14.8	21.5	23.1	16.9	21.3	22.6	827	65
LIBSB	3	Niet	2046	2688	2015	2426	14.9	21.8	22.1	16.7	22.0	22.0	838	36
MOISB	1	Niet	1994	2584	1984	2379	14.7	23.1	21.1	15.6	22.9	21.0	681	97
MOISB	2	Niet	1895	2550	1919	2406	16.0	20.9	23.0	16.9	21.3	22.6	827	65
MOISB	3	Niet	1889	2471	2015	2426	15.7	21.3	21.3	16.7	22.0	22.0	838	36
RUSB	1	Thel	1859	2256	1948	2243	14.6	22.3	19.7	15.6	22.5	20.3	*	140
RUSB	2	Thel	1871	2421	1817	2248	16.8	20.6	22.6	17.2	20.5	22.4	1257	62
RUSB	3	Thel	1875	2380	1869	2247	15.9	21.7	21.1	16.7	21.6	21.5	1177	40
SWSB	1	Groen	2085	2719	2003	2405	14.6	23.6	21.9	16.4	22.6	21.2	*	96
SWSB	2	Groen	2019	2741	1922	2431	15.4	22.0	23.4	16.4	21.3	22.6	680	49
SWSB	3	Groen	2064	2737	2011	2460	14.8	22.4	21.8	16.8	21.9	22.0	631	-

* The winter rainfall data for Groen V1 was excluded due to an abnormally low value in relation to values measured by other weather stations and the normal level of rainfall found in this area, most likely because of a technological error of the weather station and for Thel V1 due to data missing from 1 April to 23 May 2006.

¹ The assigned automatic weather station per site (refer to Fig. 6)

² The Winkler index calculated from the datalogger network data (Amerine & Winkler, 1944)

³ The Huglin index calculated from the datalogger network data (Tonietto & Carbonneau, 2004)

⁴ The Winkler index calculated from the automatic weather station network data (Amerine & Winkler, 1944)

⁵ The Huglin index calculated from the automatic weather station network data (Tonietto & Carbonneau, 2004)

⁶ The fresh night index calculated for February from the datalogger network data (Tonietto & Carbonneau, 2004)

⁷ The mean January temperature calculated from the datalogger network data (Smart & Dry, 1980)

⁸ The mean February temperature calculated from the datalogger network data (Smart & Dry, 1980)

⁹ The fresh night index calculated for February from the automatic weather station network data (Tonietto & Carbonneau, 2004)

¹⁰ The mean January temperature calculated from the automatic weather station network data (Smart & Dry, 1980)

¹¹ The mean February temperature calculated from the automatic weather station network data (Smart & Dry, 1980)

¹² The cumulative winter rainfall for the period of April to November calculated from the weather station network data

¹³ The cumulative seasonal rainfall for the period of December to March calculated from the weather station network data

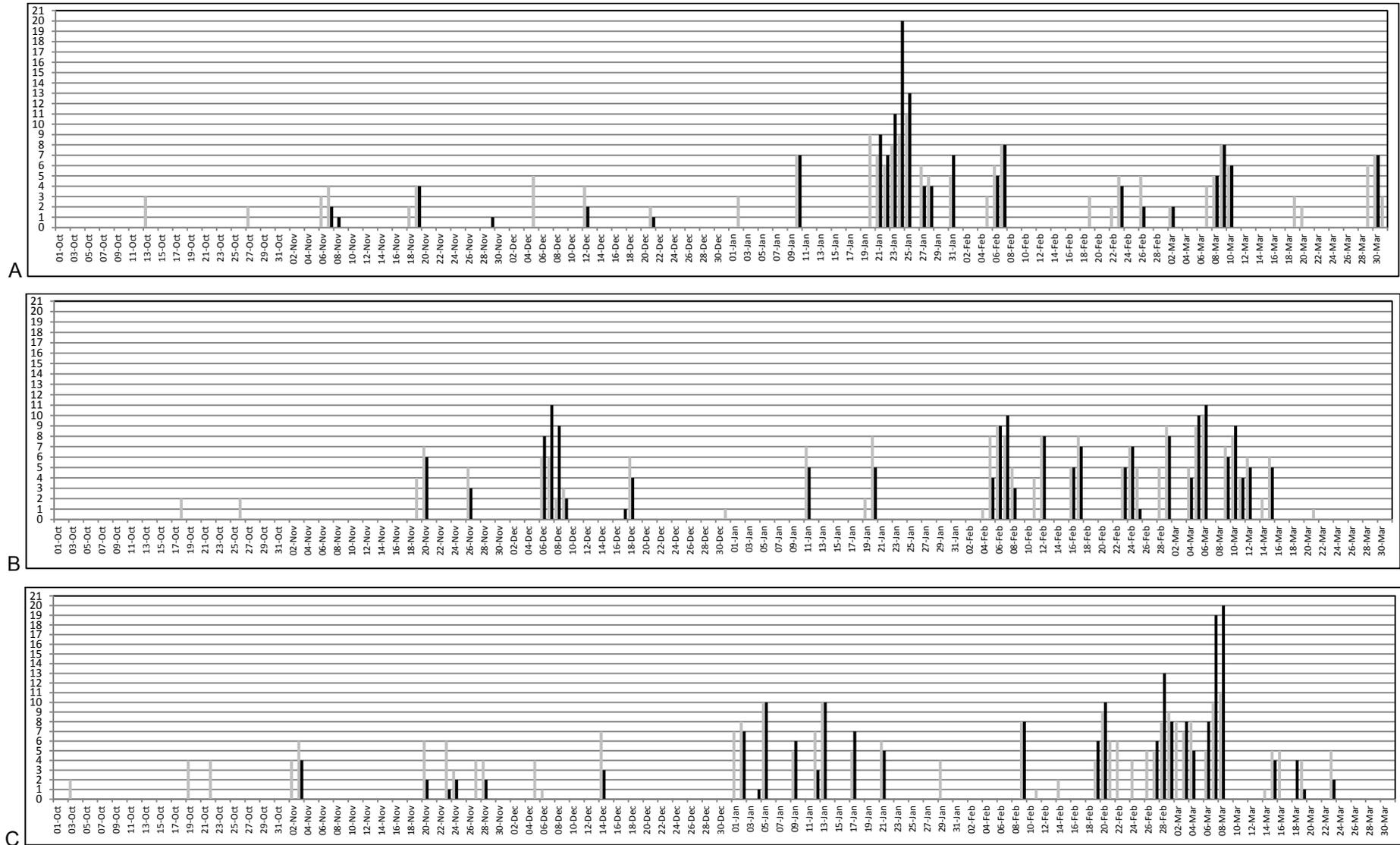


FIGURE 6

Indication of the time of occurrence of the heat waves from October to March per vintage with data collected from the AWS of Groen, indicated in grey, and Thel, indicated in black, as examples. Graph A represents vintage 1 (2006/2007), graph B vintage 2 (2008/2009) and graph C vintage 3 (2009/2010). Each column represents the sum of the number of hours per day of which the AWS registered a temperature above 30°C.

TABLE 2

An indication of the productive (yield) and vegetative (score sheet and point quadrat (PQ) indicators) differences between the experimental sites for vintage 1 (2006/2007), vintage 2 (2008/2009) and vintage 3 (2009/2010) for CS (Table A) and SB (Table B).

A	Site	Vintage	Mean yield per vine per site (kg) ¹	Mean pruning mass per vine per site (kg) ²	Yield:pruning mass index ³	Mean bunch mass per site (g) ⁴	Score sheet: Total (70) ⁵	Score sheet: % ⁶	PQ: % gaps ⁷	PQ: leaf layer number (LLN) ⁸	PQ: % shaded leaves ⁹	PQ: % shaded bunches ¹⁰
	LACS	1	0.71	0.92	0.77	83.87	55.33	79.05	19.33	1.49	17.90	53.35
	LEICS	1	2.57	0.96	2.68	128.40	55.67	79.52	9.33	2.45	38.91	65.08
	MMCS	1	1.20	1.08	1.11	88.74	61.00	87.14	9.33	2.52	35.60	70.62
	MOCS	1	0.91	0.78	1.17	65.42	49.00	70.00	26.00	0.91	9.17	22.34
	RECS	1	1.76			116.87	52.67	75.24	12.00	1.55	31.44	35.82
	RUCS [*]	1	1.31	1.19	1.09	72.52	67.00	95.71	12.00	2.05	29.66	52.49
	SWCS	1	1.47	0.75	1.97	79.58	54.33	77.62	8.67	3.25	53.19	61.48
	WATCS	1	1.91	0.47	4.05	85.17	58.00	82.86	8.67	2.65	40.69	67.65
	LACS	2	0.79			87.46	47.00	67.14	21.33	1.40	14.13	47.37
	LEICS [*]	2	1.56			91.60						
	MMCS	2	0.89			75.44	43.67	62.38	20.00	1.72	21.25	50.00
	MOCS	2	1.10	0.81	1.35	69.92	45.00	64.29	26.00	1.00	28.37	22.05
	RECS	2	1.55			122.30	45.00	64.29	18.00	1.42	29.78	37.38
	RUCS	2	1.26			80.18	41.00	58.57	22.00	1.24	17.72	40.71
	SWCS	2		0.64			42.00	60.00	6.67	3.33	50.49	71.84
	WATCS	2	1.48	1.19	1.25	91.21	49.67	70.95	10.67	2.49	34.64	64.73
	LACS	3	1.52	0.66	2.31	103.36	53.67	76.67	24.67	0.89	88.75	74.25
	LEICS	3	1.84	0.75	2.46	79.92	55.33	79.05	17.33	1.72	74.08	53.49
	MMCS	3	1.05			76.49	53.33	76.19	22.00	1.24	84.55	78.73
	MOCS	3	1.45	0.42	3.48	78.97	43.67	62.38	30.67	0.82	90.17	76.09
	RECS	3	1.74			110.23	54.00	77.14	15.33	1.15	83.69	74.40
	RUCS	3	1.39	0.89	1.57	69.74	52.33	74.76	36.00	0.94	73.23	75.28
	SWCS	3	1.41	0.57	2.50	76.55	52.67	75.24	13.33	2.15	63.94	50.10
	WATCS	3	1.21	1.18	1.02	57.16	51.67	73.81	8.67	2.87	58.01	24.88

B

Site	Vintage	Mean yield per vine per site (kg) ¹	Mean pruning mass per vine per site (kg) ²	Yield:pruning mass index ³	Mean bunch mass per site (g) ⁴	Score sheet: Total (70) ⁵	Score sheet: % ⁶	PQ: % gaps ⁷	PQ: leaf layer number (LLN) ⁸	PQ: % shaded leaves ⁹	PQ: % shaded bunches ¹⁰
ADWSB	1	2.18	0.93	2.34	116.07	57.67	82.38	14.67	2.00	30.36	68.19
AMPSB	1	2.39	0.69	3.48	87.01	56.33	80.48	4.67	2.69	33.56	81.07
DEHSB	1	1.15	1.23	0.94	69.52	55.00	78.57	10.67	2.69	38.15	71.60
HEKSB ⁺	1	1.51			88.53	55.00	78.57	20.67	1.71	22.42	64.38
LIBSB	1	2.68			93.93	45.00	64.29	8.00	3.12	43.26	87.55
MOISB	1	1.06	0.80	1.33	81.22	53.67	76.67	8.67	3.03	42.37	87.45
RUSB	1	3.37	1.25	2.69	139.24	61.00	87.14	10.67	2.00	25.25	70.30
SWSB	1	2.27	0.44	5.19	65.04	56.67	80.95	8.00	2.91	40.75	81.16
ADWSB	2	2.04	0.75	2.72	102.94	49.00	70.00	8.00	2.56	37.32	64.64
AMPSB	2	2.37	0.69	3.46	92.67	44.00	62.86	0.09	2.80	39.83	73.24
DEHSB	2	1.99	0.93	2.14	123.71	37.00	52.86	11.33	2.80	44.49	71.36
HEKSB	2	1.58			86.32						
LIBSB	2	2.70	1.36	1.98	110.88	44.33	63.33	5.33	3.55	48.29	90.31
MOISB	2	1.68			107.95	46.33	66.19	8.00	3.03	41.74	78.25
RUSB	2		0.72			47.00	67.14	10.67	2.28	34.55	46.11
SWSB	2		0.62			38.33	54.76	1.33	3.89	50.89	87.70
ADWSB	3	1.63	0.64	2.53	114.98	53.67	76.67	16.00	2.11	48.48	27.49
AMPSB	3	2.97	0.52	5.69	90.11	55.67	79.52	10.00	2.65	57.78	33.49
DEHSB	3					43.00	61.43	19.00	2.08	72.59	36.42
HEKSB	3	2.58	0.43	6.06	71.45	57.00	81.43	12.00	2.39	66.06	30.12
LIBSB	3	1.67	1.13	1.47	86.82	45.00	64.29	4.00	3.46	53.12	12.77
MOISB	3	1.18	0.49	2.42	71.02	44.00	62.86	9.33	2.77	61.56	23.10
RUSB	3	0.65	0.47	1.38	81.88	50.33	71.90	21.33	1.61	79.82	67.25
SWSB	3	2.65	0.56	4.75	90.73	43.67	62.38	2.00	3.87	46.62	17.16

* RUCS V1 underwent crop thinning; LEICS V2 underwent no canopy managements before the evaluation was performed; HEKSB V2 the leaves and lateral shoots were removed from the bunch zone

¹ Mean value of the mean mass per vine per site (n=30) determined at harvest

² Mean value of the cane mass per vine per site (n=30) determined at pruning

³ The mean yield: pruning mass value calculated by dividing the yield per vine by the cane mass per vine

⁴ Mean bunch mass per site (n=30) determined by dividing the mass per vine by the number of bunches harvested

⁵ The mean value of the total count of the score sheet per site (n=3) as shown in Table 3 (Archer, 2002)

⁶ The mean percentage determined from the total count of the score sheet per site (n=3) as shown in Table 3 (Archer, 2002)

⁷⁻¹⁰ Point quadrat (PQ) calculations:

⁷ The percentage gaps in the canopy (the number of observed gaps divided by the total number of measurements (50))

⁸ The leaf layer number (the total number of leaves observed divided by the total number of measurements (50))

⁹ The percentage shaded leaves in the canopy (number of internal leaves observed divided by the total number of leaves observed)

¹⁰ The percentage shaded bunches in the canopy (number of internal leaves observed divided by the total number of leaves observed)

TABLE 3

Example of the score sheet of Smart & Robinson (1993) and adapted for South African conditions as used in this study (Archer, 2002).

Vineyard score sheet to determine the potential for the production of quality wine grapes			
1. Canopy gaps		5. Bunch exposure	
± 30 %	= 10	± 30 %	= 10
20 - 30 %	= 8	± 20 %	= 8
30 - 40 %	= 6	± 10 %	= 6
10 - 20 %	= 4	≥ 40 %	= 2
< 10 %	= 0	< 10 %	= 2
> 50 %	= 0		
2. Leaf size		6. Shoot length	
a bit small	= 5	15 - 25 nodes	= 10
average	= 4	11 - 14 nodes	= 8
a bit large	= 3	8 - 10 nodes	= 4
very large	= 2	< 8 nodes	= 2
very small	= 2	> 30 nodes	= 2
3. Leaf colour		7. Lateral growth	
green, healthy, slightly dull and bleak	= 5	limited or none	= 10
yellowish green, healthy	= 5	only top 1/3 of shoot	= 8
dark green, shiny, healthy	= 2	top 2/3 of shoot	= 6
slight deficiency symptoms	= 2	complete shoot	= 2
unhealthy, necrosis or chlorosis	= 0		
4. Canopy density (LLN)		8. Growth tips	
± 2 - 3	= 10	≤ 5 %	= 10
± 3 - 4	= 8	± 10 %	= 8
≤ 2	= 4	± 10 %	= 6
> 4	= 0	± 10 %	= 4
		± 10 %	= 2
		≥ 50 %	= 0
Total: _____ /70 = _____ %			

¹ The percentage of canopy gaps observed in the area covered by 90% of the canopy

² The leaf size of the middle basal leaves situated on the outside of the canopy in accordance with the cultivar

³ The leaf colour of the basal leaves situated in the bunch zone

⁴ The average estimated leaf layer number (LLN) determined from side to side of the canopy in the bunch zone

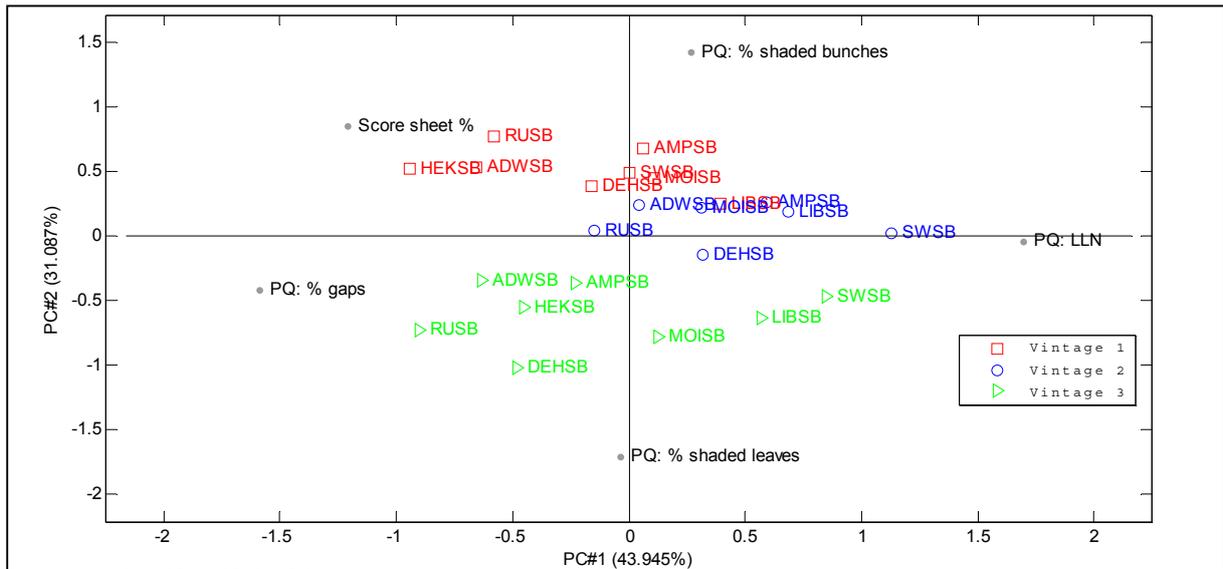
⁵ The percentage of exposed bunches on both sides of the canopy

⁶ The mean shoot length in the canopy penalising the score for abnormally long or short internodes

⁷ The lateral shoot growth observed in the canopy

⁸ The percentage of actively growing shoot tips, allowing for canopy management actions

A



B

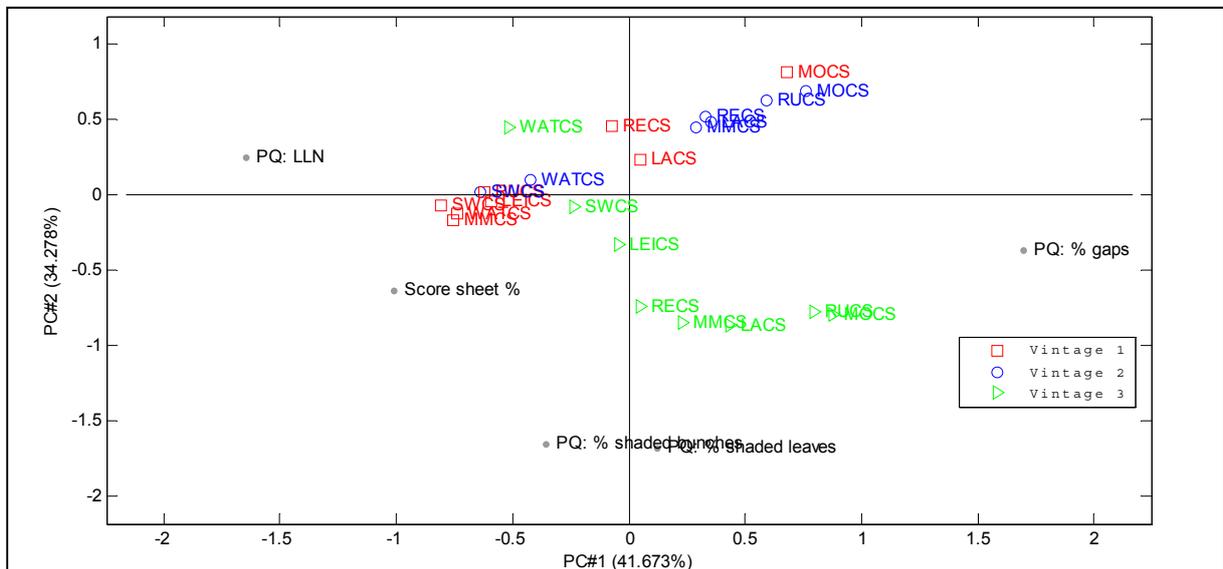


FIGURE 7

Multivariate analyses PCA bi-plots for SB (A) and CS (B) illustrating the variance of the point quadrate calculations and the shore sheet measurements between sites and indicated per vintage (n=24) with Vintage 1 (2006/2007) indicated in red, Vintage 2 (2007/2008) in blue and Vintage 3 (2009/2010) in green. The percentage variance per component is indicated on the graphs.

PQ: Point quadrate calculations (Smart & Robinson, 1993):

LLN: Leaf layer number (total number of leaves observed divided by the total number of measurements (50))

% gaps: Percentage of gaps in the canopy (number of gaps observed divided by the total number of measurements (50))

% shaded leaves: Percentage of shaded leaves in the canopy (number of internal leaves observed divided by the total number of leaves observed)

% shaded bunches: Percentage of shaded bunches (number of internal bunches observed divided by the total number of bunches observed)

Score sheet %: The score sheet percentage calculated from the total score per site (Archer, 2002)

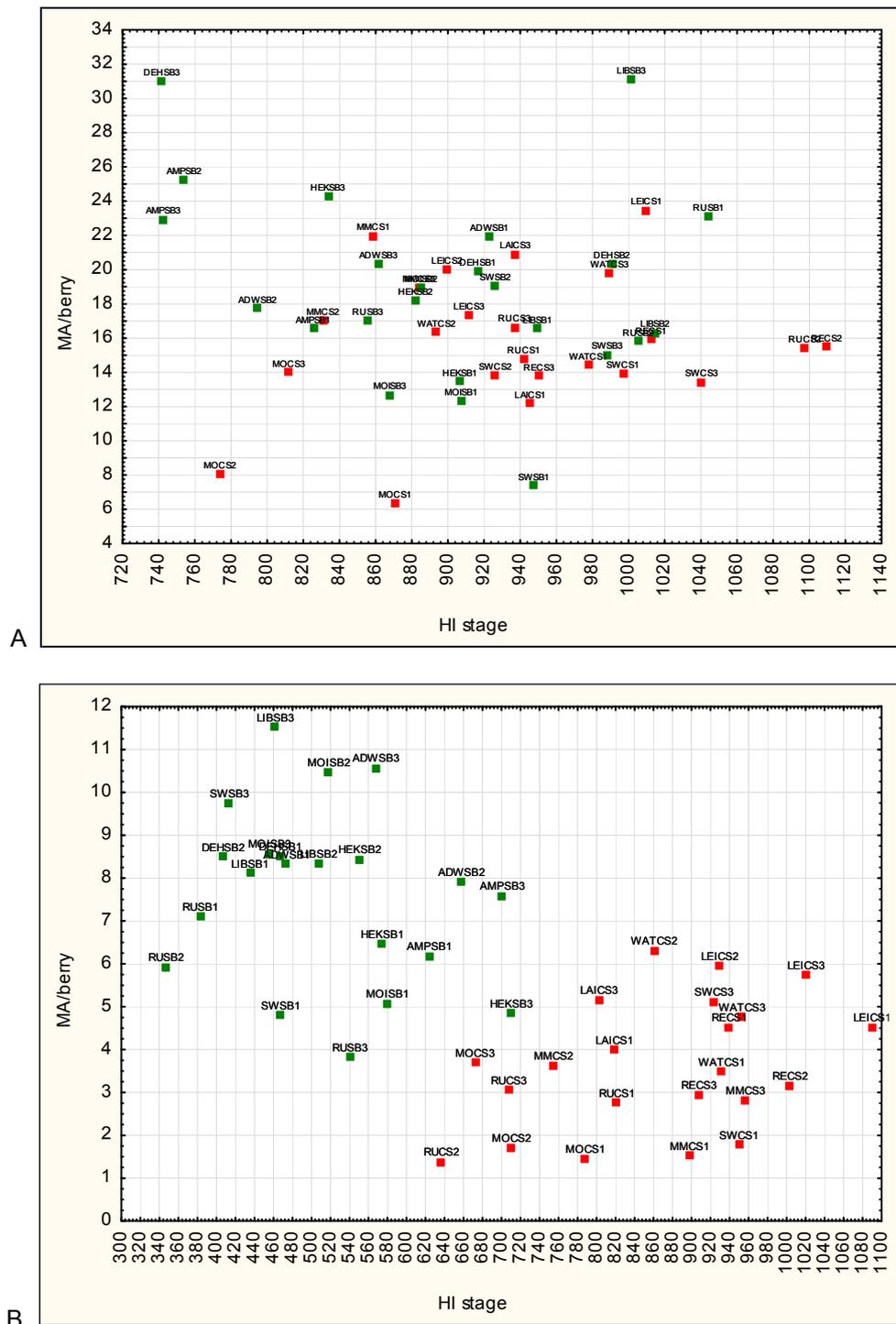


FIGURE 8

Scatterplots indicating the relationship of the malic acid per berry with the Huglin index thermal units per phenological stage at véraison (figure A) and at harvest (figure B) for Cabernet Sauvignon (red squares) and Sauvignon Blanc (green squares). Each data point is labelled with the site name and the number following the code indicates the vintage with 1 representing data for vintage 1 (2006/2007), 2 representing vintage 2 (2008/2009) and 3 representing the data for vintage 3 (2009/2010).

GENERAL SITE DESCRIPTIONS – Cabernet Sauvignon

LAICS

	
<p>Plot description: This low vigour, single arm cordon dryland plot is situated on the lower foot slopes of the Simonsberg-Stellenbosch ward.</p> <p>Aspect: NW</p> <p>Slope %: 13</p> <p>Altitude: 240 m</p> <p>Terrain description: Open, Simonsberg to the South,</p>	
Plot 1	Higher vigour than plot 2 and 3
Plot 2	
Plot 3	

LEICS

<p>No image available</p>	
<p>Plot description: This very old double cordon vineyard is situated in the midslope of the Devon Valley ward. Supplementary micro irrigation installed.</p> <p>Aspect: SW</p> <p>Slope %: 15</p> <p>Altitude: 160 m</p> <p>Terrain description: Closed. Surrounded by hills,</p>	
Plot 1	Higher vigour than plot 2 and 3
Plot 2	
Plot 3	Low vigour, high disease occurrence

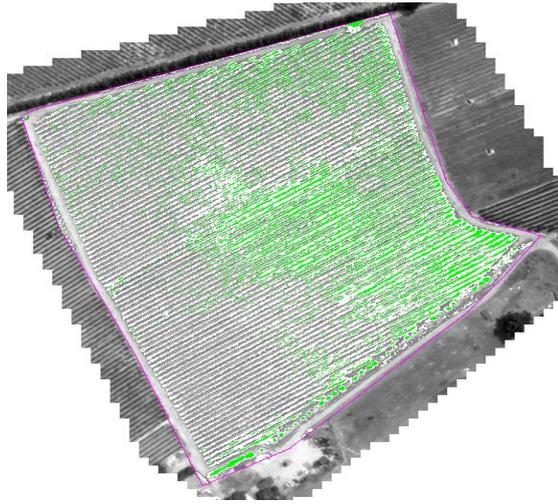
MMCS

<p>No image available</p>	
<p>Plot description: Positioned on the midslope of the Devon Valley ward. A single arm cordon, dryland vineyard. Aspect: S Slope %: 27 Altitude: 240 m Terrain description: Open, Hills to the North and South.</p>	
<p>Plot 1</p>	<p>Concave slope, aspect is SW</p>
<p>Plot 2</p>	
<p>Plot 3</p>	

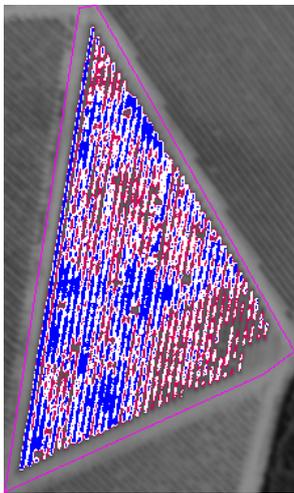
MOCS

<p>No image available</p>	
<p>Plot description: This low vigour, double cordon dryland plot is situated on the midslopes of the foot slopes of the Simonsberg-Stellenbosch ward. Aspect: W Slope %: 27 Altitude: 210 m Terrain description: Open, Simonsberg foothills to the East</p>	
<p>Plot 1</p>	
<p>Plot 2</p>	
<p>Plot 3</p>	<p>Higher vigour earlier in season due to slope and water drainage</p>

RECS

	
<p>Plot description: High vigour double cordon vineyard positioned on the lower footslopes of the Simonsberg-Stellenbosch ward. Supplementary drip irrigation installed.</p> <p>Aspect: S</p> <p>Slope %: 11</p> <p>Altitude: 210 m</p> <p>Terrain description: Open, Simonsberg to the North-East</p>	
<p>Plot 1</p>	
<p>Plot 2</p>	<p>Plot wet until anthesis</p>
<p>Plot 3</p>	<p>Extremely wet plot until deep into the growing season</p>

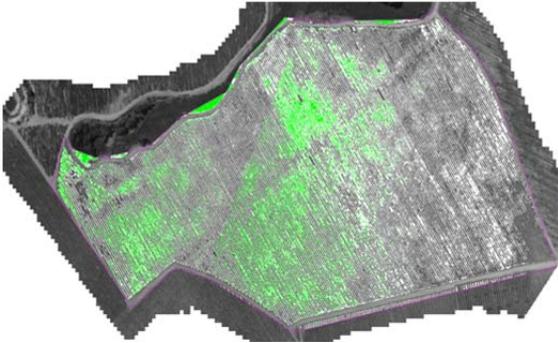
RUCS

	
<p>Plot description: This high vigour, old and diseased vineyard is situated on the midslope of the Simonsberg-Stellenbosch ward. Meticulous canopy management produces a homogeneous and thin, upright canopy with a high exposed leaf area and exposed bunches. Supplementary drip irrigation installed.</p> <p>Aspect: SW</p> <p>Slope %: 9</p> <p>Altitude: 385 m</p> <p>Terrain description: Open, Simonsberg to the North.</p>	
<p>Plot 1</p>	
<p>Plot 2</p>	
<p>Plot 3</p>	

SWCS

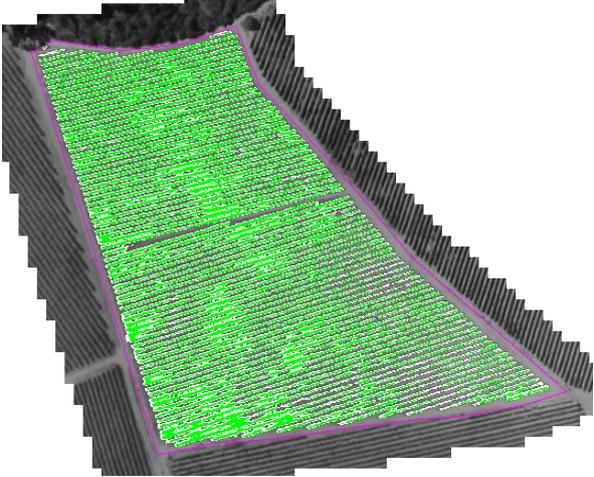
<p>No image available</p>	
<p>Plot description: This low vigour, double cordon vineyard is situated in the valley bottom of the Bottelary Hills ward. Supplementary drip irrigation installed. Aspect: W Slope %: 3 Altitude: 155 m Terrain description: Open, Bottelary Hills to the South.</p>	
<p>Plot 1</p>	
<p>Plot 2</p>	
<p>Plot 3</p>	

WATCS

	
<p>Plot description: A medium vigour double cordon vineyard situated on the midslopes of the Devon Valley ward with supplementary micro irrigation installed. Aspect: NE Slope %: 17 Altitude: 170 m Terrain description: Closed between hills of Devon Valley. Large dam to the West.</p>	
<p>Plot 1</p>	
<p>Plot 2</p>	
<p>Plot 3</p>	<p>Higher vigour than plot 1 and 2 due to the slope and water flow.</p>

GENERAL SITE DESCRIPTIONS – Sauvignon Blanc

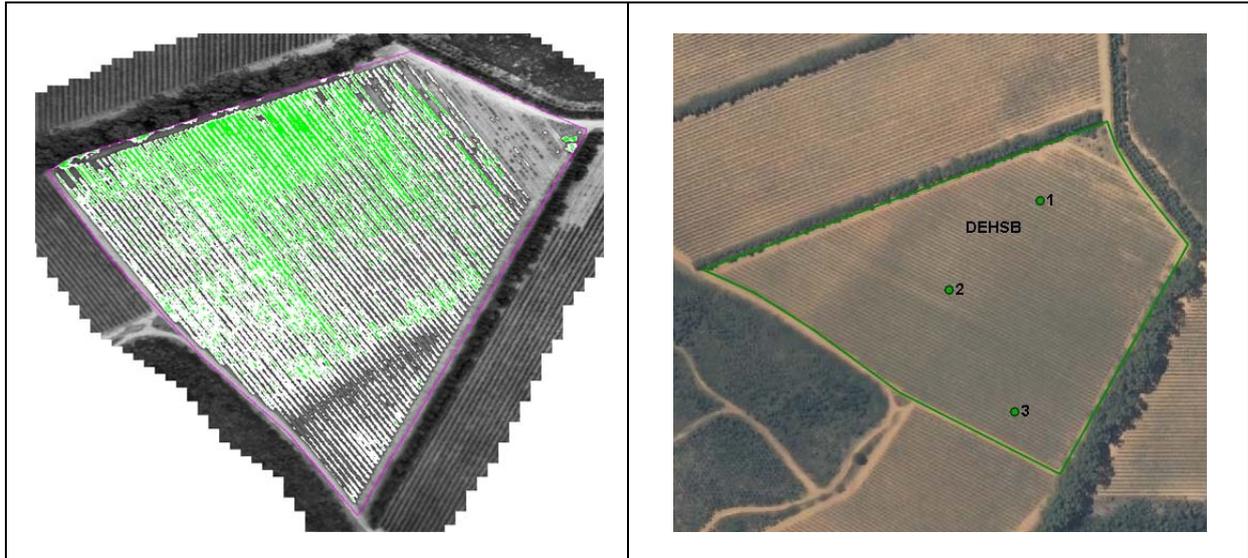
ADWSB1

	
<p>Plot description: This medium to high vigour vineyard is situated on the midslopes of the Bottelary Hills in the Polkadraai Hills ward. Supplementary drip irrigation is installed. Aspect: SSE Slope %: 20 Altitude: 200 m Terrain description: Open. Bottelary hills to the North.</p>	
<p>Plot 1</p>	
<p>Plot 2</p>	
<p>Plot 3</p>	<p>Due to the concave form the aspect is SE</p>

AMPSB

<p>No image available</p>	
<p>Plot description: This medium vigour, single arm cordon plot is situated on the midslope of the Bottelary Hills. Supplementary drip irrigation installed. Aspect: SSW Slope %: 26 Altitude: 270 m Terrain description: Open, Simonsberg to the South</p>	
<p>Plot 1</p>	<p>Due to the convex slope the aspect is SW</p>
<p>Plot 2</p>	<p>Due to the convex slope the aspect is SW</p>
<p>Plot 3</p>	<p>Due to the convex slope the aspect is SW</p>

DEHSB



Plot description: This dryland, double cordon high vigour vineyard is on the midslope of the Simonsberg-Stellenbosch ward.

Aspect: WSW

Slope %: 14

Altitude: 410 m

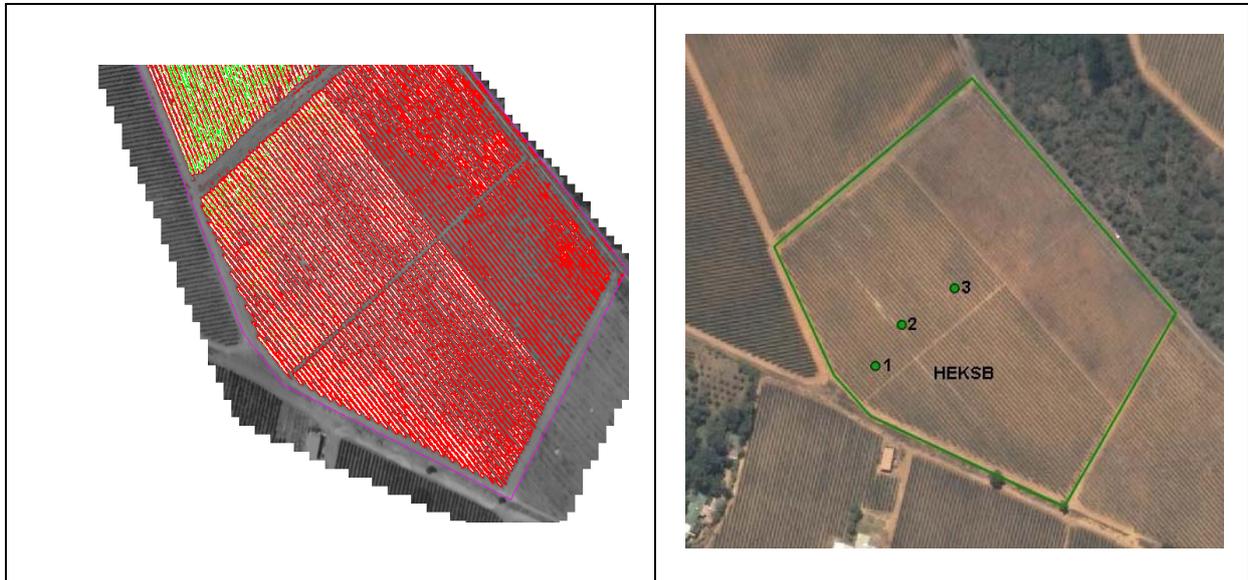
Terrain description: Enclosed by foothills of Simonsberg to the South and Simonsberg to the North.

Plot 1 | First and second season, wind damage. Last season, plot's vines renewed.

Plot 2 | Extensive wind damage in windy seasons

Plot 3 | High vigour in comparison with plot 2

HEKSB



Plot description: This low vigour, single arm cordon plot is situated on the higher footslopes of Helderberg. Supplementary drip irrigation installed.

Aspect: NNE

Slope %: 17

Altitude: 230 m

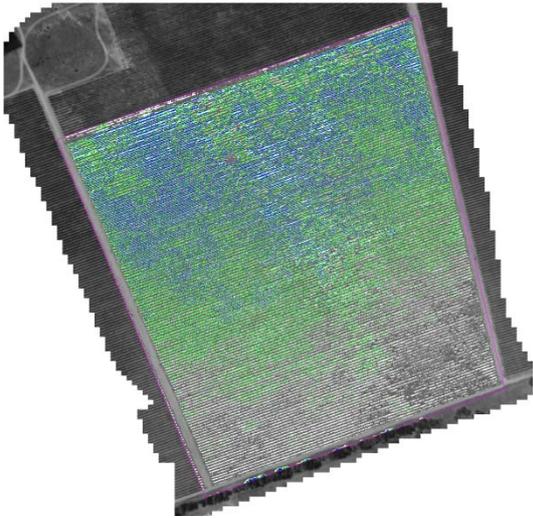
Terrain description: Semi-open. Helderberg to the East and foothills to the North.

Plot 1

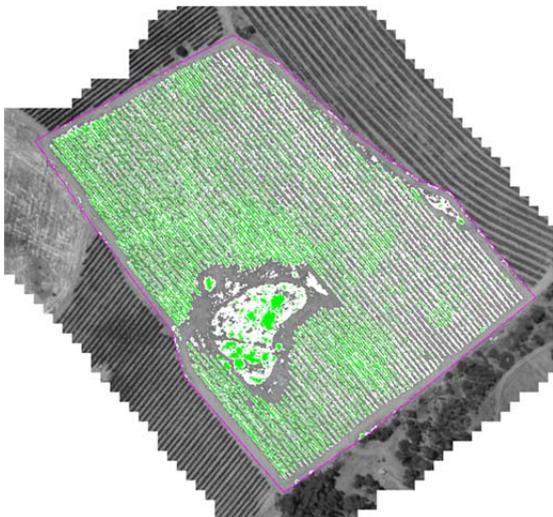
Plot 2

Plot 3

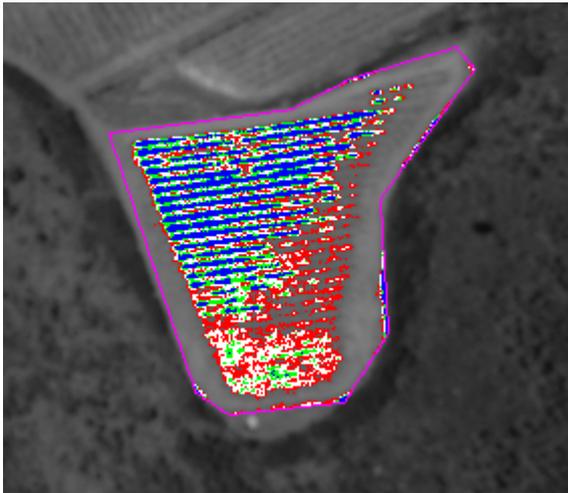
LIBSB

	
<p>Plot description: This high vigour plot is situated in the Stellenbosch Wine Growing region in the valley bottom. The double cordon plot has supplemental irrigation.</p> <p>Aspect: NNW</p> <p>Slope %: 6</p> <p>Altitude: 120 m</p> <p>Terrain description: Open</p>	
<p>Plot 1</p>	
<p>Plot 2</p>	
<p>Plot 3</p>	<p>Higher vigour than plot 1 and 2</p>

MOISB

	
<p>Plot description: This medium vigour is at the crest of the Bottelary Hills ward. The double cordon vineyard has supplemental irrigation installed.</p> <p>Aspect: N</p> <p>Slope %: 11</p> <p>Altitude: 360 m</p> <p>Terrain description: Open due to altitude except for a tree line to the South</p>	
<p>Plot 1</p>	<p>Lower vigour than 1 and 2 due to higher wind exposure</p>
<p>Plot 2</p>	<p>Lower yield than plot 1 and 2 (soil)</p>
<p>Plot 3</p>	

RUSB



Plot description: This high vigour vineyard is situated on the higher midslope of Simonsberg in the Simonsberg-Stellenbosch ward. Meticulous canopy management produces a homogeneous and thin, upright canopy with a high exposed leaf area and exposed bunches. Due to high wind exposure the vines have a low yield and stunted growth of the shoots in windy seasons. Supplementary drip irrigation installed.

Aspect: S

Slope %: 32

Altitude: 470 m

Terrain description: Open, Simonsberg to the North.

Plot 1	
Plot 2	
Plot 3	Due to the concave form of the vineyard the aspect is SE

SWSB

No image available



Plot description: This low vigour, double cordon vineyard is situated in the valley bottom of the Bottelary Hills ward. Supplementary drip irrigation installed.

Aspect: W

Slope %: 3

Altitude: 155 m

Terrain description: Open, Bottelary Hills to the South.

Plot 1	
Plot 2	
Plot 3	

Addendum: Literature cited

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