EFFECT OF VARYING LEVELS OF NITROGEN, POTASSIUM AND CALCIUM NUTRITION ON TABLE GRAPE VINE PHYSIOLOGY AND BERRY QUALITY

by

PJ Raath

Dissertation presented for the the degree of

Doctor of Philosophy (Agricultural Sciences)

at

Stellenbosch University

Department of Viticulture and Oenology, Faculty of AgriSciences

Supervisor: Prof JJ Hunter

Co-supervisor: Dr WJ Conradie

March 2012

DECLARATION

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

SUMMARY

A lack of defects is required for successful table grape marketing, which pre-suppose optimal vine performance, berry development and post-harvest quality. The supply of mineral nutrients affects vine development, physiology and berry quality. Despite a vast amount of research conducted over decades, there remain many unresolved issues regarding table grape vine nutrition to ensure optimal table grape quality and shelve-life. Unjustified fertilisation practices often include excessive applications of nitrogen (N), potassium (K) and calcium (Ca).

A four-year field trial was therefore conducted on a sandy soil in the Paarl district of South Africa, using grafted on Ramsey, and trained to a gable trellis system. Nitrogen, potassium and calcium were applied, singular or in combination, at rates up to 300% the calculated annual nutritional requirement. The effect of these excessive applications on table grape performance under typical South African cultivation conditions was investigated for *Vitis vinifera* L. cv. Prime Seedless, a very early seedless table cultivar that is produced with minimum berry diameter of 18mm, with special reference to 1) vegetative growth, 2) expression of grapevine nutrient availability through foliar analyses, 3) berry nutrient accumulation patterns of this early cultivar, 4) manipulation of berry nutrient content through soil and bunch directed applications and 5) the effect of berry nutrient content on its quality.

No definite vegetative growth responses (expressed as shoot length, leaf surface area and shoot mass) and leaf chlorophyll content differences were obtained for all the treatments. These results were obtained in a vineyard on a sandy soil where excessive N fertilisation caused a reduction of soil pH to detrimentally low levels and where the excessive N, K and Ca applications reduced mutual concentrations and that of Mg, in the soil. A lack of stimulation in vegetative growth may therefore be ascribed to the combined negative effect of these excessive applications on soil pH and vine nutrition.

Although the N content of petioles was higher for treatments where N was applied, consistent significant increases in petiole N with N fertilisation were not observed. Petiole N concentration showed a decreasing trend throughout the season. Petiole K concentrations were significantly increased by the K fertilisation at all phenological stages. None of the K fertilisation treatments, however, succeeded to raise petiole K concentrations above the accepted maximum norms and petiole K concentration at a specific sampling stage varied significantly between the four seasons. A general decrease in petiole K concentration was

found for all seasons. Calcium fertilisation did not increase soil Ca content, resulting in a lack of differences in petiole Ca concentrations between treatments. An increase in petiole Ca concentration towards harvest was obtained. Correlations between petiole nutrient concentration and berry mineral content at harvest were poor. The only way of knowing the mineral content of berries would seem to be by measuring it directly instead of deducing it from the results of leaf or petiole analyses.

The dynamics of berry growth impacted on berry nutrient concentration. Early rapid berry growth, predominantly due to cell division and cell growth, was associated with the most rapid decreases in N, P and Ca concentration. Due to mobility of K and Mg in the plant, that exceeds other nutrients, the decrease in concentration of these two mineral elements was not as pronounced as that of the others. Nutrient accumulation was most rapid during the pre-véraison period, but only Ca showed a definite termination during the early ripening period. The continued inflow of N, P, K and Mg, albeit at slower rates immediately after véraison, should be taken into consideration when fertilisation is applied. As a table grape, total accumulation of each nutrient in Prime Seedless berries also far exceeded that of other cultivars studied thus far. A particular difference is that the berry flesh:skin ratio is much higher than that of previously studied cultivars, leading to higher levels of nutrient accumulation in the flesh.

Slightly larger berry size was obtained for N applications and is ascribed to slight increases in early vegetative growth, allowing a better response to GA_3 treatments. The use of GA_3 for berry enlargement is also considered the reason why K fertilisation, resulting in increased berry K levels, did not affect berry size, as is often found for wine grapes.

Higher available NO_3^- in the soil on account of excessive N applications resulted in higher levels of berry N, despite sub-optimal soil pH regimes that were created by these treatments.

Berry K concentration and content were increased by K fertilisation. Rapid vine K uptake and translocation to the berries seem to negate the reduced vine nutritional status as observed in petioles for situations of over-fertilisation with N. Berry Ca levels were not increased by Ca fertilisation or by bunch applied Ca. The rapid rates of berry growth, together with low rates of berry Ca uptake and Ca uptake that terminates at the onset of ripening, are assumed to be the main reasons for this result.

Low levels of decay as well as a lack of consistently increased decay were obtained for N containing treatments. Nitrogen levels in the berries above which their susceptibility to fungal infection is increased, should be established. Information on specific N compounds that may lead to more susceptibility is required. Potentially increased berry browning on account of high rates of K fertilisation needs to be further investigated; indications that this may occur were observed. Neither soil applied Ca nor bunch applied Ca improved berry quality, although Ca treatments seemed to reduce decay during the only season that significant differences were obtained.

The negative effect of excessive fertilisation on soil chemistry of sandy soils has again been highlighted by this study. This annuls the fertilisation, leading to inefficient fertilisation and a lack of the desired responses.

As indicator of vine nutrient availability, petiole analysis, was proven unreliable and should be evaluated in parallel with soil analyses, taking seasonal variation into consideration. The danger of being only guided by published norms for leaf nutrient concentrations when establishing fertilisation practices has again been highlighted by this study.

This research indicated that for a very early cultivar like Prime Seedless, nutrient accumulation dynamics can already start to change during the pre-véraison period in some seasons. This is due to different edaphic and climatic conditions as well as berry size, which leads to much higher flesh:skin ratios. Future research on table grapes would need to develop an understanding of the various factors and dynamics that determine berry nutrient concentration and accumulation of early ripening, large berry sized, seedless table grape cultivars.

OPSOMMING

Suksesvolle bemarking van tafeldruiwe is ten nouste afhanklik van die beskikbaarheid van druiwe sonder defekte, wat ‘n direkte verband met optimale wingerdprestasie, korrelontwikkeling en na-oes kwaliteit inhou. Voorsiening van minerale voedingstowwe beïnvloed die stok se groei, fisiologie en korrelgehalte. Ten spyte van ‘n oorweldigende hoeveelheid navorsing wat oor dekades reeds gedoen is, is daar steeds onopgeloste kwessies aangaande bemesting van tafeldruiwe vir optimale druifgehalte en houvermoë. Die gevolg is onoordeelkundige bemestingspraktyke wat o.a. aanleiding gee tot oorbemesting met stikstof (N), kalium (K) en kalsium (Ca).

‘n Vier-jaar-lange veldproef is gevolglik op ‘n sandgrond in die Paarl distrik (Suid-Afrika) onderneem deur gebruik te maak van *Vitis vinifera* L. cv. Prime Seedless geënt op Ramsey en op ‘n dubbel-gewel prieelstelsel opgelei is. Stikstof, K en Ca is alleen, of in kombinasie, toegedien teen hoeveelhede gelykstaande aan 300% van die wingerd se jaarlikse behoefte. Die effek van hierdie oormatige toedienings op tafeldruif prestasie onder Suid-Afrikaanse verbouingstoestande is ondersoek, met spesiale verwysing na 1) vegetatiewe groei, 2) uitdrukking van voedingstofbeskikbaarheid deur blaarontledings, 3) die voedingstof akkumulasie patrone van korrels van hierdie vroeë kultivar, 4) manipulasie van korrel voedingstofinhoud deur grond en trosgerigte toedienings en 5) die effek van korrel voedingstofinhoud op kwaliteit.

Die doel van die proef was om bemestinspraktyke van Prime Seedless, ‘n baie vroeë pitlose tafeldruifkultivar met ‘n minimum korrelgrootte van 18 mm, te verfyn. Deur die akkumulasie patrone van die druiwe uit te klaar is daar ook ondersoek ingestel of oestyd en na-oes gehalte deur oormatige toediening van voedingstowwe affekteer word.

Geen duidelike verskille betreffende vegetatiewe groeireaksies (uitgedruk as lootlengte, blaaroppervlaktes en lootmassas) asook verskille in blaar chlorofilinhoud is vir die behandelings verkry nie. Hierdie resultate is verkry in ‘n wingerd op ‘n sandgrond, waar oormatige N-bemesting aanleiding gegee het tot grond pH verlagings tot die peil van nadelige vlakke. Verder het die oormatige N, K en Ca toedienings wederkerige verlagings in konsentrasies, asook op dié van Mg, in die grond teweeggebring. Die tekort aan vegetatiewe groeiresponse op die behandelings kon dus toegeskryf word aan ‘n gekombineerde effek van die oormatige toedienings op grond pH en voedingstofbalanse.

Hoewel die N-inhoud van bladstele hoër was vir behandelings wat N toediening ingesluit het, was daar nie konstante toenames in die vlakke verkry nie. Bladskyf N-konsentrasie het afgeneem deur die loop van die groeiseisoen. Vir alle fenologiese stadiums was bladskyf K-konsentrasies betekenisvol verhoog deur K-bemesting. Nie een van die K-bemestingsbehandelings het egter daarin geslaag om bladskyf K inhoud vir enige monstertyd bo die algemeen aanvaarde maksimum norms te lig nie. Verder het bladskyf K inhoud by ‘n spesifieke fenologiese stadium ook betekenisvol tussen seisoene verskil. Die K-inhoud van bladskywe het afgeneem met verloop van die seisoen. Kalsiumbemesting het nie die grond se Ca inhoud deurgans verhoog nie, wat dus die tekort aan verskille in Ca konsentrasies tussen die behandelings verklaar. ‘n Toename in Ca konsentrasie en korrel Ca inhoud is vanaf set tot oes waargeneem. Swak korrelasies tussen bladskywe se voedingstofinhoude en korrels se voedingstofinhoude is verkry. Die enigste manier waarop korrels se voedingstofinhoude dus afgelei kan word, blyk te wees deur direkte bepaling daarvan.

Voedingstofinhoude van korrels is deur groeipatrone daarvan beïnvloed. Vroeë korrelgroei, hoofsaaklik a.g.v. seldeling en selgroei, het met die vinnigste afnametempo van N, P en Ca gepaard gegaan. As gevolg van die hoër beweeglikheid van K en Mg in die plant in vergelyking met ander voedingstowwe, was die afname in konsentrasie van hierdie twee elemente nie so groot soos vir die ander nie. Voedingstofakkumulasie was die vinnigste in die periode voor deurslaan. Slegs Ca het ‘n beeïndiging van opname aan die einde van hierdie periode getoon. Die voortgesette opname van N, P, K en Mg, alhoewel stadiger kort na deurslaan, moet in ag geneem word wanneer bemesting toegedien word. Vir hierdie kultivar het die totale opname van elke bemestingstof dié van die ander kultivars wat tot hede bestudeer is, ver oorskry. ‘n Spesifieke verskil is ‘n baie hoër vleis:dop verhouding as wat vir ander kultivars verkry is. Dit gee aanleiding tot baie hoër vlakke van voedingstofakkumulasie in die vleis.

Effens groter korrelgroottes is verkry waar N toedienings gemaak is. Dit word toegeskryf aan klein toenames in vroeë vegetatiewe groei, wat dus beter reaksie op GA_3 behandelings tot gevolg gehad het. Die gebruik van GA_3 vir korrelvergroting word ook beskou as die rede waarom K-bemesting, wat tot hoër vlakke van K in die korrels aanleiding gegee het, nie korrelgrootte, soos by wyndruiwe, bevorder het nie.

Hoër NO_3^- in die grond (water), na aanleiding van N toedienings, het aanleiding gegee tot hoer vlakke van N in die korrels. Dit het plaasgevind ten spyte van sub-optimale grond pH wat deur die oormatige N toedienings veroorsaak is.

Korrel K konsentrasie en -inhoud is deur K-bemesting verhoog. Vinnige opname en translokasie van K na die korrels het ook geblyk die rede te wees waarom die verlaagde voedingstatus van die stokke a.g.v. oorbemesting met N nie die korrels se K inhoud geaffekteer het nie. Die vinnige groeitempo van die korrels, tesame met lae vlakke van Ca opname, asook korrels se Ca opname wat tydens rypwording ophou, word as die redes vir die tekorte aan behandelingseffekte beskou.

Lae vlakke van bederf, asook ‘n tekort aan betroubare tendense dat bederf deur N-bemesting verhoog word, is verkry. Daar moet vasgestel word of daar N vlakke in die korrels is waarbo hul vatbaarheid vir swaminfeksies verhoog word, en of daar spesifieke N verbindings is wat die korrels meer vatbaar maak vir bederf. Indikasies dat K-bemesting interne verbruiningsvlakke verhoog het, regverdig verdere ondersoek. Korrelkwaliteit is nie deur grond- of trosgerigte toedienings bevoordeel nie.

Die negatiewe effek van oormatige bemesting op die chemiese samestelling van sandgronde is weer deur hierdie navorsing uitgelig. Dit lei tot oneffektiewe bemesting en ‘n tekort aan die verlangde effekte.

Blaarontledings blyk onbetroubaar te wees as aanduiding van voedingstof beskikbaarheid. Dit moet evalueer word saam met grondontledings en ook seisoenale variasie in ag neem. Die gevaar om slegs deur gepubliseerde norme gelei te word wanneer bemestingspraktyke bepaal word, is weer deur hierdie navorsing uitgelig.

Voorst is daar in hierdie navorsing gevind dat voedingstof akkumulasiepatrone van ‘n baie vroeë kultivar soos Prime Seedless alreeds voor deurslaan begin verander a.g.v. omgewingstoestande en korrelgroei wat tot ‘n veel hoër vleis:dop verhouding aanleiding gee. Toekomstige navorsing op tafeldruiwe behoort die faktore en dinamika wat voedingstofkonsentrasie en -akkumulasie in korrels van vroeë, groot korrel, pitlose tafeldruifkultivars beïnvloed verder te ondersoek.

BIOGRAPHICAL SKETCH

Pieter Raath matriculated from DF Malan High School in 1984. He studied BScAgric (Soil Science and Chemistry) at Stellenbosch University, graduating in 1989. The following year he started his BScAgric Hons (Soil Science), whilst also finishing a pre-graduate course in Viticulture. He was employed by ARC Infruitec-Nietvoorbij from 1991, first as a junior researcher and then as a researcher. During this period, he completed his MScAgric degree, titled “The effect of soil management practices on N-releasing capacity of vineyard soils in the Western Cape”, and graduated in March 1994. Since then he has worked in agricultural business development as a table grape producer and consultant. He has been employed as a lecturer in viticulture at the Department of Viticulture and Oenology, Stellenbosch University, since February 2005.

ACKNOWLEDGEMENTS

I thank my Lord and Saviour Jesus Christ for helping me to be the best I can be. On Him I depend to steadfastly run the race of life.

My sincere gratitude to my promotor, Prof. Kobus Hunter, for his support, guidance, kindness and patience throughout this study.

I would like to express my appreciation to my co-promotor and long-time mentor, Dr. Kobus Conradie, for his wisdom, example, kindness and unselfish provision of valuable insights.

A special word of thanks to my previous manager, Hugh Campbell, for creating an enabling work environment and for his encouragement to see this dissertation completed.

I thank DFPT Research and the South African Table Grape Industry for financial support.

I thank my colleagues at the Department of Viticulture and Oenology for their support and understanding during my absence to finish this dissertation.

Thank you to Derick Clift for allowing me to use his vineyard for this study, his interest in research and his generous provision of assistance to make the trial a success.

Special thanks to my wife, Adri, for her love, support and encouragement.

I dedicate this dissertation to all table grape producers who seek to cultivate table grapes responsibly and who gladly use information, generated through research, to produce grapes to the best of their ability.

PREFACE

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

Chapter I **Literature review**

Chapter II **Research results**

Excessive N, K and Ca fertilisation effects on vine growth and leaf chlorophyll content of an early ripening table grape cultivar (*Vitis vinifera* L. cv. Prime Seedless), grafted onto Ramsey on a sandy soil.

Chapter III **Research results**

Excessive N, K and Ca fertilisation effects on leaf and fruit nutrient status of an early ripening table grape cultivar (*Vitis vinifera* L. cv. Prime Seedless), grafted onto Ramsey on a sandy soil.

Chapter IV **Research results**

Accumulation of macro-nutrients (N, P, K, Ca and Mg) in berries by an early ripening table grape cultivar (*Vitis vinifera* L. cv. Prime Seedless) on a sandy soil.

Chapter V **Research results**

Excessive N, K and Ca fertilisation effects on ripening, berry nutrient content and post-harvest quality of an early ripening table grape cultivar (*Vitis vinifera* L. cv. Prime Seedless), grafted onto Ramsey on a sandy soil.

Chapter VI **Research results**

General conclusions

INDEX

CHAPTER I

Introduction

The table grape (*Vitis vinifera* L.) is a non-climacteric fruit for which consumer acceptance is attained by high soluble sugar contents and berry appearance. A lack of defects, such as decay, cracked berries, browning, soft and shrivelled berries is required after transport and cold storage. Optimal vine performance, berry development and ripening as well as accurate harvest time are therefore prerequisites for successful table grape production and marketing. The supply of mineral nutrients affects plant growth and physiology, requiring a balanced mineral nutrient supply to ensure vine performance and to avoid excessive vigour or mineral deficiencies.

In the apple industry it has been established that fruit quality is dependent on its mineral composition. Fertilisation therefore started to take into account the mineral composition of the fruit and an optimal balance between different minerals, particularly potassium (K) and calcium (Ca) is sought. For grapes it is generally accepted that some aspects of fruit quality, such as solid concentration, are positively correlated to fruit K, while during storage fruit quality is favoured by low N and high Ca levels. This is why K and Ca fertilisation has become common practices in South Africa for table grapes, even where soil K and Ca are sufficient. In order to manipulate mineral content and balance in grapes, it is important to know the dynamics of nutrient accumulation in developing berries. Due to its many ascribed functions, potassium (K) is regarded by many producers as a most critical nutrient ensuring successful sugar accumulation and colour development. The growth in consumption of calcium (Ca) containing fertilisers and Ca foliar applications is furthermore evidence of the popular believe that, due to the fact that it is found in high concentrations in plant cell walls, a luxurious supply of Ca is required to ensure post-harvest berry quality.

Nitrogen, potassium and calcium in the soil

Various fertilisers are used in grapevine nutrition, of which limestone ammonia nitrate, potassium chloride and calcium nitrate are typical N, K and Ca sources (Conradie, 1994). According to Follet *et al*. (1981) LAN has no effect on soil pH, while KCl are feared by some producers to enhance salinity symptoms in soils containing high concentrations of salts.

Nitrogen, potassium and calcium in the grapevine

Mineral nutrients can be divided into two broad categories, based on their accumulation patterns: (1) those elements that continue to accumulate throughout berry growth, and (2) those elements that accumulate mostly prior to vèraison. Among the first group are N, K, phosphorus (P) and magnesium (Mg), while the second group includes Ca (Rogiers *et al*., 2006).

Nitrogen (N): Nitrogen is one of the most important nutrients as far as growth (Keller *et al*., 1998; Roubelakis-Angelakis & Kliewer, 1992), production and fruit quality of grapevines are concerned (Conradie, 1980; Keller *et al*., 1998; Porro *et al.*, 1995). It alters plant composition much more than any other mineral nutrient (Wermelinger, 1991; Roubelakis-Angelakis & Kliewer, 1992; Marschner, 1995) and a good N supply can relieve some plant stress symptoms (Miklós *et al*., 2000), e.g. N applied at berry set was found to lead to lower levels of phenolic compounds in white grapes and tannins in Merlot, but higher glutathione levels (Choné *et al*., 2006). Since about 50% of N is located in the proteins that form the light harvesting complex, there also is a high correlation between leaf N allocated in chloroplasts and the functioning of the photosynthetic system (Porro *et al*., 1995).

Since it is the plant nutrient most likely to be deficient in grapevines, N is most commonly applied to vineyards to ensure sufficient growth (Conradie, 1994; Roubelakis-Angelakis & Kliewer, 1992). The response obtained from N applications, however, depends largely on the cultivar (Neilsen *et al*., 2010). Grapevines have a high ability to take up N from the soil after bloom (Conradie, 1980; Keller *et al.*, 1998; Zapata *et al*., 2004; Choné *et al*., 2006). Most NO_3^- taken up from the soil is reduced to a useable (ammoniacal) form by nitrate reductase and nitrite reductase before it is incorporated into organic forms, which are mostly amino acids. Arginine is one of the most abundant amino acids and is of special importance in *Vitis spp*. because it is a major N-storage compound and also participates in the biosynthesis of other amino acids (Roubelakis-Angelakis & Kliewer, 1992; Zapata *et al*., 2004). Accumulation of N-reserves in the grapevine during the later part of the growing season occurs in all climatic conditions (Conradie, 1992a). Remobilisation of these N-reserves, accumulated by the grapevine during the previous season, play an important role in sustaining new growth at the start of the next season (Conradie, 1980; 1986b, Peacock *et al*., 1989; Conradie, 1992a; Millard, 1995). Partitioning of N in the grapevine has been discussed in detail by Conradie (1991), who also found that translocation and distribution of N are affected by the nutrient status of the vine. Over-application of N increases the N-content of grapes, and is also associated with increased N contents of vegetative organs and possibly excessive vigour. Furthermore, it was found that with insufficient soil N supply during the phases bud break to

bloom and end of bloom to véraison, the permanent structure will have to supply a larger fraction of the N demand of the new growth (Conradie, 1991). Conradie (1992a) established that roots, permanent wood, leaves and shoots all play an important part in satisfying the N demand of bunches, even with an adequate supply of soil N being available. This highlights the fact that N-fertilisation will only indirectly affect the N-status of bunches.

Potassium (K): Potassium is an essential nutrient (Mpelasoka *et al*., 2003) and is required for crop yield (Conradie & De Wet, 1985; Hunter *et al.,* 2000) and vine growth (Conradie & De Wet, 1985), especially when applied on soil low in available K (Kasimatis & Christensen, 1976). According to Clarkson & Hanson (1980), K has four physiological-biochemical roles, namely: (1) enzyme activation; (2) cellular membrane transport processes and translocation of assimilates; (3) anion neutralisation which is essential in maintenance of membrane potential; and (4) osmotic potential regulation, which is one of the most important mechanisms in the control of plant water relations, turgor maintenance and growth.

Active uptake, defined as ion transport against an electrochemical gradient where the concentration in the inner cell is higher than in the outer solution, is the process by which only K can be taken up (Kirkby, 1979).

Bravdo & Hepner (1987) are of the opinion that grapevines have a better ability to utilize soil K than most other plants. Saayman (1981) and also Conradie & De Wet (1985) found that the South African approach to K fertilisation on soil with more than 10% clay, i.e. to strive for 4% of CEC, is realistic and any K added in excess of this amount is unlikely to have any significant effect. The guideline of applying 3 kg of K for every ton of grapes produced is also sufficient to maintain existing K levels in soils. The impact of K fertiliser on the level of available soil K and uptake is influenced by various factors, such as the amount of fertiliser applied, the timing and frequency of application, soil characteristics (Conradie, 1994), the amount and frequency of irrigation (Mpelasoka *et al*., 2003), plant root activity (Mengel & Kirkby, 1982) and rootstock-scion combination (Wolpert *et al*., 2005). At low soil pH, however, the high H^+ inhibits K^+ uptake (Kirkby, 1979). In situations of adequate to high soil K, a growth response to K fertilisation is not clear (Iland, 1988), while in other cases K fertilisation led to stronger vegetative growth (Conradie & de Wet, 1985; Hunter *et al.*, 2000).

The accumulation of K in permanent structures of the vine can take place throughout the growing season, including the post-harvest period (Conradie, 1981a). Potassium in these pools may be mobilised to support the new roots, stems, leaves and clusters when the uptake from the soil is insufficient to meet the demand (Conradie, 1981a; Mpelasoka *et al.*,

2003). During their development, berries appear to be the strongest sink for K, especially between vèraison and harvest (Conradie, 1981a). This may be due to the high demand of the berry for K during rapid cell expansion (Storey, 1987).

Despite potential differences in root K uptake capacity between different rootstocks due to differences in rooting morphology and density in the profile, there also appear to be differences in xylem loading and translocation from roots to shoots among different rootstocks (Mpelasoka *et al*., 2003). Rühl (2000) found that xylem sap K content varied between rootstocks at high K supply levels, but not at low K supply. They assumed that at high levels of K supply active transport is not required and that K uptake is passive, possibly using K-channels, which vary in effectiveness between rootstock varieties.

Calcium (Ca): In terms of its requirements in plants, Ca is classified as a secondary nutrient (Millaway & Wiersholm, 1979) and also the most immobile macronutrient (Ferguson & Bollard, 1976; Hanger, 1979). Although growth is benefitted by Ca (Saxton 2002; Domingos *et al*., 2004), grapevines have small requirements for this nutrient (Conradie, 1981a; Follet *et al.* 1981). Calcium deficiencies are therefore rarely observed in vineyards (Conradie, 1981a; Rorison & Robinson, 1984).

Calcium uptake is passive and follows the influx of water. It, however, is concentration dependent, with diffusion being the main process responsible for uptake at higher concentrations (Kirkby, 1979). Soil pH plays an important role in Ca-uptake (Follet *et al*.,1981; Clarkson *et al*., 1984; Storey *et al*., 2003). At low soil pH (pH_{KCl}<4), aluminium (Al^{3+}) is usually the dominant cation at the soil cation exchange sites. It interferes with Ca-uptake by reducing Ca binding to cell walls of root cells (Storey *et al.*, 2003). Under these conditions, plants may be very susceptible to Ca deficiency (Clarkson, 1984). On the other hand, in non-acid soils (pH_{KCl}>4) the dominant cation at the soil cation exchange sites usually is Ca (Follet *et al*.,1981). If the [Ca^{2+}] of the soil solution is sufficient or high, there is a correlation between water flow and Ca movement to the shoot. However, at low [Ca^{2+}] in the soil, the low [Ca^{2+}] becomes the rate limiting factor in uptake because Ca^{2+} needs to compete with other cations at the exchange complex of the apoplast (Clarkson, 1984).

Kirkby & Pilbeam (1984) stated that the soil solution usually provides an adequate supply of Ca to plants. Calcium uptake can, however, be influenced by the uptake of other ions, e.g NH_4^+, K^+, and Mg^{2+} (Kirkby, 1979) and since Ca moves with water, its rate of translocation, and subsequent tissue content, is subject to the rate of transpiration (Millaway & Wiersholm, 1979; Demarty *et al.*, 1984). Rapid transpiration conditions lead to decreased Ca being

received by the fruit (Wiersum, 1979; Donèche & Chardonnet, 1992; Sen *et al.*, 2009). Avoidance of dense foliage and too much vigour therefore result in higher fruit Ca contents (Drake *et al.*, 1979). The favourable effect of summer pruning to diminish leaf surface area of apples was found to reduce bitter pit and may be an expression of this (Wiersum, 1979).

The highest amount of Ca in the plant is located apoplastic, where it has an important structural and functional role in plant cell walls (Zocchi & Mignani, 1995). About 60% of Ca in plant tissues is associated with the cell walls, while 7% with the membranes and 33% with the soluble fraction (Christiansen & Foy, 1979). Calcium is particularly located in the cell walls of xylem vessels (Demarty *et al.*, 1984), because it moves through the apoplast and because of the continuous influx *via* the xylem (Zocchi & Mignani, 1995). Failure to re-export Ca *via* the phloem leads to its accumulation in high-rate transpiring tissues (such as expanding leaves), while deficiencies in low-rate transpiring tissues (such as fruit) commonly develops (Kirkby, 1979; Millaway & Wiersholm, 1979; Kirkby & Pilbeam, 1984; Zocchi & Mignani, 1995; Bonomelli & Ruiz, 2010). Calcium movement to growing tips and fruit often decreases as the season progresses due to an increase in the exchange positions in the xylem that bind Ca (Ferguson & Bollard, 1976; Hanger, 1979); hence a continuous supply of Ca is essential (Mengel & Kirkby, 1982). Redistribution of stored Ca does, however, occur. This stored Ca is mainly found in winter canes and roots of vines (Conradie, 1981a). For trees, this stored Ca is released into the xylem sap (Wiersum, 1979). Shoots and fruit compete for available plant Ca. Because shoots have a higher demand, N stimulated shoot growth diverts the Ca sink from the fruit to the shoot. This happens in response to high N applications (Kirkby, 1979).

Easterwood (2002) is of the opinion that the role of Ca in plant nutrition is often eclipsed by interest in macro-nutrients or specific micro-nutrients. However, there is an increasing incidence of a number of physiological disorders associated with inadequate Ca nutrition in various crops grown under intensive horticulture conditions.

Use of foliar N, K and Ca concentration analyses

According to Montañés *et al.* (1995) plant analyses was first applied in France by Lagatu & Maume in 1929 as a diagnostic technique to determine the crop nutrient status. Since then, leaf analyses are commonly used as indication of the availability of nutrient elements in the soil, with the possibility to identify visually non-detectable deficiencies and to compensate for these in the fertilisation programme (Domingos *et al*., 2004). However, meaningful correlations between nutrients in the soil and leaves or fruit could not be found for apples (Marcelle, 1990a; Montañés *et al.,* 1995) or grapevines (Morris *et al*., 2003; Mpelasoka *et al*.,

2003). Montañés *et al*. (1995) stated that interpretation of foliar analyses was found to be of little value in research evaluating crop response to fertiliser applications. Furthermore, there often is little difference in the leaf composition of high- and low-yielding vineyards, leading to the conclusion that leaf analyses cannot replace soil analyses (Conradie, 1986c). This was ascribed to the fact that nutrient availability in the soil is only one factor affecting nutrient uptake and plant growth. The biggest problem, experienced world-wide, however, is a lack of reliable norms for the interpretation of analyses figures (Conradie, 1986c; Montañés *et al*., 1995). The other drawback of leaf analyses as a diagnostic tool is that, apart from the seasonal variation, it is also influenced by soil physical and chemical properties (Conradie, 1981b; Roubelakis-Angelakis & Kliewer, 1992), rootstock and scion cultivars, climate (Conradie, 1986c), the type of tissue sample, leaf age and position on the shoot (Conradie, 1981b; Roubelakis-Angelakis & Kliewer, 1992) as well as diseases and cultural practices (Conradie, 1986c). According to Conradie (1992b), the use of leaf analyses is of little practical use in healthy, good performing vineyards, while Roubelakis-Angelakis & Kliewer (1992) and Conradie & Van Huyssteen (1996) stated that the range between norms for total N that indicate deficiency and sufficiency is too small to reliably estimate the nitrogen status of grapevines. Seasonal effects also influence the nutrient content in the leaves, with the highest level of susceptibility to variation obtained for N when sampled at fruit set and for P, K, Ca & Mg when analysed at harvest time (Porro *et al*., 1995).

Considering the seasonal variations in leaf concentrations of N, P, K, Ca and Mg it appears that the most suitable organ to analyse is petiole tissue (Conradie, 1981b; Christensen, 1984; Bravdo & Hepner, 1987) and the most stable time for sampling is during the month following bloom (Conradie, 1981b). Porro *et al.* (1995) stated that low variability in nutrient levels is obtained for P, K and Mg when the leaves are analysed at fruit set. The same is true for N, Ca and B when analysed at vèraison.

The usefulness of grapevine petiole analyses for NO_3-N as a guideline for N fertilisation was investigated by Conradie & van Huyssteen (1996) because total N often shows a very narrow range between deficiently and adequately supplied vineyards. It was found that the NO_3-N content of petioles, analysed at full-bloom, could be used more effectively as a guideline for N-fertilisation, but only for specific cultivars. Christensen (1984) ascribed the wide seasonal and cultivar differences of NO_3-N levels in petioles, compared to total N, to the fact that assimilation of NO_3 and NH_4 is influenced by light, temperature and nitrate reductase activity. Nitrate in petioles is inversely related to light availability (Perez & Kliewer, 1982). Several factors influence nitrate levels in petioles. These include cultivar and rootstock, the phenological stage of the vine, irrigation and rainfall as well as temperature. This led to the

conclusion that when the climate is variable, especially during bloom, petiole nitrate may not reflect the true nitrogen status of vineyards (Perez & Kliewer, 1982).

Hunter *et al*. (2000) succeeded in raising the total N content of Sauvignon blanc/110 Richter vine leaves at harvest time on a 10% clay content soil through N applications alone. When K was applied, alone or in combination with N, leaf N content remained unaffected or decreased. Porro *et al.* (1995) found a high correlation between N levels of leaves at set and vineyard performance (i.e. yield and vigour). Conradie (1981b) and Porro *et al.* (1995) found that leaf N decreased throughout the season.

Adding potassium to the soil resulted in higher K concentration in leaf blades of both main and lateral shoots and berries at harvest for Cabernet Sauvignon grapevines (Poni *et al*., 2003). No relationship could be found between level of petiole K and vegetative growth of Concord vines (Morris *et al*., 1980).

Potassium is the major cation in the leaf and shoot xylem sap with leaf xylem sap flow being the highest in the middle leaves and lowest in the old and young leaves (Peuke, 2000). This might be a reason for differences in assimilation and transpirational activities among different leaf ages.

Morris *et al.* (1980) found that K fertilisation at increasing levels leads to a marked increase in K content of petioles and a positive correlation between petiole K and berry juice K content. The type of correlation (either linear or curvilinear), however, differed from region to region. Other authors found that a poor correlation between petiole K and berry K seem to exist (Morris *et al*., 2003; Mpelasoka *et al*., 2003). The relationship between petiole K and berry K is likely to change over the season due to changes in sink strength (Mpelasoka *et al*., 2003) as well as seasonal climatic conditions (Etchebarne *et al.*, 2009). The K content of grapevine leaf blades and shoots was found to be higher where grapevines experience shading, compared to shoots fully exposed to sunlight (Porro *et al.*, 1995). Bogoni *et al.* (1995) found that leaf K content showed a negative correlation with soil temperature.

Both petiole and leaf laminae K levels are higher at bloom and vèraison than at harvest (Wolpert *et al*., 2005), indicating a translocation of K from the leaves to the berries from vèraison onwards (Conradie, 1981a; Conradie, 1981b). The practice of comparing petiole (or laminae) analyses at bloom time with critical levels might be insufficient indicators of vine K nutritional status without taking into account the rootstock-scion combination (Wolpert *et al.,* 2005) or soil analyses (Conradie, 1986c) and vineyard canopy (Iland, 1988).

Green & Smith (1979) and Bogoni *et al*. (1995) found a positive correlation between soil Ca carbonate content and Ca in the leaves. Green & Smith (1979), Sen *et al*. (2010) and Wójcik *et al*. (2010), however, stated that soils rich in Ca, or Ca applications to soils, do not guarantee a sufficient supply of Ca to the fruit itself. Attempts to increase apple fruit Ca concentration through soil applications of $CaCO_3$ or $CaSO_4$ (Schlegel & Schönherr, 2002; Wójcik *et al*. 2010) and table grape Ca concentration through soil and foliar applications of $CaCl_2$ have been ineffective (Bonomelli & Ruiz, 2010). Kirkby & Pilbeam (1984) quoted previous research which showed that for 18 different plant species it was found that regardless whether they were grown in nutrient solutions or in the field, the Ca concentration for a given plant species did not vary greatly. However, Bogoni *et al.* (1995) found that Ca and Mg content in the leaves were positively correlated with soil temperature, whereas Green & Smith (1979) as well as Bogoni *et al*. (1995) found a positive correlation between soil Ca carbonate content and Ca in the leaves. Calcium content of grapevine leaf blades, petioles and shoots was also found to be higher where grapevines shoots are in direct sunlight compared to being shaded (Porro *et al*., 1995).

Calcium from the soil moves readily into the metabolically active tissues of expanding young leaves. When Ca supply to the roots is limited, the young leaves are unable to compete for Ca with the lignified tissues of the older leaves. Therefore, the young leaves are the first to show symptoms of Ca deficiency (Shear & Faust, 1970), while Ca concentration of mature leaves increases throughout the season (Conradie, 1981b; Porro *et al.,* 1995). The affinity of lignin for Ca may be responsible for the accumulation of Ca in mature leaves in proportion to their age (Shear & Faust, 1970).

Nitrogen, potassium and calcium in the berry

In the apple industry it has been established that fruit quality is dependent on the fruit mineral composition with an optimal balance between different minerals, and in particular K/Ca, that is sought (Marcelle, 1990a; Marcelle 1990b). For grapevines conflicting results have been obtained, where in some cases no relationship between soil nutrient status and must nutrient concentration could be found (Morris *et al*., 1982b), while Iland (1988) stated that there is a significant correlation between petiole K concentration and the K content of berry juice. It is generally accepted that some aspects of fruit quality, such as solid concentration, are positively correlated to fruit K (Rogiers *et al.,* 2006), while during storage fruit quality is favoured by low N and high Ca levels (Marcelle, 1995; Bonomelli & Ruiz, 2010). This is why K and Ca fertilisation has become common practices in South African table grape production systems, even if the soil K and Ca are sufficient.

Nitrogen: Nitrogen fixed in grapes originates directly from the uptake of nitrate from the soil solution or indirectly from mobilization of storage compounds, which means that nitrogen concentration (both total N and amino acid concentration) in the berry depends on the nitrate supply in the soil as well as the reserve N of the vine (Löhnertz *et al.,* 2000). In the grape berry, N mainly occurs as the free amino acids proline and arginine (Kliewer, 1968; Ough & Stashak, 1974). The latter is atypical of other fruit (Tagliavini, 2000). Increased application of N fertilisers to grapevines is mirrored by higher concentrations of free amino acids in berries (Ough & Stashak, 1974; Kliewer, 1977; Löhnertz *et al.*, 2000; Frank, *et al*., 2005). Conradie (1986a) is, however, of the opinion that the time of N application determines whether the N is translocated to the crop as amino acid N (soluble N) or incorporated in proteins, while both Roubelakis-Angelakis & Kliewer (1992) and Löhnertz *et al.* (2000) found that berries contain higher amounts of proteins during warm dry years, resulting in reduced amounts of amino acids. Soluble protein content also increases with maturity. Linsenmeier *et al.* (2008) indicated that higher must N concentrations were obtained from N applications at fruit set than at budbreak, while Neilsen *et al*. (2010), on the other hand, found inconsistent changes in berry N concentrations.

Vine nitrogen status has a strong influence on vine vigour, resulting in higher must acidity and sensitivity to *Botrytis* infection (Conradie, 1986; Choné *et al*., 2006). Low vine N status also limits Sauvignon blanc berry mass and titratable acidity, mainly malic acid (Choné *et al*., 2006). An abundant N supply decreases mass of grape skins and reduces sugar and acid levels of the pulp (Keller *et al*., 1998). Ruiz *et al*. (2004) found some sort of correlation between amino acid N content of berry skin and pulp of soft Thompson Seedless berries and arginine, as well as with putrescine levels in berries. The higher the contents, the softer were the berries. This can be ascribed to the fact that the amino acid concentration in berries, and in particular arginine, increases dramatically at the termination of ripening (Löhnertz *et al.*, 2000).

Potassium: The fruit is a large sink for K (Conradie, 1981a; Mpelasoka *et al*., 2003) and it is the major cation occurring in both the pulp and skin of the grape berry (Storey, 1987; Rogiers *et al.*, 2006). Berry K content generally increases over the season (Conradie, 1981a; Iland, 1988; Donèche & Chardonnet, 1992; Rogiers *et al.*, 2006) with a sharp escalation at the onset of ripening (Creasy *et al*., 1993). At harvest, clusters account for 66% of the total K content of the above-ground organs (Conradie, 1981a). From vèraison to harvest the K content accumulated in the berries exceeds the total amount taken up by the vine, while the K content in the trunk, roots, shoots and leaves decreases. This suggests that a significant

amount of K is translocated from other organs to the berries during this period (Conradie, 1981a). This remobilisation of K from other organs to the berries after vèraison is also reported in other studies (Conradie & De Wet, 1985; Williams & Biscay, 1991).

Potassium movement occurs in both xylem and phloem (Mengel & Kirkby, 1982). In grape berries the xylem is a minor route of K entry because xylem flow into the berry is low due to the low transpiration rate thereof. This especially decreases during berry growth and development due to degeneration of stomata to lenticels as well as the deposit of epicuticular wax (Blanke *et al*., 1999). Variation in K accumulation in the berry after véraison is associated with a change in berry water supply from the peripheral to the axial xylem system, and from the xylem system to the phloem system (Lang & Thorpe, 1989; Cabanne & Donèche, 2003). It has important implications for the ultimate mineral composition of the fruit, i.e. the increase in K and decrease in Ca concentration (Cabanne & Donèche, 2003). Potassium is the major cation in the leaf xylem sap and shoot xylem sap with leaf xylem sap flow being the highest in the middle leaves and lowest in the old and young leaves (Peuke, 2000). According to Iland (1988) and Conradie (1981b), potassium concentration in petioles and leaf laminae decreases as the season progresses, while it increases in the fruit. Furthermore, due to a link between leaf photosynthetic activity and K transport, any conditions that reduce leaf photosynthetic activity could contribute to increased K levels in the phloem and subsequently in the berries (Freeman *et al.*, 1982; Iland, 1988; Archer & Strauss, 1989, Esteban *et al*., 1999). Mpelasoka *et al.* (2003), however, are of the opinion that berry K concentration need not increase in dense canopies, especially in conditions where berry growth and berry K accumulation are maintained at similar rates. This implies that factors such as cultivar (berry size), crop load, climatic conditions and cultural practices that affect the rate of berry growth, would affect berry K concentration. Furthermore, increased irrigation increases berry K accumulation (Hepner & Bravdo, 1987; Iland, 1988; Esteban *et al*., 1999; Etchebarne *et al*., 2009). Variation in berry K is also caused by differences in root K uptake capacity between rootstocks as well as differences in xylem loading of K and translocation from roots to the shoots (Mpelasoka *et al*., 2003).

Related to berry growth, Mpelasoka *et al.* (2003) highlighted the role of K in cellular growth when they suggested that the cell walls in the berry skin loosens at the onset of stage III (rapid cell expansion) of berry growth. This loosening of the cell walls involves acidification of the apoplast and the activation of cell wall loosening enzymes. Kirkby & Pilbeam (1984) also showed that fast growing fruit, which are more dependent on phloem than xylem, have a higher K/Ca ratio than slower growing fruit.

Marcelle (1990b) found a positive correlation between apple fruit sugar content and the K/Ca ratio of fruit flesh. Rogiers *et al*. (2006) and Etchebarne *et al*. (2009) indicated that due to a strong correlation found between K accumulation and berry fresh mass, K plays a key role in cell expansion, and therefore berry growth. These authors as well as Mpelasoka *et al*. (2003) also found a strong relationship between berry K content and both sugar and dry mass accumulation. Shaded leaves transported more K to the berries than exposed leaves (Iland, 1988). Conradie & de Wet (1985), however, found no significant increase in berry sugar where up to 90 kg K per ha was applied on soil with a K saturation close to 4 percent. Furthermore, under conditions of low sugar production, K also accumulated in the berries. More studies were suggested to determine the relationship between berry sugar accumulation and berry K accumulation.

Donèche & Chardonnet (1992) found that both flesh and skin cells accumulate K during ripening with the flesh containing large quantities of K. Deficiency in K results in unevenly ripened berries (Mullins *et al.,* 1996). According to Iland (1988), Coombe (1992) and Mpelasoka *et al*. (2003), K concentration in the berry skin is higher than in the flesh. A high degree of difference in K concentration among berry tissues is observed (Storey, 1987; Mpelasoka *et al*., 2003; Rogiers *et al.*, 2006), which is ascribed to wide variation among varieties and among rootstock/scion combinations and seed number (Mpelasoka *et al*., 2003). The latter generally influences berry size, affecting K partitioning in the berry (Mpelasoka *et al*., 2003). For example, Storey (1987) found that skin K concentration was higher for smaller berries compared to larger berries.

Morris *et al*. (1980) and Morris *et al.* (1982a) found that K fertilisation above the adequate levels had no positive effect on total pigment content of grape juice and no improvement in colour was observed. Furthermore, Morris *et al.* (1982a) and Mpelasoka *et al*. (2003) were of the opinion that the detrimental effect that excessive K fertilisation had on colour quality and acidity of grape juice, can also be expected in intact grapes. They speculated that a substitution of K^+ cations for H^+ in the grape tissue would increase the pH despite high acidity. This high pH would then reduce the colour of the berries. Furthermore, high juice K precipitates tartaric acid, which is a significantly stronger acid than malic acid (Rühl, 2000), in salt form so that the free tartrate decreases, leading to reduced tartaric acid:malic acid ratios (Mpelasoka *et al*., 2003). This can affect the taste of the berries significantly, because tartrate has a more fresh crisp acid taste than malate (Rühl, 2000).

Adding potassium to the soil resulted in higher K concentration in berries at harvest of Cabernet Sauvignon grapevines (Poni *et al*., 2003). Potassium fertilisation on soil containing

sufficient K (4 percent of the CEC) significantly increased berry titratable acidity content, increased berry size, suppressed the N content of grape juice and appeared to increase resistance against *Botrytis* rot, which was ascribed to the fact that K suppressed the uptake of N (Conradie & De Wet, 1985).

Calcium: In all fruits there is a decline in Ca influx during growth. This results not only from an increase in solute influx *via* the phloem during fruit ripening, but also from a decline in cell division rate, reduced formation of new binding sites for Ca, and a increase in volume/surface area, with a reduction in transpiration per unit weight of fruit (Kirkby & Pilbeam, 1984).

Neither liming nor $CaCl_2$ soil application successfully increases apple fruit Ca concentration (Green & Smith, 1979; Sen *et al*., 2010). This was ascribed to the fact that xylem Ca transport is mainly directed to leaf tissues with only 5-10% of absorbed Ca being transported to fruit tissues (Wójcik *et al*., 2010). Mason (1979) and Terblanche *et al*. (1979), however, reported that $CaNO_3$ applications to a heavy soil with high K and Mg saturation controlled bitter pit.

Although the most active uptake of Ca by the grapevine is between the period bud burst to vèraison, the grapes have a very narrow window for calcium uptake (bloom to véraison) (Conradie, 1981a), which is a six to seven week period. Various other studies (Donèche & Chardonnet, 1992; Schaller *et al.,* 1992; Ollat & Gaudillère, 1996; Rogiers *et al*., 2001; Cabanne & Donèche, 2002) showed that grape berries accumulate Ca throughout their development. This increase was found to be the fastest during the first stage of berry growth, while an increase post-vèraison was exclusively due to Ca accumulation in the seeds (Cabanne & Donèche, 2003; Etcherbane *et al*., 2009). On the other hand, other research indicated that calcium accumulation stops when green berries start to soften, often even before vèraison, which is also related to a decrease in xylem flow (Possner & Kliewer, 1985; Creasy *et al*., 1993). Donèche & Chardonnet (1992) ascribed the decrease in Ca and Mg concentration after vèraison in grape berries to dilution that occurs as a result of volume increase brought about by cell growth. In apples, fruit size was also found to affect the Ca concentration, with larger fruit having lower Ca concentrations (Drake *et al*., 1979; Perring, 1979).

Calcium content of the pericarp increases until vèraison, and then decreases (Cabanne & Donèche, 2003). Etchebarne *et al*. (2009) found that Ca transport to skin cells occurs during ripening and, because of this, a dramatic reduction in the concentration of Ca in cells of the flesh occurs. The Ca content in the seed increases throughout the development of the

berries, including ripening (During *et al*., 1987). Calcium is the second most abundant cation in the skin, occurring mainly as crystalline needle-shaped deposits, in contrast to K, which does not exist as crystalline deposits, but rather in a soluble form in the vacuoles of hypodermal cells (Storey, 1987).

The level of Ca increase in the berry during the active uptake period depends on the weather conditions before vèraison. Cool dry weather between flowering and vèraison reduces calcium uptake (Saxton, 2002, Etchebarne *et al*., 2009). The average Ca concentration of young apples was found to be much higher than mature apples and the amount of Ca retained by the young fruits is often too small (Schlegel & Schönherr, 2002). Moreover, the Ca content of grape berries depends on biological (cultivar & rootstock), edaphic (available soil cations and water content) and climatic (Boselli *et al*., 1998; Esteban *et al*., 1999) factors. Consequently, the evolution of the Ca content in the various berry compartments can vary from year to year or with gapevine cultivar (Cabanne & Donèche, 2003; Rogiers *et al*., 2006; Etchebarne *et al*., 2009).

Calcium has a major effect on membrane integrity (Fuller, 1976; Poovaiah, 1979) and the activity of membrane-bound enzymes (Poovaiah *et al*., 1988), in that it mediates membrane continuity with regards to cell organic constituents (Christiansen & Foy, 1979). It also activates membrane-bound ATPase that mediates K movement (Christiansen & Foy, 1979). A loss of membrane bound Ca, either by replacement with K^+ or through chelation, increases membrane permeability. Calcium binds anionic groups of the membrane structure to form bridges between structural components, thereby maintaining a selective permeability by pore radius or surface charge relations as well as membrane structural integrity. Ripening is caused by an increase in membrane permeability and dissolving of the middle lamella, both processes favoured by low levels of Ca (Kirkby & Pilbeam, 1984). Calcium also maintains mitochondrial integrity, the endoplasmic reticulum and other cytoplasmic membranes (Christiansen & Foy, 1979).

Ripening (and senescence) is a prerequisite to softening in fruit. Ripening is caused by changes in the permeability properties of cell membranes. In the case of climacteric fruit, membrane leakage increases prior to climacteric rise in respiration. Calcium decreases the hydraulic permeability in apple fruit (Poovaiah *et al*., 1988; Casero *et al.,* 2010). Therefore, maintenance of relatively high Ca concentrations, associated with relatively low K concentrations in fruit tissue, reduces rates of respiration, reduces ethylene production and slows down softening of fruit flesh (Marcelle, 1990a). Because various Ca-deficient plants showed extensive disintegration of mitochondria, ER and cytoplasmic membranes, it was

suggested that Ca has a major role in maintaining membrane integrity (Poovaiah *et al*., 1988).

Cell and cell wall structure are affected by Ca, K and P nutrition (Yang *et al*., 1997; Cabanne & Donèche, 2001; Saxton, 2002). With rapid growth (which includes berry expansion after véraison) the structural integrity of plant tissues is strongly coupled with Ca availability (Easterwood, 2002). If there is a deficiency of Ca, intracellular Ca takes precedence, so the amounts of Ca in the cell walls decrease (Saxton, 2002) and cell wall integrity reduces. Since it renders the substrate less accessible to polygalacturonase due to the intermolecular cross-links within the pectic polysaccharide matrix, Ca reduces cell wall breakdown (Poovaiah *et al*., 1988). Tagliavini *et al*. (2000), however, stated that all tissues subjected to fast volume expansion showed a low Ca requirement and that Ca has an inhibitory effect on cell growth.

Donèche & Chardonnet (1992) referred to the profound modifications that occur in cell structure of berry flesh during ripening, stating that it is due to the solubilisation of pectins brought about by migration of Ca from the flesh. The result is a net degradation of the cell wall takes place during ripening (Poovaiah *et al.*, 1988). Cell cohesion seems to play an important role in the textural quality of fruit (Poovaiah *et al*., 1988). This is obtained by cell-to-cell contact especially with regard to the middle lamella, which is rich in pectinaceous materials and which contributes to cell cohesiveness. This area is an important site for Ca interaction. So, with pectic polysaccharides being particularly abundant in the middle lamellar region, the bridges that form with Ca between these polymers, ensure flesh firmness. Pectic substances, cross-linked inter- and intra-molecular by Ca, are thought to be largely responsible for tissue rigidity (Cabanne & Donèche, 2001). This correlates with Casero *et al*. (2010) who ascribed to Ca a key role in the retention of apple fruit firmness.

Foliar application of Ca, for whatever reason, has been practiced in agriculture for more than 100 years. Today, foliar application of Ca as $Ca(NO_3)$ or $CaCl_2$ or other products, is a well-established practice to prevent bitter pit, cork spot and storage softening in apple fruit (Schlegel & Schönherr, 2002). Foliar applied Ca, however, is relatively immobile (Hanger, 1979) and under South African conditions it has been found that these sprays have on average only 16% effectiveness on apples (Terblanche *et al.*, 1975). Using a chelated Ca-product, Wójcik *et al.* (2010) found that although the Ca concentration of the fruit was increased slightly, they only found increases in fruit firmness and titratable acidity where continuous high application rates were applied from fruit set to harvest. Not one of the various types of foliar Ca treatments increased grape berry firmness after cold storage (Del Solar *et al.*, 2000). Likewise, Schlegel & Schönherr (2002) as well as Bonomelli & Ruiz

(2010) stated that even numerous spray applications of Ca did not achieve the desired effect. On the other hand, reports that Ca as foliar applications can increase yield and berry size of Italia grapes (Colapietra & Alexander, 2006) and also enhance sugar berry accumulation (Sen *et al.,* 2009) have been found. Post-harvest dips of apples and pears in Ca solutions to prevent storage losses are also common (Millaway & Wiersholm, 1979) and the most widely used control method for bitter pit and cork spot in apples remains to be foliar applications of $CaCl_2$ or $Ca(NO_3)_2$ (Drake *et al*., 1979; Gallerani *et al.*, 1990). Drake & Spayd (1983) and Sen *et al*. (2009) also published data showing that through $CaCl_2$ foliar applications firmness of apples is better retained.

The many studies about the effects of Ca applications on fruit quality, especially for apples, include different ways of delivery (soil vs. foliar), different chemical forms, and timing. The results are contradictory, and for table grapes, very limited.

Conclusions and objectives

Optimal vine performance and berry development are required for production of table grapes without defects. As the world-wide volumes of table grapes escalate, increased levels of production and larger berry size are being sought, early ripening cultivars are incorporated, and more rigorous fruit quality assessments are done. The potential of manipulated vine nutrition to maintain and extend the market value of South African grapes must therefore be elucidated. Although research, conducted over decades, has provided various insights regarding vine response to the nutrients discussed above, various unresolved issues remain. A project was therefore proposed to address a few of them, namely:

- To establish the nutrient accumulation patterns of Prime Seedless (*Vitis vinifera* L.), a very early seedless table grape cultivar that is produced with a minimum berry diameter of 18mm.
- To establish whether it would be beneficial to berry quality to apply K or Ca in addition to the established nutritional requirements.
- To establish whether berry Ca levels could be elevated and whether it benefits fruit quality.
- To understand the interaction of N, K and Ca in uptake and translocation to the berries as well as other potential negative outcomes from excessive applications of any of these nutrients.

Literature cited

Archer, E. & Strauss, H.C., 1989. Effect of shading on the performance of *Vitis vinifera* L. cv. Cabernet Sauvignon. S. Afr. J. Enol. Vitic. 10, 74 - 77.

Blanke, M.M., Pring, R.J. & Baker, E.A., 1999. Structure and elemental composition of grape berry stomata. J. Plant Physiol. 154, 477 - 481.

Bogoni, M., Panont, A., Valenti, L. & Scienza, A., 1995. Effects of soil physical and chemical conditions on grapevine nutritional status. Acta Hort. 383, 299 - 311.

Bonomelli, C. & Ruiz, R., 2010. Effects of foliar and soil calcium application on yield and quality of table grape cv. “Thompson Seedless”. J. Plant Nutr. 33, 299 - 314.

Boselli, M., Di Vaio, C. & Pica, B., 1998. Effect of soil moisture and transpiration on mineral content in leaves and berries of Cabernet Sauvignon grapevine. J. Pl. Nutrition 21, 1163 - 1178.

Bravdo, B. & Hepner, Y., 1987. Irrigation management and fertigation to optimize grape composition and vine performance. Acta Hort. 206, 49 - 67.

Cabanne, C. & Donèche, B., 2001. Changes in polygalacturonase activity and calcium content during ripening of grape berries. Am. J. Enol. Vitic. 52, 331 - 335.

Cabanne, C. & Donèche, B., 2003. Calcium accumulation and redistribution during the development of grape berry. Vitis 42, 19 - 21.

Casero, T., Benavides, A.L. & Recasens, I., 2010. Interrelation between fruit mineral content and pre-harvest calcium treatments on “Golden Smoothee” apple quality. J. Plant Nutr. 33, 27 - 37.

Choné, X., Lavigne-Cruège, V., Tominaga, T., van Leeuwen, C., Castagnède, C., Saucier, C. & Dubourdieu, D., 2006. Effect of vine nitrogen status on grape aromatic potential: Flavour precursors (S-cysteine conjugates), glutathione and phenolic content in *Vitis vinifera* (L.) cv. Sauvignon blanc grape juice. J. Int. Sci. Vigne Vin 40, 1 - 6.

Christensen, L. P., 1984. Nutrient level comparisons of leaf petioles and blades in twenty-six grape cultivars over three years (1979 through 1981). Am. J. Enol. Vitic. 35, 124 - 133.

Christiansen, M.N. & Foy, C.D., 1979. Fate and function of calcium in tissue. Commun. Soil Sci. Plant Anal. 10, 427 - 442.

Clarkson, D.T., 1984. Calcium transport between tissues and its distribution in the plant. Plant, Cell & Environ. 7, 449 - 456.

Clarkson, D.T. & Hanson, J.B., 1980. The mineral nutrition of higher plants. Annual Rev. Plant Physiol. 31, 239 - 298.

Colapietra, M. & Alexander, A., 2006. Effect of foliar fertilization on yield and quality of table grapes. Acta Hort. 721, 213 - 218.

Conradie, W.J., 1980. Seasonal uptake of nutrients by Chenin blanc in sand culture: I. Nitrogen. S. Afr. J. Enol. Vitic. 1, 59 - 65.

Conradie, W.J., 1981a. Seasonal uptake of nutrients by Chenin blanc in sand culture: II. Phosphorus, potassium, calcium and magnesium. S. Afr. J. Enol. Vitic. 2, 7 - 13.

Conradie, W.J., 1981b. Nutrient consumption by Chenin Blanc grown in sand culture and seasonal changes in the chemical composition of leaf blades and petioles. S. Afr. J. Enol. Vitic. 2, 15 - 18.

Conradie, W.J., 1986a. Nitrogen nutrition of the grapevine (*Vitis vinifera* L.), PhD Thesis, Univ. of Stellenbosch.

Conradie, W.J., 1986b. Utilisation of nitrogen by the grapevine as affected by time of application and soil type. S. Afr. J. Enol. Vitic. 7, 76 - 83.

Conradie, W.J., 1986c. Norms for leaf analyses of vines. Farming in South Africa: Viticulture and Oenology. E24.

Conradie, W.J., 1991. Translocation and storage of nitrogen by grapevines as affected by time of application. In: J.M. Rantz (ed) Proc. Int. Symp. On Nitrogen in Grapes & Wine. 18-19 June 1991, Seattle, Washington, USA. pp. 32 - 42.

Conradie, W.J., 1992a. Partitioning of nitrogen in grapevines during autumn and the utilisation of nitrogen reserves during the following growing season. S. Afr. J. Enol. Vitic. 13, 45 - 51.

Conradie, W.J., 1992b. Die nut van blaarontledings by wingerd. Wynboer, Nov., 75 - 76.

Conradie, W.J., 1994. Wingerdbemesting. Handleiding van die werksessie oor wingerdbemesting, Nietvoorbij, 30 September, ARC Research Institute for Fruit, Vine and Wine, Private Bag X 5026, Stellenbosch, 7600, R.S.A.

Conradie, W.J. & de Wet, T., 1985. The effect of potassium fertilisation of grapevines on yield and quality. Proc. Potassium Symp. Dept. Agric. & Water Supply, Pretoria, 1 - 3 October 1985, 181 - 183.

Conradie, W.J. & van Huyssteen, I., 1996. Nitrate content of grapevine petioles as a guideline for nitrogen fertilization. 76th General Assembly of the OIV, Cape Town, 10 - 18 November 1996.

Coombe, B.G., 1992. Research on development and ripening of the grape berry. Am. J. Enol. Vitic. 43, 101 - 110.

Creasy, G.L., Price, S.F. & Lombard, P.B., 1993. Evidence of xylem discontinuity in Pinot noir and Merlot grapes: Dye uptake and mineral composition during berry maturation. Am. J. Enol. Vitic. 44, 187 - 192.

Del Solar, C., Depallens, D., Neubauer, L., Pizarro, U. & Soza, J.A., 2000. Efectos de fitorreguladores, cacao, magnesio y anillardo sobre la calidad y condicion en uva de mesa cvs. (Thompson Seedless y Red Globe). Pharos, 7, 19 - 41.

Demarty, M., Morvan, C. & Thellier, M., 1984. Calcium in the cell wall. Plant, Cell & Environ. 7, 441 - 448.

Domingos, I., Silva, T., Correia, P.J., Pestana, M. & de Varennes, A., 2004. Effects of fertiliser practices on the growth and quality of two table grape cultivars: 'Çardinal' and 'D. Maria'. Acta Hort. 652, 241 - 247.

Donèche, B. & Chardonnet, C., 1992. Evolution et localisation des principaux cations au cours du développement du raisin. Vitis 31, 175 - 181.

Drake, M., Bramlage, W.J. & Baker, J.H., 1979. Effects of foliar calcium on McIntosh apple storage disorders. Commun. Soil Sci. Plant Anal. 10, 303 - 309.

Drake, S.R. & Spayd, S.E., 1983. Influence of calcium treatment on “Golden Dlicious” apple quality. J. Food Sci. 48, 403 - 405.

Düring, H., Lang, A. & Oggionni, F., 1987. Patterns of water flow in Riesling berries in relation to developmental changes in their xylem morphology. Vitis 26, 123-131.

Easterwood, G.W., 2002. Calcium’s role in plant nutrition. Fluid Journal 2, 1 - 3.

Esteban, A., Villanueva, J. & Lissarrague, J.R., 1999. Effect of irrigation on changes in berry composition of Tempranillo during maturation. Sugars, organic acids, and mineral elements. Am. J. Enol. Vitic. 50, 418 - 434.

Etchebarne, F., Ojeda, H. & Deloire, A., 2009. Influence of water status on mineral composition of berries in ‘Grenache Noir’ (*Vitis vinifera* L.). Vitis 48, 63 - 68.

Ferguson, I.B. & Bollard, E.G., 1976. The movement of calcium in woody stems. Ann. Bot. 40, 1057 - 1065.

Follet, R.H., Murphy, L.S. & Donahue, R.L., 1981. Fertilizers and soil amendments. Prentice-Hall Inc., London, UK.

Frank, D., Gould, I. & Millikan, M., 2005. Browning reactions during storage of low-moisture Australian sultanas: Effects of vine nitrogen nutrition on subsequent arginine-mediated Maillard reactions during storage of dried fruit. Austr. J. Grape Wine Res. 11, 29 – 35.

Freeman, B.M., Kliewer, W.M. & Stern, P., 1982. Research note: Influence of windbreaks and climatic region on diurnal fluctuation of leaf water potential, stomatal conductance, and leaf temperature of grapevines. Am. J. Enol. Vitic. 33, 233 - 236.

Fuller, M., 1976. The ultrastructure of the outer tissues of cold-stored apple fruits of high and low calcium content in relation to cell breakdown. Ann. Appl. Biol. 83, 299 - 304.

Gallerani, G., Pratella, G.C., Bertolini, P. & Marchi, A., 1990. Lack of relationship between total calcium of apple fruit and a calcium deficiency related disorder (Bitter pit): A four year report. Acta Hort. 274, 141 - 148.

Green, G.M. & Smith, C.B., 1979. Effects of calcium and nitrogen sources on corking of apples. Commun. Soil Sci. Plant Anal. 10, 129 - 139.

Hanger, B.C., 1979. The movement of calcium in plants. Commun. Soil Sci. Plant Anal. 10, 171 - 193.

Hepner, Y. & Bravdo, B., 1985. Effect of crop level and drip irrigation scheduling on the potassium status of Cabernet Sauvignon and Carignane vines and its must and wine composition and quality. Am. J. Enol. Vitic. 36, 140 - 147.

Hunter, J.J., Volschenk, C.G. & Stevens, S., 2000. Effect of N and K fertilisation on organic acid accumulation and stability in grapes and must. Abstract: 2nd Int. Viticulture & Enology Congress, Cape Town, 8 - 10 November.

Iland, P.G. 1988. Grape berry ripening: the potassium story. Aust. Grapegrower & Winemaker 289, 22 - 24.

Kasimatis, A.N. & Christensen, L.P., 1976. Response of Thompson Seedless grapevines to potassium application from three fertilizer sources. Am. J. Enol. Vitic. 27, 145 - 149.

Keller, M., Arnink, K.J. & Hrazdina, G., 1998. Interaction of nitrogen availability during bloom and light intensity during veraison. I. Effects on grapevine growth, fruit development, and ripening. Am. J. Enol. Vitic. 49, 333 - 340.

Kirkby, E.A., 1979. Maximizing Ca uptake by plants. Commun. Soil Sci. Plant Anal. 10, 89 - 113.

Kirkby, E.A. & Pilbeam, D.J., 1984. Calcium as a plant nutrient. Plant, Cell & Environ. 7, 397 - 405.

Kliewer, W.M., 1968. Changes in the concentration of free amino acids in grape berries during maturation. Am. J. Enol. Vitic. 19, 166 – 174.

Kliewer, W.M., 1977. Influence of temperature, solar radiation and nitrogen on coloration and composition of emperor grapes. Am. J. Enol. Vitic. 28, 96 - 103.

Lang A. & Thorpe, M.R., 1989. Xylem, phloem and transpiration flows in a grape: Application of a technique for measuring the volume of attached fruits to high resolution using Archimedes' principle. J. Expt. Bot. 40, 1069 - 1078.

Linsenmeier, A.W., Loos U. & Löhnertz, O., 2008. Must composition and nitrogen uptake in a long-term trial as affected by timing of nitrogen fertilization in a cool-climate Riesling vineyard. Am. J. Enol. Vitic. 59, 255 - 264.

Löhnertz, O., Prior, B., Bleser, M. & Linsenmeier, A., 2000. Influence of N-supply and soil management on the nitrogen composition of grapes. Acta Hort. 512, 55 - 64.

Marcelle, R.D., 1990a. Comparison of mineral composition of leaf and fruit in apple and pear cultivars. Acta Hort. 274, 315 - 320.

Marcelle, R.D., 1990b. Predicting storage quality from preharvest fruit mineral analyses: A review. Acta Hort. 274, 305 - 313.

Marcelle, R.D., 1995. Mineral nutrition and fruit quality. Acta Hort. 383, 219-226.

Marschner, H., 1995. Mineral nutrition of higher plants. Second edition. Academic Press. London, UK.

Mason, J.L., 1979. Increasing calcium content of calcium-sensitive tissues. Commun. Soil Sci. Plant Anal. 10, 349 - 371.

Mengel, K. & Kirkby, E.A., 1982. Principles of plant nutrition. Int. Potash Institute., Bern, Switzerland.

Miklós, E., Szegletes, Z. & Erdei, L., 2000. Nitrate and chloride transport interaction in grapevine. Acta Hort. 526, 249 - 254.

Millard, P., 1995. Internal cycling of nitrogen in trees. Acta Hort. 383, 3 - 14.

Millaway, R.M. & Wiersholm, L., 1979. Calcium and metabolic disorders. Commun. Soil Sci. Plant Anal. 10, 1 - 28.

Montañés, L., Monge, E., Val, J. & Sanz, M., 1995. Interpretative possibilities of plant analysis by the DOP index. Acta Hort. 383, 165 - 170.

Morris, J.R., Cawthon, D.L. & Fleming, J.W., 1980. Effects of high rates of potassium fertilization on raw product quality and changes in pH and acidity during storage of Concord grap juice. Am. J. Enol. Vitic. 31, 323 - 328.

Morris, J.R., Sims, C.A. & Cawthon, D.L., 1982a. Excessive potassium fertilisation destroys grape juice quality. ARstHortSoc. 103, 106 - 108.

Mpelasoka, B.S., Schachtman, D.S., Treeby, M.T. & Thomas, M.R., 2003. A review of potassium nutrition in grapevines with special emphasis on berry accumulation. Aust. J. Grape and Wine Res. 9, 154 - 168.

Mullins, M.G., Bouquet, A. & Williams, L.E., 1996. Biology of the grapevine. Cambridge University press, Cambridge, USA.

Neilsen, G.H., Neilsen, D., Bowen, P., Bogdanoff, C. & Usher, K., 2010. Effect of timing, rate, and form of N fertilization on nutrition, vigor, yield, and berry yeast-assimalable N of grape. Am. J. Enol. Vitic. 61, 327 - 336.

Ollat, N. & Gaudillère, J.P., 1996. Investigation of assimilate import mechanisms in berries of *Vitis vinifera* Var. Cabernet Sauvignon. Acta Hort. 427, 141 - 149.

Ough, C.S. & Stashak, R.M., 1974. Further studies on proline concentration in grapes and wines. Am. J. Enol. Vitic. 25, 7 - 12.

Peacock, W.L., Christensen, L.P. & Broadbent, F.E., 1989. Uptake, storage and utilization of soil-applied nitrogen by Thompson Seedless as affected by time of application. Am. J. Enol. Vitic. 40, 16 - 20.

Perez, J.R. & Kliewer, W.M., 1982. Influence of light regime and nitrate fertilization on nitrate reductase activity and concentrations of nitrate and arginine in tissues of three cultivars of grapevines. Am. J. Enol. Vitic. 33, 86 - 93.

Perring, M.A., 1979. The effects of environment and cultural practices on calcium concentration in the apple fruit. Commun. Soil Sci. Plant Anal. 10, 279 - 293.

Peuke, A.D., 2000. The chemical composition of xylem sap in Vitis vinefera L. cv. Riesling during vegetative growth on three different Franconian vineyard soils and as influenced by nitrogen fertilizer. Am. J. Enol. Vitic. 51, 329 - 339.

Poni, S., Quartieri, M. & Tagliavini. M., 2003. Potassium nutrition of Cabernet Sauvignon grapevines (*Vitis vinifera* L.). Plant & Soil 253, 341 - 351.

Poovaiah, B.W., 1979. Role of calcium in ripening and senescence. Commun. Soil Sci. Plant Anal. 10, 83 - 88.

Poovaiah, B.W., Glenn, G.M. & Reddy, A.S.N., 1988. Calcium and fruit softening: physiology and biochemistry. Hort. Reviews 10, 107 - 152.

Porro, D., Steffanini, M., Failla, O. & Stringari, G., 1995. Optimal leaf sampling time in diagnosis of grapevine nutritional status. Acta Hort. 383, 135 - 142.

Possner, D.R.E & Kliewer, W.M., 1985. The localisation of acids, sugars, potassium and calcium in developing grape berries. Vitis 24, 229 - 240.

Rogiers, S.Y., Greer, D.H., Hatfield, J.M., Orchard, B.A. & Keller, M., 2006. Mineral sinks within ripening grape berries (*Vitis vinifera* L.). Vitis 45, 115 - 123.

Rogiers, S.Y., Smith, J.A., White, R., Keller, M., Holzapfel, B.P. & Virgona, J.M., 2001. Vascular function in berries of *Vitis vinifera* (L) cv. Shiraz. Aust. J. Grape Wine Res. 7, 46 - 51.

Rorison, I.H. & Robinson, D., 1984. Calcium as an environmental variable. Plant, Cell & Environ. 7, 381 - 390.

Roubelakis-Angelakis, K.A. & Kliewer, W.M., 1992. Nitrogen metabolism in grapevine. Hort. Reviews. 14, 407 - 452.

Rühl, E.H., 2000. Effect of rootstocks and K^+ supply on pH and acidity of grape juice. Acta Hort. 512, 31 - 37.

Ruiz, R., Moyano, S. & Navia, T., 2004. Accumulation of nitrogen compounds as related to the “soft berry” problem in table grapes. Agricultura Téchnica Chile 64, 426 - 430.

Saayman, D., 1981. Wingerdvoeding. In: J.D. Burger & J. Deist (eds). Wingerdbou in Suid-Afrika. VORI, 7600, Stellenbosch, R.S.A. pp. 343-383

Saxton, V., 2002. Calcium and the vine. Wine Ind. J. 17, 59 - 62.

Schaller, K., Löhnertz, O. & Chikkasubbanna, V., 1992. Calcium absorption by the grape berries of different cultivars during growth and development. Vit. Enol. Sci. 47, 62 - 65.

Schlegel, T.K. & Schönherr, J., 2002. Penetration of calcium chloride into apple fruits as affected by stage of fruit development. Acta Hort. 594, 527 - 533.

Sen, K., Karacali, I., Irget, M.E., Elmaci, O.L. & Tepecik, M., 2010. A new strategy to enrich calcium nutrition of fruit: synergistic effects of postharvest foliar calcium and boron sprays. J. Plant Nutr. 33, 175 - 184.

Shear, C.B. & Faust, M., 1970. Calcium transport in apple trees. Plant Physiol. 45, 670 - 674.

Storey, R., 1987. Potassium localization in the grape berry pericarp by enery-dispersive X-ray microanalyses. Am. J. Enol. Vitic. 38, 301 - 309.

Storey, R., Jones, R.G.W., Schachtman, D.P. & Treeby, M.T., 2003. Calcium-accumulating cells in the meristematic region of grapevines root apices. Functional Pl. Biol. 30, 719 - 727.

Tagliavini, M., Zavalloni, C., Romolà, A.D., Quartieri, M., Malaguti, D., Mazzanti, F., Millard, P. & Marangoni, B., 2000. Mineral nutrient partitioning to fruits of deciduous trees. Acta Hort. 512, 131 - 140.

Terblanche, J.H., Pienaar, W.J. & DeWall, D.R., 1975. Efficacy of calcium sprays for controlling bitter pit in apples. Decid. Fruit Grow. 25, 304 - 306.

Terblanche, J.H., Wooldridge, L., Hesebeck, I. & Joubert, M., 1979. The redistribution and immobilization of calcium in apple trees with special reference to bitter pit. Commun. Soil Sci. Plant Anal. 10, 195 - 215.

Wermelinger, B., 1991. Nitrogen dynamics in grapevine: physiology and modeling. Proc. Int. Symp. Nitrogen in Grapes and Wine. (Ed. J.M. Rantz), Seattle, USA. pp. 23 - 31.

Wiersum, L.K., 1979. Effects of environment and cultural practices on calcium nutrition. Commun. Soil Sci. Plant Anal. 10, 259 - 278.

Williams, L.E. & Biscay, P.J., 1991. Partitioning of dry weight, nitrogen, and potassium in Cabernet Sauvignon grapevines from anthesis until harvest. Am. J. Enol. Vitic. 42, 113 - 117.

Wójcik, P., Gubbuk, H., Akgül, H., Gunes, E., Ucgun, K., Koçal. H. & Küçükyumuk, C., 2010. Effect of autumn calcium spray at a high rate on “Granny Smith” apple quality and storability. J. Plant Nutr. 33, 46 - 56.

Wolpert, J.A. Smart, D.R. & Anderson, M., 2005. Lower petiole potassium concentration at bloom in rootstocks with Vitis berlandieri genetic backgrounds. Am. J. Enol. Vitic. 56, 163 - 169.

Yang, Y.S., Wu, Y.R., Kuo, Y.K., 1997. Effects of cytokinins and calcium application on the fruit firmness of Honey red grapes. In: Special Publication, Tai’chung District Agricultural Improvement Station, 38, 151-168, Nat. Chung Hsing Univ., Dep of Hort., Taiwain.

Zapata, C., Deléens, E., Chaillou, S. & Magné, C., 2004. Mobilisation and distribution of starch and total N in two grapevine cultivars differing in their susceptibility to shedding. Funct. Pl. Biol. 31, 1127 - 1135.

Zocchi, G. & Mignani, I., 1995. Calcium physiology and metabolism in fruit trees. Acta Hort. 383, 15 - 23.

CHAPTER II

Excessive N, K and Ca fertilisation effects on vine growth and leaf chlorophyll content of an early ripening table grape cultivar (*Vitis vinifera* L. cv. Prime Seedless), grafted onto Ramsey on a sandy soil

ABSTRACT

A four-year field trial was conducted on a sandy soil in the Paarl district of South Africa, using cv. Prime Seedless (*Vitis vinifera* L.) grapevines, grafted onto rootstock Ramsey, and trained to a gable trellis system. The effect of excessive applications of N, K and Ca on table grape performance under typical South African cultivation conditions, with special reference to vegetative growth, was investigated. Nitrogen, potassium and calcium were applied, singular or in combination, at rates equal to 300% the calculated annual nutritional requirement, with the Control treatment having received 70 kg N/ha/year, 60 kg K/ha/year and 10 kg Ca/ha/year. Vine shoot length, leaf surface area and chlorophyll content were determined and are discussed in parallel to the impact of the treatments on soil chemical composition and leaf nutrient content. Neither shoot growth nor leaf chlorophyll content was affected according to a clear pattern by the treatments. Excessive N fertilisation caused a reduction of soil pH to detrimental levels. The excessive N, K and Ca applications also reduced mutual concentrations, and that of Mg, in the soil. The lack of vegetative growth responses and chlorophyll content differences is therefore ascribed to the combined negative effect that these excessive applications had on soil pH and soil nutrient content, thereby causing imbalanced vine nutrition.

INTRODUCTION

Nitrogen (N), potassium (K) and calcium (Ca) are essential elements for plant nutrition that are commonly applied on an annual basis to table grape vineyards. Nitrogen is described as the most essential element of plant growth, with chlorophyll content that is approximately proportional to leaf N content (Shaahan *et al*., 1999). Quoting various authors, Conradie (2001) pointed out that although a magnitude of responses to N fertilisation, depending on soil, cultivar, cultivation practices and climatic conditions, can be obtained, many studies found no positive responses to high N fertilisation rates. He concluded, however, that in low-vigour vineyards fairly heavy applications of N at budbreak, should be beneficial.

Potassium affects vine growth through its involvement in enzyme activation, cellular membrane transport and osmotic potential regulation (Clarkson & Hanson, 1980). According to Kasimatis & Christensen (1976) crop yield and vine growth increases when K fertilisation is applied to soil that is low in available K. On the other hand, Poni *et al*. (2003) found that although K fertilisation increased soil exchangeable K significantly, vegetative growth was not affected. Excessive K fertilisation of apples resulted in decreased Mg contents in the trees resulting in reduced vegetative growth and yields (Sadowski *et al*., 1988).

Calcium deficiencies are rarely observed in the vineyard (Conradie, 1981) and Ca applications are therefore intended to utilise the positive effect of Ca on membrane integrity (Fuller, 1976; Poovaiah, 1979) and cell wall structure (Poovaiah, 1979; Demarty *et al*., 1984) to enhance fruit quality. However, translocation of Ca within the plant is slow and favours tissues with the highest transpiration rate (Mengel & Kirkby, 1982). Although Ca is required for plant growth due to its role in mitoses (Takagi *et al*., 1990), various enzyme systems in plants are also inhibited by Ca; low cellular levels of Ca is thus maintained (Kirkby & Pilbeam, 1984; Macklon, 1984; Trewavas, 1999) by active transport across the plasmalemma by a Ca transporting ATPase or by a Ca^{2+}/H^{+} anti-port across the tonoplast into the vacuole, where it is precipitated (Macklon, 1984; Trewavas, 1999). The addition of Ca as fertiliser or foliar applications, in addition to already sufficient levels, therefore seems superfluous.

In view of the above considerations a study was undertaken under typical South African table grape cultivation conditions on a nutrient poor sandy soil to investigate whether excessive applications of N, K and Ca might benefit grapevine performance, with special reference to vegetative growth, leaf chlorophyll content, grapevine nutrient status, berry nutrient

accumulation and increased fruit quality. This article (the first in a series of four) deals with the effect of high rates of fertilisation on vegetative growth and leaf chlorohyll content.

MATERIALS AND METHODS

Vineyard site, experimental design and treatments

The trial was conducted over four seasons (2006/07 to 2009/10) in a micro-irrigated Prime Seedless (*Vitis vinifera* L.)/Ramsey commercial vineyard at De Hoop Farm in Paarl (33°45'S, 18°58'E). The soil in question was a Clovelly soil (Soil Classification Working Group, 1991) with a fine sandy texture containing less than 5% clay (Table 1). Soil topography was almost level. The vineyard was planted in 2002. The grapevines were trained to a gable system, spaced 1.8 m x 3 m apart, head trained and cane pruned to eight buds. Standard cultural practices for the cultivar and region were followed as described in Anonymous (2007). It entailed shoot tipping and crop control after set, combined with removal of leaves that are in close proximity of the retained bunches. Bunch preparation entailed an application of 1 mg/L gibberellic acid (GA_3) at bloom for bunch (flower cluster) thinning, shortening of bunches to 8 cm length at set, dipping bunches in 20 mg/L GA_3 for berry enlargement when they were 8 - 10 mm in diameter and again at 10 - 12 mm diameter. Finally, hand-thinning of bunches was done just before véraison. Irrigation scheduling was based on soil water content measurements done with tensiometers at 30 cm and 60 cm depth. Mid-season irrigation averaged two applications of 20mm per week. Long-term annual winter rainfall is 630 mm and summer rainfall is 130 mm.

The experiment was laid out as a completely randomised block design. Each experimental unit consisted of four grapevines in four rows (16 grapevines), with only the central two grapevines in the middle rows being used for experimental purposes. Each treatment was replicated five times. The treatments consisted of combinations of different levels of soil applied nitrogen (N), potassium (K) and calcium (Ca), up to 300% of the annual nutritional requirement of the vineyard, while the control treatment represented the standard fertilisation practices applied by the producer for commercial production purposes (70 kg N/ha/year, 60 kg K/ha/year, 10 kg Ca/ha/year) (Table 2). An additional treatment, i.e. bunch applied Ca, was also included. Except for the control (where N was applied in two instalments, before set and post-harvest, while all the K was applied after set), fertiliser was applied by hand in six instalments throughout the growing season, two times prior to flowering, three times from set to véraison and once after harvest. Instalment size was calculated from the total intended seasonal application of each nutrient, divided as a percentage of the seasonal requirement during each phenological period (Conradie,1980; Conradie, 1981). Treatments were applied

each year to the same plants. Due to a lack of clear treatment effects, only the extreme treatments, i.e. treatments 1 (Control), 2 (Ca(Bunch)), 5 (N), 8 (K), 11 (Ca), and 14 (KCa) were repeated in the fourth year. Furthermore, only these treatments and treatment 17 (NCa) are discussed with respect to the first three years, when it was applied and sampled.

Plant measurements and tissue analyses

A uniform vine canopy for all treatments was maintained as far as possible to eliminate variation in vine canopy density as an additional factor affecting nutrient balances and translocation in the vine. It was assumed that this could be achieved through proper canopy management (tipping, topping, and removal of leaves and lateral shoots), typical of table grape management strategies. However, vegetative growth responses that may have occurred on account of the different fertilisation levels received (Table 2) were monitored throughout the growing season by means of shoot growth, leaf surface area, the determination of dry mass of the plant material removed, and cane mass (in winter).

Vine shoot length, leaf surface area and topped material

One bearer shoot (shoot with a bunch) was removed from each of the four experimental grapevines per plot at early flowering [37 days after budbreak (DAB)], 15 - 16 mm berry size (82 DAB), véraison (96 DAB) and first harvest (114 DAB) during the 2006/07 season; at 15 - 16 mm berry size (78 DAB), véraison (94 DAB) and first harvest (114 DAB) during the 2007/08 season; and at véraison (96 DAB), two weeks before harvest (106 DAB) and first harvest (119 DAB) during the 2008/09 season. Primary and lateral shoot lengths were recorded. All primary and lateral leaves were removed, the leaf surface area measured immediately after removal (for only the 2006/07 and 2007/08 seasons), and the leaves put in separate paper bags according to shoot type. It was kept in the dark at 4^{o}C and 90% relative humidity until chlorophyll analyses could be done (not more than 7 days after sampling). A Delta-T area meter (Delta-T devices, Cambridge, England) was used for leaf area determinations. Leaf area was expressed as the amount of leaf area (m^2) per vine, determined by multiplying the mean total leaf area per shoot by the mean number of shoots per grapevine. The average number of shoots per vine, as recorded at berry set, was 45.

In the 2009/2010 season all the plant material removed through canopy management, mainly the topped part of the shoots, were collected, dried in an oven at 80^{o}C and weighed.

Table 1. Analyses of the fine sandy soil, determined before the treatments commenced (sampled on 8 September 2006 at De Hoop, Paarl).

Soil depth (mm)	Clay (%)	Silt (%)	Sand (%)	pH (KCl)	Resistance (ohm)	P (mg/kg)	K (mg/kg)	Exchangeable cations (cmol (+)/kg)				Organic C (%)
								Na	K	Ca	Mg	
0 -300	2	12	86	5.5	4 680	126	45	0.03	0.115	1.54	0.26	0.34
300-600	3	15	82	5.6	5 727	131	45	0.03	0.115	1.47	0.22	0.22
600-900	4	18	78	5.5	8 329	90	37	0.02	0.095	1.23	0.20	0.18

Table 2. Fertilisation treatments applied to the Prime Seedless/Ramsey (*Vitis vinifera* L.) micro-irrigated commercial vineyard, De Hoop, Paarl.

Treatment[1]	Total annual nutrient application (kg/ha)		
	N	K	Ca
1 (Control)	70	60	10
2 (Control-Bunch)[2]	70	60	10
3	140	60	10
4	175	60	10
5 (N)[3]	210	60	10
6	70	120	10
7	70	150	10
8 (K)[4]	70	180	10
9	70	60	100
10	70	60	125
11 (Ca)[5]	70	60	150
12	70	120	100
13	70	150	125
14 (KCa)[6]	70	180	150
15	140	60	100
16	175	60	125
17 (NCa)[7]	210	60	150

[1] Legends used for the different treatments are indicated in brackets.
[2] A mixture of 8L/ha Stopit plus 5 L/ha Caltrac, applied directly to bunches every two weeks from berry set to vèraison (three applications). A total of 10 kg Ca/ha was therefore applied.
[3] LAN (28%) was used as nitrogen source.
[4] KCl was used as K source.
[5] Gypsum ($CaSO_4$) was used as Ca source for the 2006/07, 2007/08 and 2008/09 seasons. In the 2009/10 season, $CaCl_2$ was used.
[6] A combination of KCl and $CaSO_4$ was used as K and Ca sources in the 2006/07, 2007/08 and 2008/09 seasons, while $CaCl_2$ was used instead of $CaSO_4$ in the 2009/10 season.
[7] A combination of $CaNO_3$, LAN and $CaSO_4$ was used as N and Ca source in the 2006/07, 2007/08 and 2008/09 seasons, while $CaCl_2$ was used instead of $CaSO_4$ in the 2009/10 season.

Leaf chlorophyll content

Chlorophyll determinations were done according to the method described by Hunter & Visser (1989). A representative fresh leaf sample of 5 g was cut into pieces of 1 cm^2. The leaf material was added to 100 cm^3 80% aqueous acetone containing 0.1 g $CaCO_3$ and macerated with a Junke & Kunkel IKA Ultra-Turrax T-25 macerator at room temperature for 60 s at 10 000 rpm. The homogenate was left to settle in the dark at 4°C for 24 h, after which the sediment was completely discoloured. The final volume was adjusted to 100 cm^3. The density of the extracts was measured in a 10 mm cell with a LKB Ultrospec spectrophotometer at 663 nm and 645 nm. The equations used for determination of chlorophyll concentration were as follows (Arnon, 1949):

Chlorophyll *a* (mg/dm^3) = $12.7A_{663} – 2{,}69A_{645}$

Chlorophyll *b* (mg/dm^3) = $22.9A_{645} – 4.68A_{663}$

Leaf petiole analyses

Leaf petiole samples were taken at various phenological stages for chemical analyses by sampling 30 mature leaves randomly from bearing shoots of the experimental vines at close proximity to the bunches. In this article only the sample taken at 15 mm berry size [78 - 82 days after budbreak (DAB)], that coincides with the leaf chlorophyll samples, are discussed. After sampling, leaf blades and petioles were separated immediately. The petioles were ashed at 480^{o}C, shaken up in a 50:50 HCl (32%) solution and the cation content measured with a Varian ICP-OES optical emission spectrometer. Total N content in the ash was determined through total combustion in a Leco N-analyser.

Shoot mass

The mass of winter pruned canes was measured in the 2007/08 and 2008/09 seasons in the vineyard, using a spring balance.

Soil and soil water extract analyses

Soil samples for chemical analyses were taken from the 0-30 cm layer with an auger, mixing soil sampled from between the two central experimental vines of both experimental vine rows in each plot. The soil was air dried, sieved through a 2 mm sieve and analysed for pH (1.0 M KCl), P (Bray II) and total extractable cations, namely K, Ca, Mg and Na (extracted at pH = 7 with 0.2 M ammonium acetate) and organic matter by means of the Walkley-Black method (The Non-affiliated Soil Analyses Work Committee, 1990). The extracted solutions was analysed with a Varian ICP-OES optical emission spectrometer.

Soil water samples were taken from wetting front detectors (Stirzaker, 2005), whenever sufficient water accumulated therein to make an extraction possible (normally after an irrigation event exceeding the soil water holding capacity and leading to free water draining from the soil). Soil water was extracted from the wetting front detector using a tube and syringe. The sample was then diluted 10 times to obtain a large enough sample for analyses. Calcium and K concentrations were determined on a Varian ICP-OES optical emission spectrometer, while NH_4^+ and NO_3^- concentrations were determined colorimetrically with a Seal Technicon III auto-analyser as described by The Non-affiliated Soil Analyses Work Committee (1990).

Statistical procedures

Standard analyses of variance were performed for each season separately, using Genstat 5 release 1.2 and SAS (SAS, 1990). Student's t-test was used to test for significant differences between treatment means. The Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965).

RESULTS AND DISCUSSION

Vine growth

Vine total shoot length, measured during the first three trial years, is reported in Figure 1. Although Prime Seedless is not a vigorous cultivar (Anonymous, 2007), the goal is that primary shoots reach the top (third) wire of the trellis by flowering. Growing shoot tips of the primary shoots are then typically removed after set (± 43 DAB), stimulating some development of lateral shoots. Thereafter, primary shoot length was kept constant by cutting it back at intervals which was determined by vigour of the vineyard. Lateral shoots were removed to maintain canopy density at levels that allow 20% direct sunlight penetration to the vineyard floor. This foliage management approach resulted in a stabilisation of primary shoot length, apical lateral shoot length as well as basal lateral shoot length after 90 DAB (Figure 1). With the exception of the N treatment that showed significantly longer apical lateral shoots in the 2006/07 season at harvest, the fertilisation treatments had no effect on the length of any of the shoot types. The longer primary shoot length obtained for the Ca treatment in the 2006/07 season is ascribed to variance in application of topping practices between labourers. In general, as demonstrated in Figure 1, foliage management effectively maintained total vine shoot length at the required norm for all the treatments.

The average effect of each element on vine growth was therefore expected to be expressed in the amount of removed plant material. This is indicated for the 2009/10 season in Figure 2. Again, when compared to the control treatment, no vine growth response to fertilisation treatments was obtained. The lack of a vegetative growth response to the N treatment was unexpected. This can partly be ascribed to the fact that Prime Seedless is not a vigorous cultivar and therefore do not react strongly to N fertilisation. More vigorous cultivars might have reacted more strongly to the excessive levels of N fertilisation. The reason for reduced amount of removed shoot material obtained for the Ca treatment, indicating reduced shoot growth, is also not clear. It cannot be ascribed to a reduction in N utilisation since data discussed in following chapters indicate otherwise. A contributing factor might be that the bunches become strong sinks already from an early stage and because of that a large part of their development occurs in the cool early summer, thereby suppressing shoot growth.

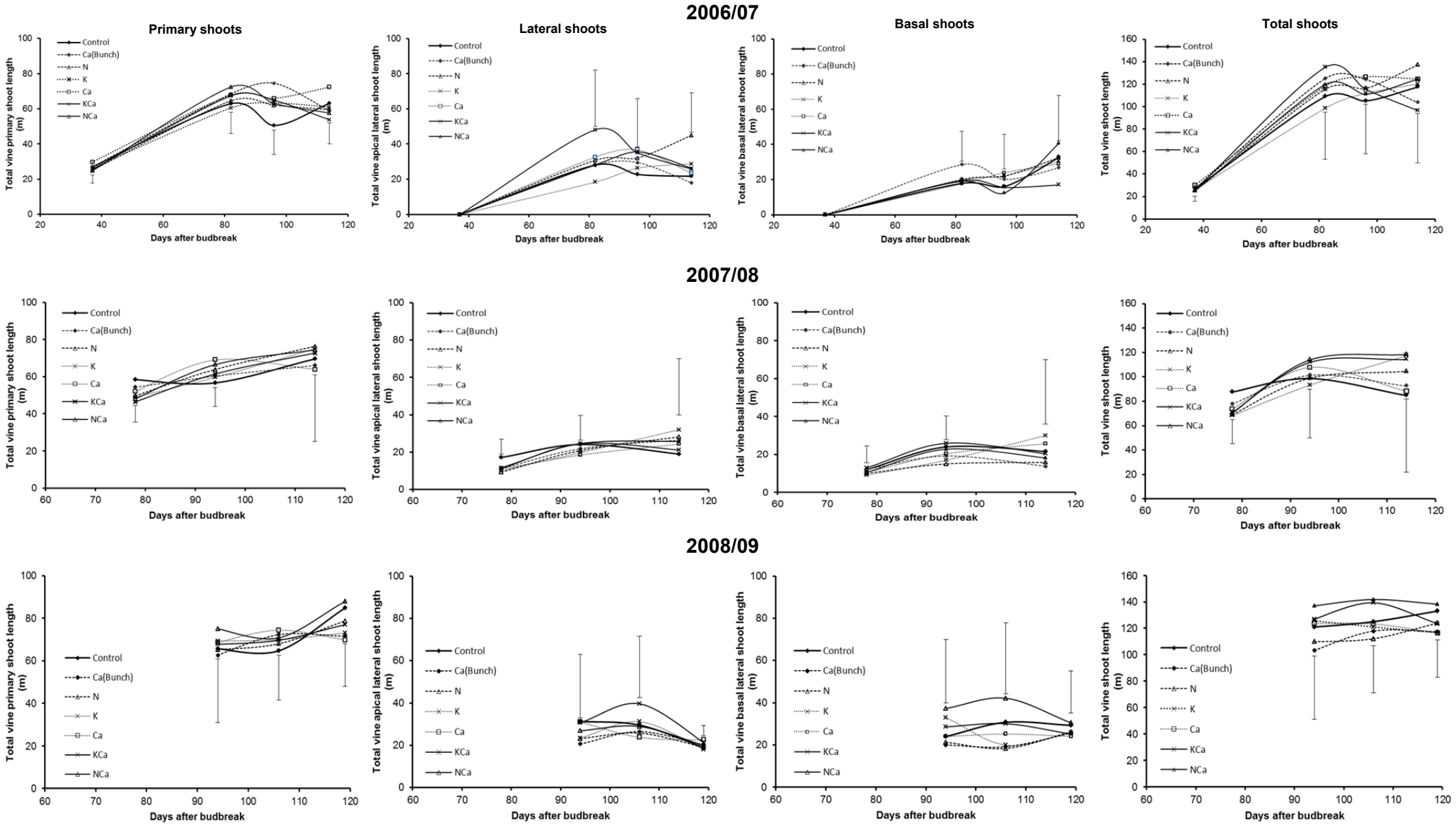

Figure 1. Shoot length of commercially cultivated Prime Seedless/Ramsey vines as affected by varying levels of N, K and Ca on a sandy soil in Paarl, over three consecutive seasons. Vertical bars indicate the standard deviation ($p \leq 0.05$) for each sampling time.

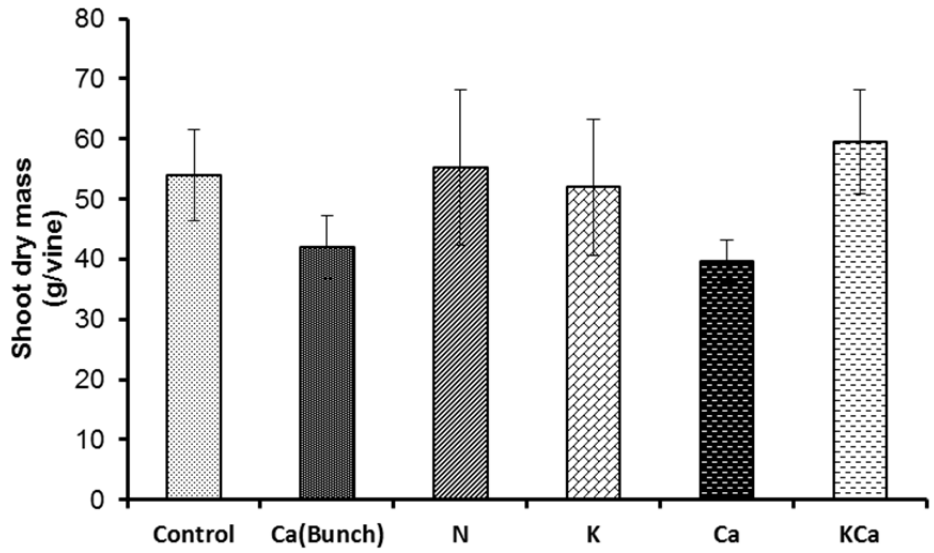

Figure 2. Treatment average dry mass of topped shoots and removed lateral shoots of differently fertilised Prime Seedless/Ramsey vines on a sandy soil in Paarl during the 2009/10 season. Vertical bars show the standard error ($p \leq 0.05$). NCa was not applied in 2009/10.

The average mass of winter pruned shoots (cane mass) for each treatment was also measured for the 2007/08 and 2008/09 seasons, i.e. during the winters of 2008 and 2009 (Figure 3). Compared to the control, none of the treatments showed any significant difference in pruning mass. Although not significant, the NCa treatment had a heavier average pruning mass for the 2007/08 season than the control. For the 2008/09 season NCa was also the only treatment that did not have a lower average pruning mass than the control. Although not significant, it also showed the longest average basal lateral and total shoot length over the whole season. The combination of N and Ca therefore seems to benefit shoot thickness compared to the other treatments of over-fertilisation. Keller *et al.* (1998) also found that an increased N supply at bloom led to increased vine vigour. The lower average pruning mass obtained for the K treatment in the 2008/09 season also indicate possible effects obtained from lower chlorophyll levels in the leaves (discussed below), resulting in less growth.

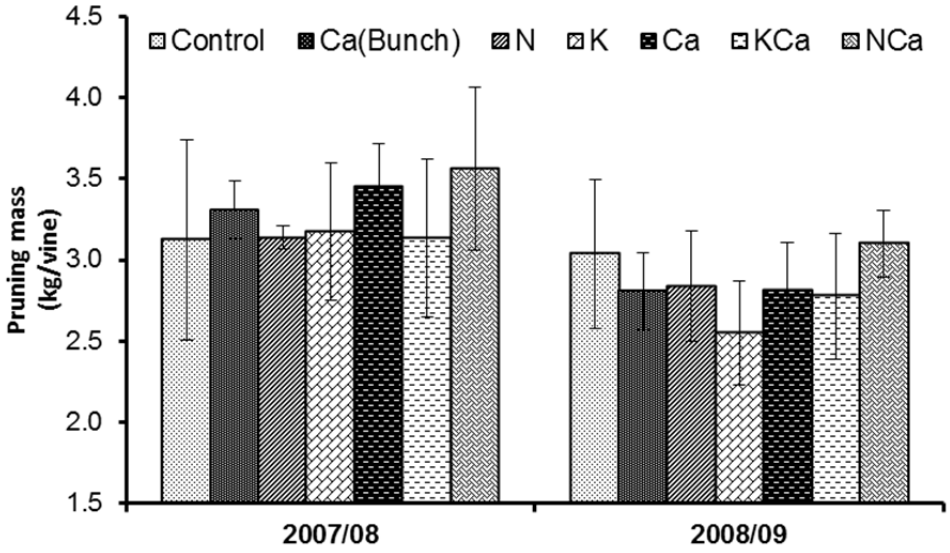

Figure 3. Treatment average mass of winter pruned shoots, expressed per vine, measured after each of the 2007/08 and 2008/09 seasons. Vertical bars show the standard error ($p \leq 0.05$).

Total vine leaf surface area, as measured in the 2006/07 and 2007/08 seasons is presented in Figure 4. The slightly higher vigour obtained for the Ca treatment in the 2006/07 season is also reflected in the larger leaf surface area by harvest on the primary shoots, while the N treatment shows a larger leaf surface area at harvest on the apical lateral shoots. Similar to shoot length, this tendency was not retained during the next season, and neither did the leaf surface area expand from véraison onwards. This is due to vine foliage management. In fact, vine leaf surface area actually decreased from véraison to harvest during the 2008/09 season. The reduction was most prominent in the apical lateral shoot positions, where more shoots are normally suckered before harvest than in other positions to ensure proper sunlight penetration. This was also observed in the reduced total apical lateral shoot length of 2008/09 for this period (Figure 1). The leaf surface area results again demonstrate that, due to foliage management, the treatments had no significant effect on the vine canopy.

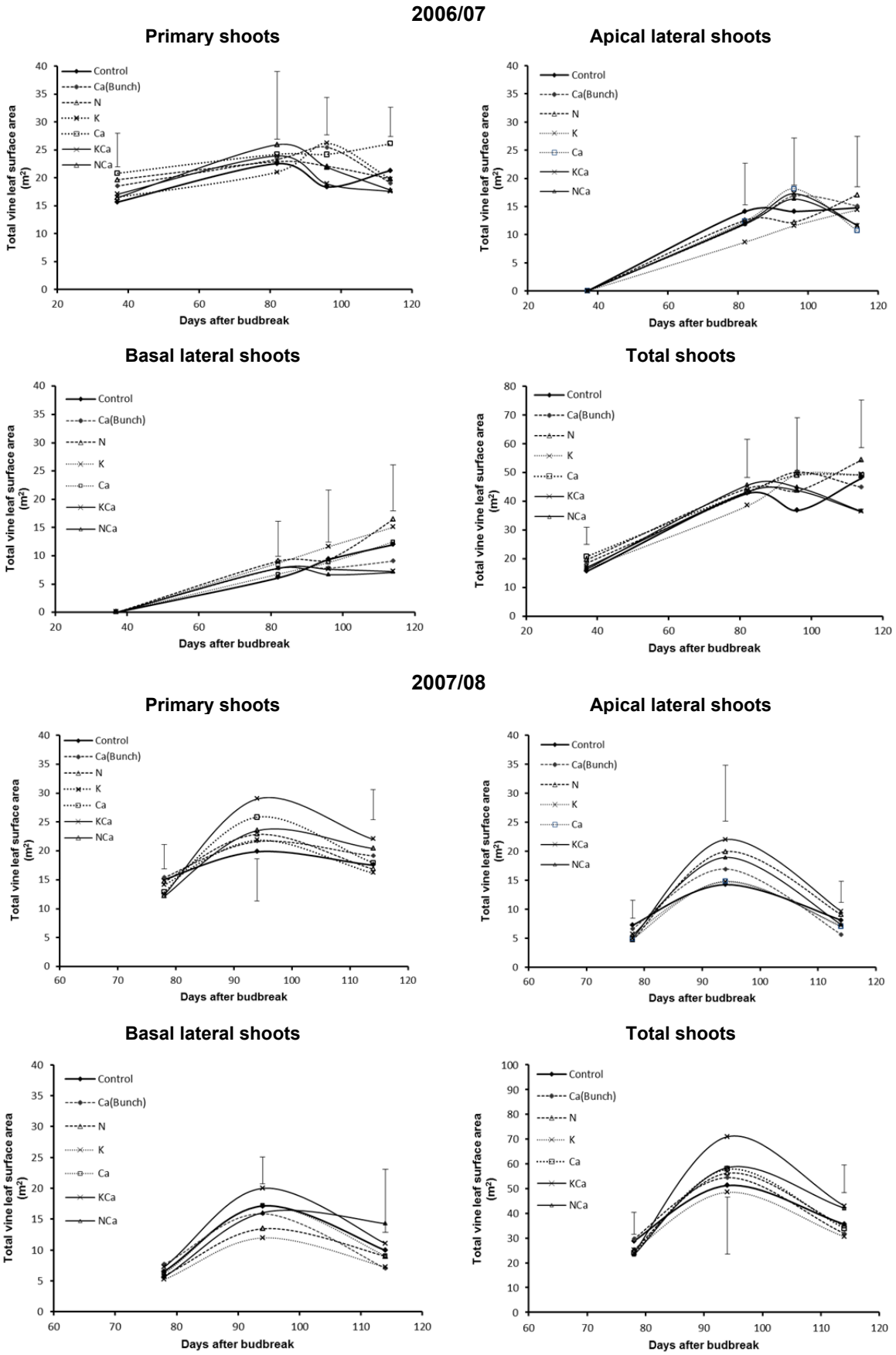

Figure 4. Leaf surface area of commercially cultivated Prime Seedless/Ramsey vines as affected by varying levels of N, K and Ca on a sandy soil in Paarl, as measured during the 2006/07 and 2007/08 seasons. Vertical bars indicate the standard deviation ($p \leq 0.05$) for each sampling time.

The effect of the treatments on the soil chemical composition is presented in Table 3. From the first season (sampled in winter 2007) the K content of the soil was significantly increased, while the Ca (mainly free Ca due to the low clay content) only started increasing from the second season (sampled winter 2008); it, however, never increased to levels significantly higher than the control soil. Furthermore, the N concentration of soil water extracts of treatments N and NCa reflected higher N availability (Table 4). The lack of response in vegetative growth by the vines on account of the fertilisation treatments, especially N, can therefore not be explained by a lack of (increased) availability of the applied nutrients.

However, a high rate of leaching-loss of K and Ca from the soil seems to have been stimulated by the application of excessive amounts of N (treatments N & NCa). This is illustrated by the excessively low K content in the soil of treatments N and NCa in winter 2008 and for treatment N by the end of the trial (winter 2010). Significantly lower Ca was also found in the soil of treatment N from winter 2008 onwards (Table 3). Barak (1997), Cakmak *et al.* (2010) and Ring *et al.* (2011) also found that an increase in exchangeable acidity associated with N fertilization was accompanied by a decline in exchangeable base cations. Mineral weathering, including weathering of the clay minerals themselves and formation of nonexchangeable hydroxy-Al complexes, leads to a reduction in cation exchange capacity (CEC), which are given as a possible explanation for reduction of base cation content due to soil acidification.

Furthermore, the Mg content of the soil was also reduced significantly for all the treatments, except Ca-Bunch, and especially for the treatments containing K. Sadowski *et al.* (1988) reported that excessive fertilisation of apple trees with K resulted in reduced leaf Mg contents which caused reduced vegetative growth and yields. Poni *et al*. (2003) also found that although K fertilisation increased soil exchangeable K significantly, vegetative growth was not affected. The reduction in the availability of essential nutrients like K, Ca and Mg to deficient levels, as well as a reciprocal suppression in their uptake (data presented in Chapters III and IV) therefore seems to explain the lack of significant responses in vegetative growth to the excessive applications of N, K and Ca.

The reduced soil pH of the soil over time, but especially of treatment N and NCa, to levels detrimental to vine root growth and plant performance, i.e. $pH_{KCl} < 5.6$ (Conradie, 1994), were obtained. This is also considered as a contributing factor for the lack in vegetative growth response obtained to excessive N applications. Bates *et al*. (2002) found that 'Concord' (*Vitis labruscana* L.) showed a reduction in root and shoot biomass below soil pH_{water} of 4.5 ($pH_{KCl} \approx 3.5$).

Table 3. The effect of varying levels of N, K and Ca fertilisation on the chemical composition of the 0-30 cm fine sandy soil layer of the trial vineyard in Paarl over the four experimental seasons. Values designated by the same letter do not differ significantly (p ≤ 0.05) for each season.

Year of sampling	Treatment	pH (KCl)	P (mg/kg)	K (mg/kg)	Extractable cations (cmol (+)/kg)				Organic C (%)
					Na	K	Ca	Mg	
2007	Control	5.6a	112b	72b	0.020a	0.185b	1.400a	0.175ab	0.33ab
	Ca-Bunch	5.3ab	140a	59c	0.020a	0.150c	1.850a	0.260a	0.43a
	N	4.6c	128ab	48cd	0.013ab	0.120cd	1.400a	0.117b	0.34ab
	K	5.1bc	123ab	93a	0.015ab	0.240a	1.445a	0.170ab	0.28ab
	Ca	5.3ab	83c	43d	0.010b	0.108d	1.492a	0.142b	0.22b
	KCa	5.3ab	86c	76b	0.013ab	0.193b	1.747a	0.163ab	0.37ab
	NCa	4.8bc	130ab	47cd	0.010b	0.123cd	1.423a	0.113b	0.26ab
	LSD (≤ 0.05)	0.50	21	14	0.007	0.038	0.465	0.109	0.19
2008	Control	5.4a	110c	44cd	0.016ab	0.112cd	1.762ab	0.304a	0.29ab
	Ca-Bunch	5.8a	149bc	52c	0.020ab	0.130c	1.895ab	0.255a	0.27ab
	N	4.3b	135c	20d	0.022a	0.052d	0.942c	0.182b	0.28ab
	K	5.6a	168ab	160a	0.018ab	0.408a	1.574abc	0.184b	0.27ab
	Ca	5.7a	169ab	26d	0.012ab	0.068d	2.168a	0.134b	0.34a
	KCa	5.6a	150b	79b	0.016ab	0.198b	1.868ab	0.140b	0.21b
	NCa	4.2b	177a	19d	0.010b	0.048d	1.074bc	0.088b	0.18b
	LSD (≤ 0.05)	0.44	21	17	0.010	0.044	0.856	0.054	0.12
2010	Control	5.0a	119	32c	0.038b	0.084c	2.150a	0.224a	0.39a
	Ca-Bunch	5.0a	146	37c	0.042ab	0.096c	1.098ab	0.184ab	0.28bc
	N	3.8b	158	13c	0.022c	0.034c	0.380b	0.116bc	0.31ab
	K	5.5a	142	357a	0.040b	0.914a	1.126ab	0.134abc	0.33ab
	Ca	5.6a	172	37c	0.046ab	0.094c	2.618a	0.104bc	0.28bc
	KCa	4.7a	114	249b	0.053a	0.637b	1.153ab	0.080c	0.20c
	NCa	-	-	-	-	-	-	-	-
	LSD (≤ 0.05)	0.93	68[1]	65	0.013	0.166	1.651	0.095	0.10

[1] Not significant at p ≤ 0.05.

Table 4. Average soil water chemical composition of a sandy vineyard soil in Paarl, as affected by N, K and Ca fertilisation treatments over four production seasons (2006/07 to 2009/10).

Treatment	Sampling depth (cm)	Nutrient concentration (mg/L)			
		K^+	Ca^{2+}	NH_4^+	NO_3^-
Control	30	3.5	8.7	0.9	11.1
	60	5.1	13.0	0.9	19.2
N	30	4.6	13.2	3.9	49.2
	60	3.3	14.7	1.0	15.8
K	30	15.5	16.9	0.6	5.8
	60	10.6	8.7	1.0	1.7
Ca	30	3.5	68.5	0.5	10.6
	60	4.3	47.4	0.3	3.5
KCa	30	20.8	75.1	0.7	8.0
	60	28.5	70.6	1.0	4.0
NCa	30	2.5	31.0	0.2	20.9
	60	2.0	16.5	0.1	14.0

Furthermore, the lower the pH the higher the solubility of Al^{3+} in the soil, reaching toxic levels at pH_{KCl} < 4.2 (Follet *et al*., 1981), which is the case for treatments N and NCa in 2008 and N in 2010. Increased soluble Al^{3+} also retards Ca-movement from the root to the shoots (Hanger, 1979), with Ca required for plant growth because it is essential to various cellular processes such as mitosis, cytoplasmic streaming and stomatal functioning (Storey *et al.,* 2003). The results are in accordance with work done by Sadowski *et al.* (1988) who reported that excessive N fertilisation (with ammonia nitrate) of apple trees led to soil pH being drastically reduced and ascribed the lack of vegetative growth obtained for the N fertilised plots to toxic levels of Al^{3+} and Mn^{2+} in the soil. Conradie (1983) also found that vegetative growth of Chenin blanc grapevines increased by 27% and 87% when limed to pH_{KCl} 5.0 and 6.0 respectively from pH_{KCl} 4.1. Mainly LAN and $CaNO_3$ were used as fertilisers, which is the least acidifying N source. Despite this, the N containing treatments resulted in the largest decrease in soil pH. This is ascribed to NO_3- having been leached rapidly from the sandy soil due to winter rain, while the NH_4^+ remained, resulting in the pH reduction

Leaf chlorophyll content

The effect of the N, K and Ca fertilisation treatments on leaf chlorophyll is shown in Figure 5. The pattern of treatment effects was dissimilar for the three consecutive years when chlorophyll analyses were done. During 2006/07, the leaves of treatments KCa and NCa had significantly more chlorophyll *a* and *b* than the control treatment and these treatments also had the highest total chlorophyll content. The N content of the petioles sampled at the same stage (Table 5) showed that treatment NCa contained higher N than the control, but not higher than KCa. For 2007/08, treatment Ca leaves contained the lowest chlorophyll *b* and lowest total chlorophyll contents, albeit not significantly lower than that of the control or any

of the fertilisation treatments. Petioles of the same treatment contained the lowest N and K. During the 2008/09 season, none of the treatments affected the chlorophyll content of leaves when compared to the control. However, both the chlorophyll *a* and *b* contents of treatment K were significantly lower than those of treatment N, although petiole N content of the two treatments was very similar. According to Shaahan *et al.* (1999) chlorophyll density in plant leaves can theoretically be used as a tool to determine the nutritional status of N, Ca and Mg. They however found that Mg appears to accumulate in leaves of plants with low N-levels, which complicates the correlations.

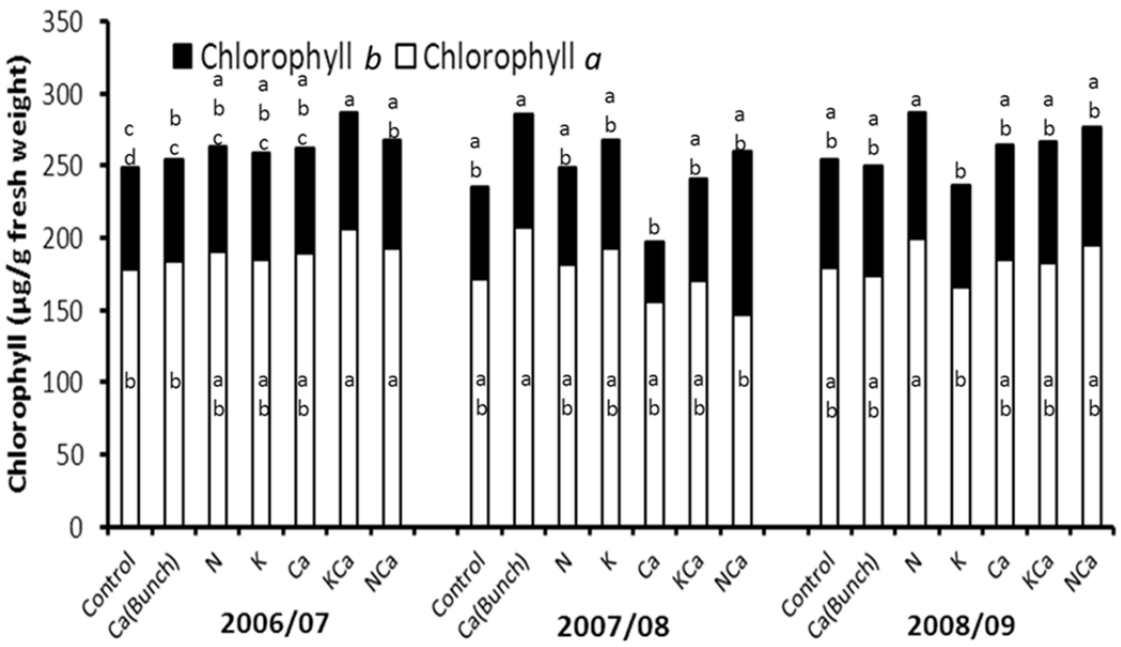

Figure 5. The effect of varying levels of N, K and Ca fertilisation on leaf chlorophyll content at 15 mm berry size (± 82 days after budbreak). Vertical bar values designated by the same letter do not differ significantly ($p \leq 0.05$) for each season.

Data of the 2008/09 season also indicate that K content of the leaves does not affect chlorophyll content. Petioles of treatments K and KCa contained significantly higher K than treatments N, Ca-Bunch, Ca and NCa but their total chlorophyll contents were lower than (in the case of treatment K *vs*. N), or comparable to, the other treatments. The lack of response to K fertilisation treatments is indirectly in agreement with work done by Poni *et al.* (2003), who found that the rate of leaf photosynthesis was only increased when leaf K was raised from levels where deficiency occurred.

Although fertilisation treatments affected petiole nutrient composition (discussed in Chapter III), and that about 50% of plant N is located in the proteins that form the chloroplasts (Porro *et al*., 1995), no clear relation between petiole N and leaf chlorophyll

content could be found. Tam & Magistad (1935) found that in the context of N deficiency, there is an increase in the chlorophyll *a* and *b* content of pineapple plants with increasing amounts of N fertilisation. However, a lack of this correlation was found where deficiency of other nutrients, such as magnesium and iron, existed or where sufficient N was supplied to the control treatments. The lack of an increase in chlorophyll with the N fertilisation treatments therefore seemed to have resulted from the fact that the vine N nutritional status of the control treatment was sufficient, as indicated by the petiole analyses (Table 5).

Table 5. The effect of varying levels of N, K and Ca fertilisation on the chemical composition of the leaf petioles in the trial vineyard at 15 mm berry size over the four experimental seasons. Values designated by the same letter do not differ significantly ($p \leq 0.05$) for each season.

Season		Treatments							
2006/07		Control	Ca (Bunch)	N	K	Ca	KCa	NCa	LSD (p≤ 0.05)
Nutrient content (% dry mass)	N	0.97b	0.93bc	0.94bc	0.95ab	0.84c	0.91bc	1.04a	0.105
	K	1.59ab	1.62ab	1.70ab	1.73ab	1.25b	1.97a	1.77a	0.483
	Ca	1.45	1.53	1.50	1.36	1.52	1.33	1.48	0.229[1]
	Mg	0.57a	0.44ab	0.44ab	0.34b	0.42ab	0.35b	0.47ab	0.16
2007/08		Control	Ca (Bunch)	N	K	Ca	KCa	NCa	LSD (p≤ 0.05)
Nutrient content (% dry mass)	N	0.79ab	0.82a	0.86a	0.73b	0.72b	0.89a	0.92a	0.087
	K	1.50b	1.55b	1.40bc	1.67ab	1.24c	1.99a	1.28c	0.346
	Ca	1.20	1.33	1.22	1.24	1.42	1.28	1.39	0.220[1]
	Mg	0.39b	0.44ab	0.42ab	0.35b	0.43ab	0.38b	0.50a	0.108
2008/09		Control	Ca (Bunch)	N	K	Ca	KCa	NCa	LSD (p≤ 0.05)
Nutrient content (% dry mass)	N	0.84ab	0.79ab	0.83ab	0.81ab	0.70b	0.83ab	0.88a	0.176
	K	2.15ab	1.78bc	1.70bc	2.57a	1.14c	2.75a	1.50bc	0.701
	Ca	0.68	0.73	0.69	0.77	0.75	0.75	0.72	0.098[1]
	Mg	0.24	0.28	0.26	0.20	0.20	0.22	0.24	0.130[1]

[1] Not significant at $p \leq 0.05$.

It is also known that Ca reduces the rate of chlorophyll breakdown and protein degradation (Ferguson, 1984), but again, no clear relationship between vine petiole Ca content and the chlorophyll content of the same leaves was found. Furthermore, potassium fertilisation treatments (K and KCa) reduced petiole Mg contents significantly during the 2006/07 season, whereas in the 2007/08 season it was decreased compared to treatments Ca-Bunch, N, Ca and NCa. Since Mg is bound as the central atom of the porphyrin ring of chlorophyll *a* and *b*

(Bohn, 2004), a reduction in leaf Mg content was expected to lead to marked reductions in leaf chlorophyll content. This, however, was not observed for either of the two seasons. The absence of a reduction of leaf chlorophyll content when the N, Ca or Mg content of petioles of a particular treatment was reduced in comparison to other treatments, is ascribed to the complex manner in which the treatments affected vine nutrition.

Although the concentration of a particular nutrient was increased by elevated application, it affected the concentration of other nutrients in the soil. A comparative nutritional status of the vines could therefore not be generated, since an increased concentration in the soil leads to a reduced concentration of another nutrient in both the soil and vine. This may have contributed to the lack of treatment effects on vine vegetative growth and leaf chlorophyll content obtained in this trial.

CONCLUSIONS

In this study, N, K and Ca fertilisation, at rates of 300% in excess of the vine nutritional requirement, did not affect shoot growth within the context of standard canopy management practices. Likewise, leaf chlorophyll content was also not affected according to a clear pattern. However, these results were obtained in a vineyard with a sandy soil where excessive N fertilisation caused a reduction of soil pH to detrimental levels and where the excessive N, K and Ca applications reduced mutual concentrations and that of Mg, in the soil. A lack of stimulation in vegetative growth may therefore be ascribed to the combined negative effect that these excessive applications had on soil pH and balanced vine nutrition.

LITERATURE CITED

Anonymous, 2007. Guidelines for preparing export table grapes. Capespan Exports (Pty) Ltd., Bellville.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24, 1 - 15.

Barak, P., Jobe, B.O., Krueger, A.R., Peterson, L.A. & Laird, D.A., 1997. Effects of long-term soil acidification due to nitrogen fertilizer inputs in Wisconsin. Plant & Soil 197, 61 - 69.

Bates, R., Dunst, R.M., Taft, T. & Vercant, M., 2002. The vegetative response of 'Concord' *(Vitis labruscana* L.) grapevines to soil pH. Hortscience 37, 890 - 893.

Bohn, T., Walczyk, T., Leisibach, S. & Hurrell, R.F., 2004. Chlorophyll-bound magnesium in commonly consumed vegetables and fruits: Relevance to magnesium nutrition. J. Food Sci. 69, 347 - 350.

Cakmak, D., Saljnikov, E., Perovic, V., Jaramaz, D. & Mrvic, V., 2010. Effect of long-term nitrogen fertilization on main soil chemical properties in Cambisol. Proc. 19th World Congress Soil Sci. E.T. Craswell, PLG Vlek & H. Tiessen (eds.), 1 - 6 August, Brisbane, Australia.

Clarkson, D.T. & Hanson, J.B., 1980. The mineral nutrition of higher plants. Ann. Rev. Plant Physiol. 31, 239 - 298.

Conradie, W.J., 1980. Seasonal uptake of nutrients by Chenin blanc in sand culture: I. Nitrogen. S. Afr. J. Enol. Vitic. 1, 59 - 65.

Conradie, W.J., 1981. Seasonal uptake of nutrients by Chenin blanc in sand culture: II. Phosphorus, potassium, calcium and magnesium. S. Afr. J. Enol. Vitic. 2, 7 - 13.

Conradie, W.J., 1983. Liming and choice of rootstocks as cultural techniques for vines in acid soils. S. Afr. J. Enol. Vitic. 4, 39 - 44.

Conradie, W.J., 1994. Wingerdbemesting. Handleiding van die werksessie oor wingerdbemesting, Nietvoorbij, 30 September, ARC Research Institute for Fruit, Vine and Wine, Private Bag X 5026, Stellenbosch, 7600 R.S.A.

Conradie, W.J., 2001. Timing of nitrogen fertilisation and the effect of poultry manure on the performance of grapevines on sandy soil. I. Soil analyses, grape yield and vegetative growth. S. Afr. J. Enol. Vitic. 22, 53 - 59.

Demarty, M., Morvan, C. & Thellier, M., 1984. Calcium in the cell wall. Plant, Cell & Environ. 7, 441 - 448.

Ferguson, I.B., 1984. Calcium in plant senescence and fruit ripening. Plant, Cell & Environ. 7, 477 - 489.

Follet, R.H., Murphy, L.S. & Donahue, R.L., 1981. Fertilizers and soil amendments. Prentice-Hall Inc., London, UK.

Fuller, M., 1976. The ultrastructure of the outer tissues of cold-stored apple fruits of high and low calcium content in relation to cell breakdown. Ann. Appl. Biol. 83, 299 - 304.

Hanger, B.C., 1979. The movement of calcium in plants. Commun. Soil Sci. Plant Anal. 10, 171 - 193.

Hunter, J.J. & Visser, J.H., 1989. The effect of partial defoliation, leaf position and developmental stage of the vine on leaf chlorophyll concentration in relation to the photosynthetic activity and light intensity in the canopy of *Vitis vinifera* L. cv. Cabernet sauvignon. S. Afr. J. Enol. Vitic. 10, 67 - 73.

Keller, M., Arnink, K.J. & Hrazdina, G., 1998. Interaction of nitrogen availability during bloom and light intensity during veraison. I. Effects on grapevine growth, fruit development, and ripening. Am. J. Enol. Vitic. 49, 333 - 340.

Kasimatis, A.N. & Christensen, L.P., 1976. Response of Thompson Seedless grapevines to potassium application from three fertilizer sources. Am. J. Enol. Vitic. 27, 145 - 149.

Kirkby, E.A. & Pilbeam, D.J., 1984. Calcium as a plant nutrient. Plant, Cell & Environ. 7, 397 - 405.

Macklon, A.E.S., 1984. Calcium fluxes at plasmalemma and tonoplast. Plant, Cell & Environ. 7, 407 - 413.

Mengel, K. & Kirkby, E.A., 1982. Principles of plant nutrition. Int. Potash Institute., Bern, Switzerland.

Poovaiah, B.W., 1979. Role of calcium in ripening and senescence. Commun. Soil Sci. Plant Anal. 10, 83 - 88.

Poni, S., Quartieri, M. & Tagliavini, M., 2003. Potassium nutrition of Cabernet sauvignon grapevines (*Vitis vinifera* L.) as affected by shoot trimming. Plant & Soil 253, 341 - 351.

Porro, D., Steffanini, M., Failla, O. & Stringari, G., 1995. Optimal leaf sampling time in diagnosis of grapevine nutritional status. Acta Hort. 383, 135 - 142.

Ring, E., Jacobson, S. & Högbom, L., 2011. Long-term effects of nitrogen fertilization on soil chemistry in three Scots pine stands in Sweden. Can. J. For. Res. 41, 279 - 288.

Sadowski, A., Scibisz, K., Tomala, K., Kozanecka, T. & Kepka, M., 1988. Negative effects of excessive nitrogen and potassium fertilization in a replanted apple orchard. Acta Hort. 233, 85 - 94.

SAS, 1990. SAS/STAT user's guide, version 8, first edition, volume 2. SAS Institute Inc., Campus drive, Cary NC 27513.

Shaahan, M.M., El-Sayed, A.A. & Abou El-Nour, E.A.A., 1999. Predicting nitrogen, magnesium and iron nutritional status in some perennial crops using a portable chlorophyll meter. Scientia Hort. 82, 339 - 348.

Shapiro, S.S. & Wilk, M.B., 1965. An analyses of variance test for normality (complete samples). Biometrika 52, 591 - 611.

Soil Classification Working Group, 1991. Soil classification: A taxonomic system for South Africa. Soil and Irrigation Research Institute, Dept. of Agric. Development, Pretoria. pp. 262.

Stirzaker, R., 2005. Managing irrigation with a wetting front detector. UK Irrigation 33, 22 - 24.

Storey, R., Jones, R.G.W., Schachtman, D.P. & Treeby, M.T., 2003. Calcium-accumulating cells in the meristematic region of grapevine root apices. Funct. Pl. Biol. 30, 719 - 727.

Takagi, S., Yammamoto, K.T., Furuyu, M. & Nagi, R., 1990. Cooperative regulation of cytoplasmic streaming and Ca^{2+} fluxes by Pfr and photosynthesis in Vallisneria mesophyll cells. Plant Physiol. 94, 1702 - 1708.

Tam, R.K. & Magistad, O.C., 1935. Relationship between nitrogen fertilisation and chlorophyll content of pineapple plants. Plant Physiol. 10, 159 - 168.

The Non-Affiliated Soil Analyses Work Committee, 1990. Handbook of standard soil testing methods for advisory purposes. Soil Sci. Soc. S.A., P.O. Box 30030, Sunnyside, Pretoria.

Trewavas, A., 1999. Le calcium, c'est la vie: Calcium make waves. Plant Physiol. 120, 1 - 6.

CHAPTER III

Excessive N, K and Ca fertilisation effects on leaf and fruit nutrient status of an early ripening table grape cultivar (*Vitis vinifera* L. cv. Prime Seedless), grafted onto Ramsey on a sandy soil

ABSTRACT

A four-year field trial was conducted on a sandy soil in the Paarl district of South Africa, using cv. Prime Seedless (*Vitis vinifera* L.) grapevines, grafted onto Ramsey, and trained to a gable trellis system. The effect of excessive applications of N, K and Ca on table grape performance under typical South African cultivation conditions, with special reference to leaf and fruit nutrient status, was investigated. Nitrogen, potassium and calcium were applied, singular or in combination, at rates equal to 300% the calculated annual nutritional requirement. The control treatment entailed annual applications of 70 kg N/ha, 60 kg K/ha and 10 kg Ca/ha). Excessive N fertilisation caused reduction of soil pH to detrimental levels. A lack of consistently significant increases in petiole N with N fertilisation occurred. Petiole N concentration showed a decreasing trend throughout the season.

At all phenological stages petiole K concentration increased significantly due to the K fertilisation. None of the K fertilisation treatments, however, succeeded to raise petiole K concentrations above the accepted maximum norms. Petiole K concentration at a specific sampling stage varied significantly between the four seasons. A general decrease in petiole K concentration was however found for all seasons, which correlated with the decreased soil K levels. Calcium fertilisation did not increase soil Ca content, resulting in a lack of differences in petiole Ca concentrations between treatments. An increase in petiole Ca concentration towards harvest was, however, obtained.

Reliable correlations between petiole nutrient concentration and berry mineral content at harvest could not be established. It is concluded that the only way of knowing the mineral content of berries would be by measuring it directly instead of deducing it from the results of leaf or petiole analyses. As indicator of vine nutrient availability, petiole analysis must be evaluated in parallel with soil analyses, taking seasonal variation into consideration. The danger of being only guided by published norms for leaf nutrient concentrations when establishing fertilisation practices, has again been highlighted by this study.

INTRODUCTION

For grapevines, the value of foliar analyses as indicator of vine nutrient status is complex. Under comparable conditions there is usually very little difference in the leaf composition of high and low-yielding vineyards (Conradie, 1986; Domingos *et al*., 2004). Apart from the nutrient level in the soil (Bogoni *et al.*, 1995; Hunter *et al*., 2000), cultivar and rootstock, stage of vine growth, cultural practices and seasonal variation are important factors that affect foliar nutrient concentrations (Christensen, 1984; Conradie, 1986; Christensen *et al.*, 1990; Porro *et al*. 1995; Conradie & van Huyssteen, 1996). Since results of one year may be misleading, wide year-to-year nutrient concentration fluctuations require repetitive annual sampling (Christensen, 1984; Marcelle 1990). Porro *et al.* (1995) stated that low variability in nutrient levels is obtained for P, K and Mg when the leaves are analysed at fruit set and for N, Ca and B when analysed at vèraison. High variation was obtained for P, K, Ca & Mg when analysed at harvest time. Considering the seasonal variations in leaf nutrient concentrations, Conradie (1981b) suggested that the most suitable tissue to analyse would be the petiole, and the most stable time for sampling is during the month following bloom. Furthermore, Conradie (1986) also argued that older leaves picked at véraison are often badly damaged and it is then too late to make adjustments to fertilisation practices during the same season. Many table grape producers also remove leaves in the bunch zone, which are the appropriate position for sampling (Conradie 1986), leaving too few for representative sampling.

This study was conducted to determine how petiole nutrient concentration of an early table grape cultivar, like Prime Seedless, changes in response to high rates of N, K and Ca fertilisation, applied singular and in combination. In view of the uncertainties raised above, the intention was also to establish the value of petiole analyses as diagnostic tool for evaluation of the effect of fertiliser applications on grapevine nutrition.

MATERIALS AND METHODS

Vineyard site, experimental design and treatments

A detailed description of the experiment vineyard, treatments and trial layout was given in Chapter II. The trial was conducted over four seasons (2006/07 to 2009/10) on Prime Seedless/Ramsey (*Vitis vinifera* L.) grapevines in a micro-irrigated commercial vineyard of De Hoop Farm in Paarl (33°45'S, 18°58'E), planted in 2002. Vines were grown in a Clovelly soil (Soil Classification Working Group, 1991) with a fine sandy texture containing less than

5% clay, optimal pH (pH_{KCl} = ±5.6), low K (<45 mg/kg), low Mg (<0.3 cmol/kg) and low organic C content (<0.4%) (see Table 1 in Chapter II).

The experiment was laid out as a completely randomised block design where each treatment was replicated five times. The treatments consisted of combinations of different levels of soil applied nitrogen (N), potassium (K) and calcium (Ca), up to 300% of the annual nutritional requirement of the vineyard (Table 1). Fertiliser was applied in six instalments throughout the growing season, two times prior to flowering, three times from set to véraison and once after harvest. The control treatment received fertilisation as required for commercial production and applied by the producer, i.e. 70 kg N/ha/year & 10 kg Ca/ha/year, both split in two instalments before set and post-harvest and 60 kg K/ha/year applied after set.

Table 1. Fertilisation treatments applied to a Prime Seedless/Ramsey (*Vitis vinifera* L.) micro-irrigated commercial vineyard.

Treatment[1]	Total annual nutrient application (kg/ha)		
	N	K	Ca
Control	70	60	10
N[1]	210	60	10
K[2]	70	180	10
Ca[3]	70	60	150
KCa[4]	70	180	150
NCa[5]	210	60	150

1. LAN (28%) was used as nitrogen source.
2. KCl was used as K source.
3. Gypsum ($CaSO_4$) was used as Ca source for the 2006/07, 2007/08 and 2008/09 seasons. In the 2009/10 season, $CaCl_2$ was used.
4. A combination of KCl and $CaSO_4$ was used as K and Ca sources in the 2006/07, 2007/08 and 2008/09 seasons, while $CaCl_2$ was used instead of $CaSO_4$ in the 2009/10 season.
5. A combination of $CaNO_3$, LAN and $CaSO_4$ was used as N and Ca source in the 2006/07, 2007/08 and 2008/09 seasons, while $CaCl_2$ was used instead of $CaSO_4$ in the 2009/10 season.

Instalment size was calculated from the total intended seasonal application of each nutrient, divided as a percentage of the seasonal requirement during each phenological period (Conradie,1980; Conradie, 1981a). Treatments (Control, N, K, Ca, KCa, NCa) were applied each year to the same plants with respect to the first three years, while NCa was not applied in the final (fourth) year.

Measurements

Leaf petiole analyses

Leaf samples were taken at various phenological stages (Table 2) for chemical analyses during the four seasons by removing 30 leaves from randomly selected bearing shoots of the experimental vines at close proximity to bunches. Leave blades and petioles were separated immediately after sampling and petioles were mostly used for experimental purposes. Petiole sampling times included fruit-set, 15 mm berry size, véraison and first harvest for all seasons. Petioles were ashed at 480°C, mixed in a 50:50 HCl (32%) solution, and the

phosphorus (P), cation [K, Ca, magnesium (Mg)], sodium (Na) and micro nutrient [iron (Fe), sink (Zn), manganese (Mn), boron (B)] concentration measured with a Varian ICP-OES optical emission spectrometer. Total N content in the ash was determined using total combustion with a Leco N-analyser (CNS-2000 Macro Elemental Analyzer; Leco Corp, St. Joseph, MI, USA).

Table 2. Petiole sampling times, indicated as days after budbreak (DAB), of a fertilisation trial conducted in a Prime Seedless/Ramsey (*Vitis vinifera* L.) micro-irrigated commercial vineyard.

Phenological stage	Season			
	2006/07	2007/08	2008/09	2009/10
Shoots at top wire (>80cm)	-	43 DAB	-	-
Bloom	-	51 DAB	52 DAB	-
Fruit-set to pea-size berries	63 DAB	71 DAB	73 DAB	66 DAB
15 mm berry size	83 DAB	85 DAB	87 DAB	87 DAB
Véraison	96 DAB	100 DAB	100 DAB	102 DAB
First Harvest	114 DAB	121 DAB	120 DAB	121 DAB
Two weeks after harvest	-	131 DAB	-	-

Berry analyses

Berry samples were taken (cut at the pedicel base) for chemical analyses at various phenological stages during the four seasons by removing three berries, respectively at the top, middle and bottom of four randomly selected bunches per experimental vine, giving a sample of at least 48 berries. Berries were rinsed with distilled water, peeled and the skin and flesh separately frozen at -20^{o}C until analysis for N, P, cations and micro-nutrients. For this purpose, the fresh and dry mass was determined. The latter were obtained after oven-drying of two 10 g duplicate samples at 80^{o}C to constant mass. Total N content was then determined on one of the samples, using total combustion on a Leco N-analyser (CNS-2000 Macro Elemental Analyzer; Leco Corp, St. Joseph, MI, USA), whereas the other sample was used to determine the mineral elements (K, Ca, Mg, Fe, Mn, Cu, Zn) as well as P and B by means of ICP-OES, after extraction with 0.5 M HCl (Isaac & Johnson, 1998). Results of only the harvest sampling time are reported.

Soil and soil water extract analyses

Soil samples for chemical analyses were taken from the 0-30 cm layer with an auger, mixing soil sampled between the two central experimental vines on both experimental vine rows in each plot. The soil was air dried, sieved through a 2 mm sieve and analysed for pH (1.0 M

KCl), P (Bray II) and total extractable cations, namely K, Ca, Mg and Na (extracted at pH = 7 with 0.2 M ammonium acetate) and organic matter by means of the Walkley-Black method (The Non-affiliated Soil Analyses Work Committee, 1990). The extracted solutions were analysed with a Varian ICP-OES optical emission spectrometer.

Soil water samples were taken from wetting front detectors (Stirzaker, 2005), whenever sufficient water accumulated to make an extraction (normally after an irrigation event exceeding the soil water holding capacity and leading to free water draining through the soil). Extraction of soil water from the wetting front detector was done using a tube and syringe. The sample was diluted 10 times to obtain a large enough volume for analyses. Calcium and K concentrations were determined with a Varian ICP-OES optical emission spectrometer. A Seal auto-analyser (AA3) was used to colorimetrically determine NH_4^+ and NO_3^- concentrations with the Na-salisilate and Cd-reduction methods respectively as described in The Non-affiliated Soil Analyses Work Committee (1990).

Statistical procedures

Standard analyses of variance were performed for each season and over all seasons, using Genstat 5 release 1.2 and SAS (SAS, 1990). Student's t-test was used to test for significant differences between treatment means and seasons. The Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965).

RESULTS AND DISCUSSION

Soil and Soil Water

Interpretation of foliar analyses cannot be conducted in isolation of soil analyses (Conradie, 1994). The effect of fertilisation treatments on soil chemical composition and nutrient availability should be taken into account when foliar analyses are interpreted. The impact of the fertilisation treatments on soil chemical as well as soil water extract composition was discussed in detail in Chapter II. Treatments containing K significantly increased the soil K content, while Ca fertilisation did not have a significant effect on the soil Ca concentration. The N concentration of soil water extracts of treatments N and NCa reflected higher N availability (Chapter II). High rates of N applications (treatments N & NCa) stimulated leaching-losses of K and Ca from the soil. Furthermore, the Mg content of the soil was also reduced significantly for all the treatments. These shifts, however were not clearly reflected in the soil water extract composition. Soil pH of treatment N and NCa decreased to levels detrimental to vine root growth and plant performance, i.e. $pH_{KCl} < 5.6$ (Conradie, 1994).

Nitrogen

The effect of the N, K and Ca fertilisation treatments on the N concentration of petioles over the four consecutive seasons is illustrated in Figures 1 a & b. Average petiole N content was generally increased by N fertilisation. There is, however, not a consistent trend in the extent of the response to the N treatment, with only the berry set and harvest sampling time of 2006/07 and véraison samples of 2007/08 that showed significant ($p \leq 0.05$) increases. In view of the fact that a total of 210 kg N per ha was applied annually for treatments N and NCa, as well as the increased NO_3^--N content of the soil water, the lack of significant increases in petiole N is difficult to explain. Similar results were, however, obtained by Conradie (2005) who applied up to 120 kgN/ha N to a Barlinka vineyard in sandy soil with low organic C content before obtaining response in vigour and production. The low uptake of soil applied N also explains why vegetative growth was not significantly increased by the high rate of N applications, as discussed in Chapter II. The decreasing effect of N fertilisation on the soil pH might have reduced root activity progressively, explaining the lack of treatment effects in the latter two seasons. However, Conradie & Saayman (1989) also found that fertilisation of up to 96 kg N per ha per year resulted in only marginal increases in the N contents of both blades and petioles of Chenin blanc vines. Similarly, Porro *et al.* (1995) found that véraison was the only sampling time when levels of N in leaves might correlate with soil fertility.

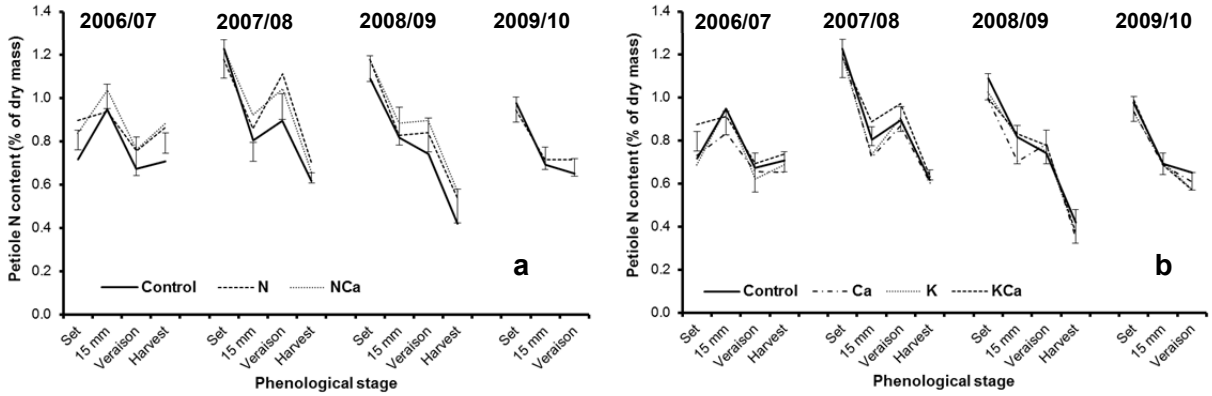

Figure 1. Petiole N concentrations of Prime Seedless vines on a sandy soil in Paarl as (a) affected by excessive fertilisation with N containing fertilisers and (b) excessive fertilisation with Ca and K respectively, or in combination.

Excessive Ca, K and a combination thereof did not affect petiole N concentration significantly or in a consistent manner (Figure 1 b). Hunter *et al.* (2000) succeeded in raising the N content of Sauvignon blanc/110 Richter vine leaves on a 10% clay content soil through N applications alone. However, when K was applied, singular or in combination with N, leaf N content remained unaffected or decreased.

At set the petiole N concentration was highest, except for the 2006/07 season. Throughout the season a decreasing trend, that became more pertinent in the latter two experimental seasons, was obtained (Figure 1). The norms used for fruit-set and véraison petiole samples, as published by Conradie (1986), range between 0.60 and 0.98 % and 0.50 and 0.95 % of dry mass respectively. For both the 2007/08 and 2008/09 seasons all the treatments exceeded the fruit-set norms (Figure 1). At véraison of 2007/08 both treatments that received N fertilisation exceeded the norms. In comparison to the norms published for fruit-set and véraison, this trial showed only once (véraison 2007/08) in four years a response to excessive N fertilisation. This is in accordance with Bravdo & Hepner (1987) who found a poor correlation between total N content in grapevine leaf blades and petioles at harvest and the total amount of N applied to the soil for Cabernet Sauvignon on a very clayey soil.

Compared to the control, the significant increase in petiole N of both N fertilisation treatments at harvest of 2006/07 and of treatment N in 2007/08, points to a possibility to use this time for setting of N nutritional norms. This notion is supported by the consistent trend of the N fertilisation treatments to increase petiole N concentration at harvest. Harvest is not regarded as a reliable sampling time for foliar analyses (Conradie, 1981b). However, in the light of the rapid development of Prime Seedless (harvest is 120 days after budbreak), harvest petiole sampling of this variety might indeed have value. The latter phenological stage may have to be determined by a specific berry maturity level, otherwise it will be difficult to set comparable norms, even for a specific block from year to year.

Changes in petiole N concentration over the season, calculated as average of all the treatments, are indicated in Figure 2 for each of the four experimental seasons. Although the first two seasons showed fluctuating values between consecutive sampling times, a generally declining concentration for all the seasons is observed towards harvest. This observation was also made by Conradie (1981b), Christensen (1984), Porro *et al.* (1995) & Romero *et al.* (2010). Although petiole N concentration at set and véraison in 2007/08 is statistically higher than in the other seasons, no clear pattern in vine N nutritional status can be distinguished between the seasons. This is mainly due to the opposing fluctuating patterns of 2006/07 and 2007/08 seasons. Christensen (1984) investigated the N levels of petioles of 26 grape cultivars at various phenological stages over three consecutive seasons up to véraison. He found no statistical differences in total N concentration of the petioles between the seasons, but the trend between the seasons also differed, as found in this study. Considering petiole NO_3 concentrations of individual cultivars, Christensen (1984) also found a fluctuating pattern that differed extensively between the seasons and per cultivar.

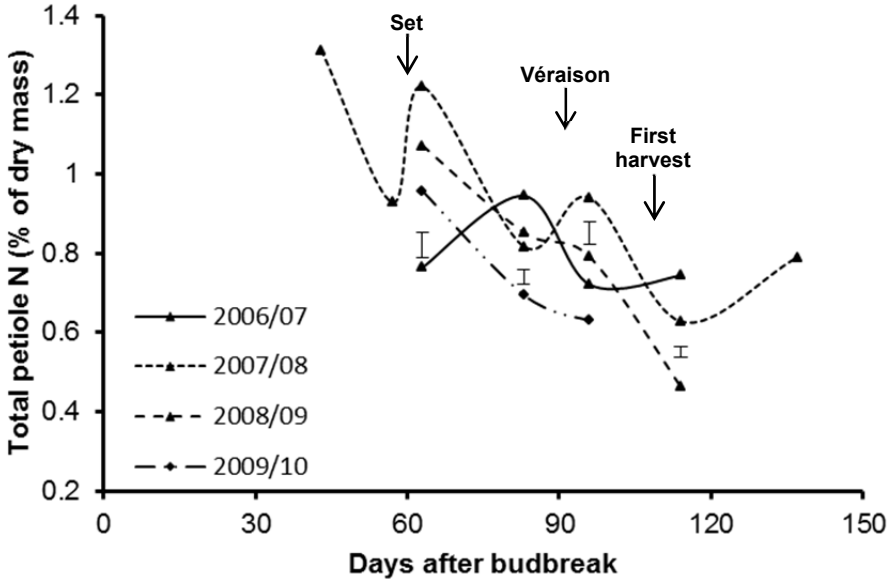

Figure 2. Seasonal changes in petiole N concentration of a Prime Seedless vineyard on a sandy soil in Paarl during four experimental years. Means of all the fertilisation treatments are indicated. Vertical bars represent least significant differences between years at p ≤ 0.05.

The opposing patterns of change in seasonal petiole N concentrations of 2006/07 and 2007/08, whilst they seemed similar in 2008/09 and 2009/10, indicate that conclusions regarding the N nutritional status of the vines cannot be made during the first two seasons after changes in fertilisation were made. This is ascribed to the carry-over effect of vine N nutrition from one season to another which is connected to vine carbohydrate and N reserve accumulation (Walker & Winter, 2006).

Potassium

The effect of the N, K and Ca fertilisation treatments on the K concentration of petioles over the four consecutive seasons is illustrated in Figures 3 a & b. Petiole K concentration was increased by K fertilisation at all phenological stages. This increase was significant for all the sampling times from véraison in 2007/08 and onwards, except for the K treatment at 15mm in 2008/09 and for KCa at 15 mm in 2009/10. In contrast to N, the 180 kg K per ha applied annually, therefore led to significant increases in petiole K. This is ascribed to the significant increases in soil K content obtained for treatments K and KCa (Table 3 in Chapter II) as well as the higher levels of K concentration in the soil water extracts (Table 4 in Chapter II), the preferential uptake of K from the soil (Kirkby, 1979) and its rapid translocation within the plant (Conradie & de Wet, 1985).

Excessive N and Ca did not suppress petiole K concentration significantly, but a consistent trend to lower K levels in the petioles was observed (Figure 3 b). This indicates a reduction in K uptake caused by N fertilisation, which is partially explained by the stimulated leaching-

losses of K from the soil under conditions of high rates of N applications (treatments N & NCa) (Table 3 in Chapter II). Last mentioned is caused by a loss of basic cations when soil pH reduces and K^+ that is displaced by NH_4^+ on the exchange complex. The shifts in K availability were, however, not clearly demonstrated in the soil water extract composition (Table 4 in Chapter II), which may explain the lack of statistically significant results. In support of abovementioned results, Conradie (1992) also stated that K deficiency can be induced by excessive N availability. The reduced petiole K due to Ca fertilisation is explained by competition that exists between K and Ca for uptake by the roots (Geraldson, 1979), but the lack of significant results are due to K uptake being more efficient than Ca uptake (Kirkby & Pilbeam, 1984). In the 2009/10 season, the suppression of K uptake by both N and Ca led to petiole K concentrations declining to levels where it became deficient (< 0.9%) (Figure 3b). It is therefore important to note that despite K fertilisation being applied in accordance to nutritional requirements, excessive N and Ca applications can progressively induce K deficiencies on a sandy soil.

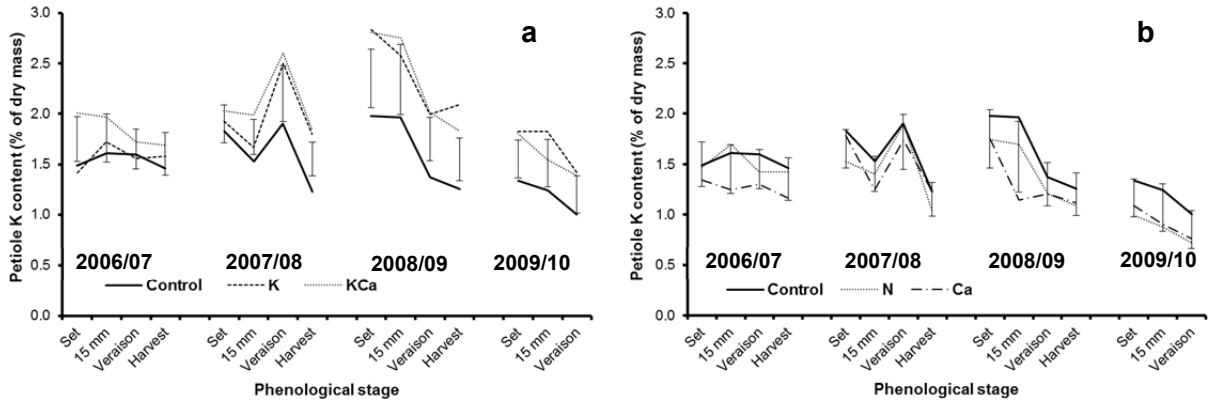

Figure 3. Petiole K concentrations of Prime Seedless vines grown on a sandy soil in Paarl as (a) affected by excessive fertilisation with K containing fertilisers and (b) excessive fertilisation with N and Ca respectively.

Over the four seasons, petiole K concentration of Control treatments varied between 1.01% and 1.98% (Figure 3), being within the acceptable ranges published by Conradie (1986) for fruit set, i.e. 1.00 – 2.90%, and exceeded the véraison norm, which is 0.90 - 1.80%. None of the K fertilisation treatments succeeded to raise petiole K concentrations above the maximum norms as provided by Conradie (1986). From these data it seems that by comparing petiole K analyses to our existing norms will not necessarily reflect an excessive K nutritional status or at least conditions of excessive K supply. A true reflection of the uptake and effectiveness of K fertilisation might therefore only be obtained through seasonal, parallel analyses of petioles from K fertilised vines and those that were not fertilised.

Changes in petiole K concentration over the season, calculated as average of all the treatments, are indicated in Figure 4 for each of the four experimental seasons. Varying

patterns in the change of petiole K concentration between phenological stages occurred for the four experimental seasons. Christensen (1984) also found that petiole K concentration varied significantly between three seasons for a specific sampling stage. This was, however, not true for leaf blade analyses (data not shown). The varying pattern obtained for petioles in this trial cannot be ascribed to the applications of fertiliser shortly before sampling, since all treatments showed similar trends, although their levels varied (as can be seen in Figure 3). The different patterns are therefore ascribed to seasonal differences in climate and possibly soil water contents.

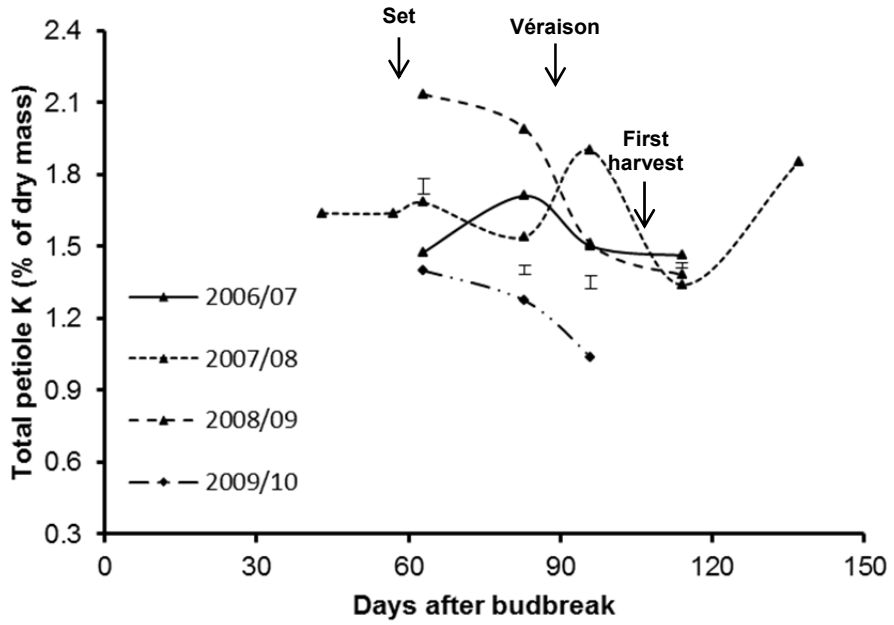

Figure 4. Seasonal changes in petiole K concentration of a Prime Seedless vineyard grown on a sandy soil in Paarl during four experimental years. Means of all the fertilisation treatments are indicated. Vertical bars represent least significant differences between years at $p \leq 0.05$.

Calcium

The effect of the N, K and Ca fertilisation treatments on the Ca concentration of petioles over the four consecutive seasons is illustrated in Figures 5 a & b. Fertilisation with Ca at rates of 150 kg per ha per year increased the petiole Ca concentration slightly only in 2007/08 and 2009/10, but it was not significant (Figure 5 a). Chiu & Bould (1976) found that leaf total-Ca is not a reliable index for predicting fruit-Ca deficiency of tomatoes. Likewise, Kirkby & Pilbeam (1984) quotes previous research which shows that for 18 different plant species the Ca concentration for a given plant species did not vary greatly, regardless whether they were grown in nutrient solutions or in the field.

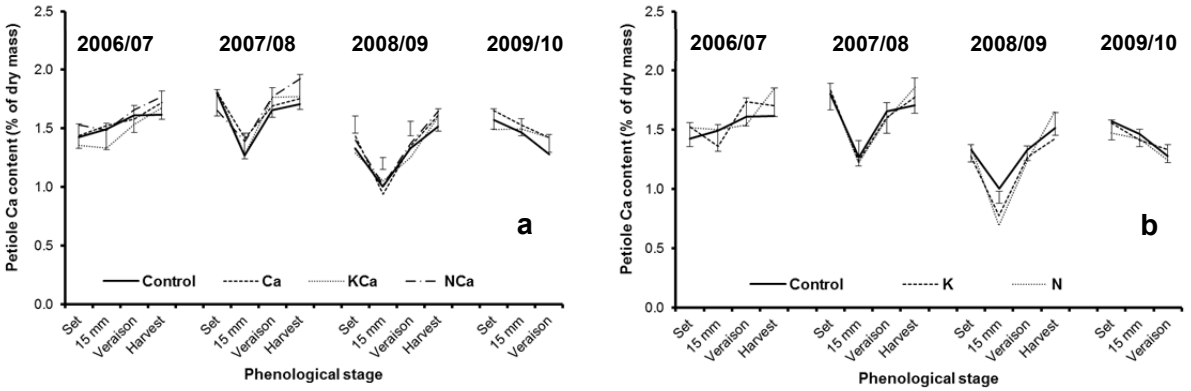

Figure 5. Petiole Ca concentrations of Prime Seedless vines grown on a sandy soil in Paarl as (a) affected by excessive fertilisation with Ca containing fertilisers and (b) excessive fertilisation with N and K respectively.

Excessive levels of N and K fertilisation also did not reduce Ca uptake significantly, with the exception of the samples taken at 15 mm berry size in 2008/09 when the petiole Ca concentration of treatments K and N was significantly lower (Figure 5b). On the one hand, Ca fertilisation (treatments Ca, KCa and NaCa) did not significantly increase soil Ca content (see Table 3 in Chapter II) (explaining the lack of significant increases in petiole Ca concentration on account of Ca applications), while on the other hand, significantly lower Ca was found in the soil of treatment N from winter 2008 onwards, which explains the reduced petiole Ca of treatment N in 2008/09 (Figure 5b). The sub-optimal low soil pH that resulted from treatment N might also have resulted in higher soluble Al^{3+}, which is toxic to vine roots. Hanger (1979) found that high concentrations of Al^{3+} in the soil retard Ca-movement from roots to shoots. Furthermore, the previously mentioned competition for uptake that exists between Ca and K explains the slightly reduced petiole Ca concentrations of treatment K from 2008/09 onwards, despite the fact that the Ca of soil water extracts was not lower than that of the Control.

Over the four seasons, petiole Ca concentration of Control treatments varied between 1.00% and 1.80% (Figure 5). This was within, or exceeded, the acceptable ranges published by Conradie (1986) for fruit set, i.e. 0.60 – 1.40% as well as for véraison, which is 1.10 - 1.90%. Even petiole concentrations of the K and N treatments, sampled at 15 mm berry size in 2008/09, that were significantly lower than the control, remained above the minimum norms (Figure 5b). The reduced Ca concentrations of these treatments, together with low soil pH in the case of treatment N (Table 3 of Chapter II) on the one hand, and the fact that petiole Ca remained above the minimum Ca norms published by Conradie (1986), on the other hand, indicate to the grapevine having very low Ca nutritional requirements. From the data of this trial it seems that a comparison of petiole Ca analyses and our present norms, would not necessarily reflect a low Ca nutritional status or at least conditions of low Ca supply. A true reflection of the uptake and effectiveness of Ca fertilisation can also not be obtained through seasonal, parallel analyses of petioles from Ca fertilised vines and those that were not

fertilised. According to Follet *et al*. (1981), Ca deficiency in field crops is seldom encountered, yet it leads to reduced growth, especially of young leaves and growing tissues. Using young leaves, of which the best sampling time would probably be before tipping or topping (before set), as indicator of Ca nutritional status, should be investigated.

Changes in petiole Ca concentration over the season, calculated as average of all the treatments, are indicated in Figure 6 for each the four experimental seasons. Also for Ca, varying patterns in petiole Ca concentration between phenological stages occurred for the four experimental seasons. An increasing trend in petiole Ca concentration from 15 mm berry size (± 83 DAB), however, occurred towards harvest for three (2006/07, 2007/08 & 2008/09) of the four seasons. Conradie (1981b), Porro *et al.* (1995) & Romero *et al*. (2010) also showed found that leaf Ca concentration increased throughout the season.

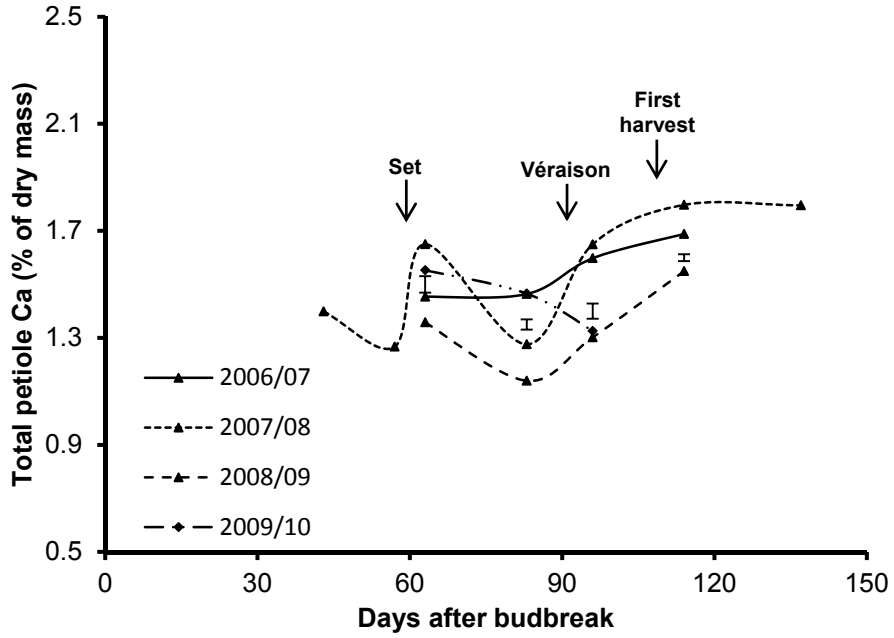

Figure 6. Seasonal changes in petiole Ca concentration of a Prime Seedless vineyard grown on a sandy soil in Paarl during four experimental years. Means of all the fertilisation treatments are indicated. Vertical bars represent least significant differences between years at p ≤ 0.05.

Magnesium

Due to the known effect of especially K and Ca to suppress Mg uptake, when applied at high rates (Kirkby & Pilbeam, 1984), the effect of the treatments on petiole Mg concentration was also investigated, and is presented in Figure 7. Both K and Ca fertilisation indeed suppressed Mg concentration in petioles, being significant from véraison 2007/08 onwards. This is in accordance with Sadowski *et al.* (1988) who reported that excessive fertilisation of apple trees with K resulted in reduced leaf Mg contents and is explained by the significant reduction of Mg content of the soil for all the treatments (Table 3 in Chapter II). Potassium

fertilisation actually increased soil water Mg content, probably due to exchange reactions, but the high K concentrations in the soil water suppressed uptake of the Mg, as stated by Conradie (1992).

Excessive applications of N had no effect on petiole Mg concentration (Figure 7).

Except for the 2009/10 season, Mg concentration in vine petioles increased from véraison to harvest. In contrast to petiole K concentration that decreases during this period due to translocation to the berries (Iland, 1988), Mg seems to accumulate.

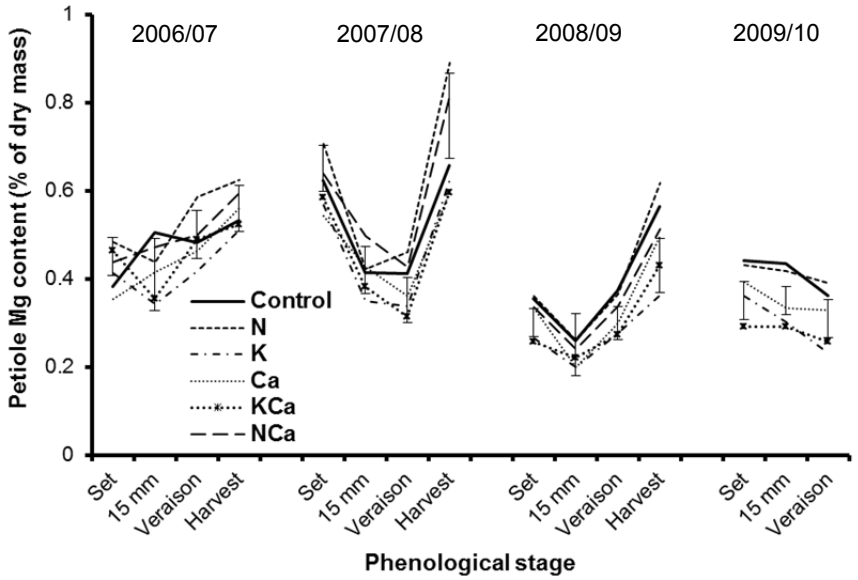

Figure 7. Petiole Mg concentrations of Prime Seedless vines grown on a sandy soil in Paarl as affected by excessive fertilisation with N, K and Ca containing fertilisers. Vertical bars represent least significant differences between years at $p \leq 0.05$.

Ratio of petiole nutrient concentrations

Another approach to evaluate nutritional status of plants is to consider the ratios of nutrient concentrations in leaf petioles. According to Fregoni (1984) nutrient ratios are better indicators of the nutritional status of a plant than normal concentrations based on dry matter, and they are better correlated with yield than absolute contents. In view of the lack of significant responses in petiole nutrients to excessive N and Ca fertilisation, and the insignificant suppression of K uptake by excessive N and Ca uptake, treatment effects on the four year average ratios of K and N (K:N ratio), Ca (K:Ca ratio), and Mg (K:Mg ratio) as well as Ca:N and Ca:Mg ratios of the different treatments were calculated for four phenological stages. The results are presented in Table 3.

Table 3. The four year average K:N, K:Ca, K:Mg, Ca:N and Ca:Mg ratio of petioles of Prime Seedless in Paarl at different phenological stages as affected by various fertilisation treatments.

Treatment	Set	15 mm berry size	Véraison	Harvest
		K:N ratio		
Control	1.79 b	2.02 b	2.10 b	2.39 b
N	1.35 c	1.55 c	1.51c	1.73 c
K	2.11 a	2.46 a	2.67 a	3.62 a
Ca	1.55 bc	1.53 c	1.67 c	2.34 b
KCa	2.17 a	2.49 a	2.65 a	3.39 a
NCa	1.50 c	1.59 c	1.57 c	1.58 c
LSD ($p \leq 0.05$)	0.27	0.29	0.32	0.59
		K:Ca ratio		
Control	1.19 bc	1.64 ab	1.09 b	0.85 b
N	0.94 d	0.98 d	0.88 bc	0.66 b
K	1.37 ab	1.84 a	1.38 a	1.15 a
Ca	0.97 cd	1.20 cd	0.83 c	0.69 b
KCa	1.51 a	1.98 a	1.35 a	1.09 a
NCa	1.08 cd	1.43 bc	0.88 bc	0.63 c
LSD ($p \leq 0.05$)	0.24	0.39	0.24	0.23
		K:Mg ratio		
Control	4.27 b	5.22 b	4.08 b	2.41b
N	3.13 c	3.25 c	2.94 c	1.74 b
K	5.96 a	7.28 a	6.39 a	4.15 a
Ca	3.89 bc	4.03 bc	3.61bc	2.18 b
KCa	6.47 a	7.25 a	6.39 a	3.66 a
NCa	3.78 bc	4.45 bc	3.55 bc	1.86 b
LSD ($p \leq 0.05$)	1.11	1.46	1.13	1.11
		Ca:N ratio		
Control	1.57 ab	1.48 c	1.96 ab	2.95 ab
N	1.49 b	1.69 ab	1.80 b	2.69 b
K	1.65 a	1.54 abc	2.03 ab	3.10 ab
Ca	1.69 a	1.71 a	2.14 a	3.44 a
KCa	1.49 b	1.52 c	2.05 ab	3.15 ab
NCa	1.45 b	1.24 d	1.83 b	2.64 b
LSD ($p \leq 0.05$)	0.14	0.16	0.25	0.52
		Ca:Mg ratio		
Control	3.56 b	3.17 d	3.68 cd	2.77 a
N	3.25 b	3.29 dc	3.38 d	2.56 d
K	4.08 a	4.06 a	4.68 a	3.46 8a
Ca	4.00 a	3.62 bc	4.29 ab	3.11 abc
KCa	4.19 a	3.95 ab	4.70 a	3.36 ab
NCa	3.41 b	3.04 d	3.92 bc	2.91 bcd
LSD ($p \leq 0.05$)	0.35	0.41	0.54	0.46

Compared to the Control, the K:N ratio was significantly reduced by N applications for all the phenological stages. This illustrates a definite response in the vine's nutritional balance of K relative to N with N fertilisation. Treatments containing N did not significantly affect the Ca:N ratio, although this ratio was consistently reduced. The lack of significant changes is ascribed to the mutually synergistic effect of these two nutrients on the uptake of each other (Kirkby, 1979).

The dramatic increase of the K:N ratio due to K fertilisation above the ratio of the control treatment, illustrates how K fertilisation leads to a reduction of the N to K nutritional balance of the vine (Table 3). The impact that K fertilisation has on Ca and Mg uptake is also illustrated in the significantly increased K:Ca and K:Mg ratios obtained for treatments K and even KCa. Morris *et al*. (1980) also found that petiole Ca and Mg showed a negative correlation with K under conditions of high rates of K fertilisation.

Fertilisation with Ca did not significantly change the Ca:N ratio, while it increased the Ca:Mg ratio significantly. The effect of K, but not of Ca, on the Mg status of the plant is also evident from the significantly increased Ca:Mg ratios of K containing treatments.

If the ratios are compared to norms published by Fregoni (1984) for K:N (0.42 – 0.53), K:Ca (2.20-2.22) and K:Mg (3.0-7.0) for wine grapes in Italy, the following is observed: (a) much higher K:N ratios were found in this trial, (b) lower K:Ca ratios were found in this trial (which are again ascribed to much less K fertilisation applied to wine grape vineyards), and (c) similar K:Mg ratios were found, despite the fairly low Mg contents in the soil of the trial vineyard. All three ratios, being used as norms, which were published by Fregoni (1984) were probably set in a context of low rates of K fertilisation in wine grape vineyards, rendering these ratios irrelevant to table grapes.

Correlation between petiole and fruit nutrient content

The correlations between vine petiole N, K, Ca and Mg concentration at véraison and their concentration in the grape berry at first harvest are presented in Table 4. Correlations between petiole samples taken at first harvest and berry analyses are also indicated in Table 5. From the four seasons, a significant correlation between petiole N at véraison was found for both berry skins and flesh only in the 2008/09 season.

Potassium in the petioles at véraison correlated only with berry skin K concentration for both 2008/09 and 2009/10 seasons, but not 2006/07 and 2007/08. Table 4 also shows that only in 2008/09 a correlation between leaf blade N at harvest and either skin or berry flesh could be obtained. In both 2008/09 and 2009/10 a correlation between leaf blade K at harvest and berry skin K concentration was obtained.

Table 4. Correlation between N, K, Ca and Mg concentrations in petioles of Prime Seedless grapevines in Paarl, sampled at véraison, and their berries at first harvest.

Season	2006/07		2007/08		2008/09		2009/10	
Berry tissue	Skin	Flesh	Skin	Flesh	Skin	Flesh	Skin	Flesh
N	NS[1]	NS	NS	NS	0.05[2]	0.01	NS	NS
K	NS	NS	NS	NS	0.05	NS	0.01	NS
Ca	NS	NS	NS	NS	NS	NS	NS	NS
Mg	NS	NS	NS	NS	NS	NS	NS	NS

[1] NS: not significant
[2] The two-tailed significance level of the correlation coefficient (r^2) for df = 83, except 2009/10 where df=28.

Table 5. Correlation between N, K, Ca and Mg concentrations in petioles of Prime Seedless grapevines in Paarl, sampled at first harvest, and their berries.

Season	2006/07		2007/08		2008/09		2009/10	
Berry tissue	Skin	Flesh	Skin	Flesh	Skin	Flesh	Skin	Flesh
N	NS[1]	NS	NS	NS	0.01[2]	0.01	NS	NS
K	NS	NS	0.05	0.02	NS	NS	0.05	0.05
Ca	NS	NS	NS	NS	NS	NS	NS	NS
Mg	NS	NS	NS	NS	NS	NS	NS	NS

[1] NS: not significant
[2] The two-tailed significance level of the correlation coefficient (r^2) for df = 83, except 2009/10 where df=28.

Comparisons of the mineral composition of leaves and fruit for apple and pear cultivars, done over 12 years by Marcelle (1990), also indicated that the correlations are rare and weak. Iland (1988) also mentioned that a response in vine growth and berry K content to K fertilisation would only be observed in vineyards with a K deficiency. It is therefore evident that reliable correlations cannot be established and the only way of knowing the mineral content of berries is by measuring it directly instead of deducing it from the results of leaf or petiole analyses.

CONCLUSIONS

Excessive N fertilisation caused reduction of soil pH to detrimental/unacceptably low levels. The lack of consistently significant increases in petiole N on account of N fertilisation indicated that the applied N could not be effectively utilised by the grapevines. Given the fact that accumulation of reserves, including N reserve compounds, takes place throughout the season from flowering onwards, the decreasing trend of petiole N concentration throughout

the season may have been evidence of a progressive translocation of N to reserve compartments in the permanent wood as the leaves age.

Significant increases in soil K content were obtained for the K fertilisation treatments. In response, petiole K concentrations were significantly increased by the K fertilisation at all phenological stages. This illustrates how readily, and preferentially, K is taken up and might also be effectively translocated to perennial parts. None of the K fertilisation treatments, however, succeeded to raise petiole K concentrations above the presently acceptable maximum norms, which indicates that maximum norms used for K nutrition that may probably be too high, or that it is difficult to reach the maximum norms on sandy soils. On the other hand, the variation of petiole K concentration between the four seasons, for a specific sampling stage, might indicate that petiole K concentration norms are difficult to define, hence the wide range.

With Ca that typically ranges between 60-80% of the cations in most vineyard soils in South Africa, it is not unexpected that Ca fertilisation had little effect on the soil's total Ca content. The lack of increased Ca concentration in the petioles, on account of Ca fertilisation, is therefore not surprising. The continuously increasing trend in petiole Ca concentration up to harvest is in agreement with the fact that Ca is mainly transported *via* the xylem, which would occur as long as the leaves transpires.

Reliable correlations between petiole nutrient concentration and berry mineral content could not be established. It is therefore concluded that the only way of knowing the mineral content of berries would be by measuring it directly instead of deducing it from the results of leaf or petiole analyses.

This study highlights the fact that petiole nutrient concentrations must be interpreted with much caution. It should only be used as indicator of vine nutrient availability if it is evaluated in parallel with soil analyses and by taking seasonal variation into consideration. If foliar analyses are to be used as a diagnostic tool, annual sampling times would have to be fixed and an individual set of norms would have to be developed per vineyard block. For Prime Seedless in Paarl, véraison and harvest seem to be the most suitable times for analyses to evaluate the grapevine N nutritional status achieved form past fertilisation practices. Most sampling times seem to be suitable for K, but 15 mm berry size and véraison were found to be the most sensitive.

LITERATURE CITED

Bravdo, B. & Hepner, Y., 1987. Irrigation management and fertigation to optimize grape composition and vine performance. Acta Hort. 206, 49 - 67.

Bogoni, M., Panont, A., Valenti, L. & Scienza, A., 1995. Effects of soil physical and chemical conditions on grapevine nutritional status. Acta Hort. 383, 299 - 311.

Chiu, T. & Bould, C., 1976. Effect of shortage of calcium and other cations on 45Ca mobility, growth and nutritional disorders of tomatoe plants (Lycoperscion esculentum). J. Sci. Food Agric. 27, 969-977.

Christensen, P., 1984. Nutrient level comparisons of leaf petioles and blades in twenty-six grape cultivars over three years (1979 through 1981). Am. J. Enol. Vitic. 35, 124 - 133.

Christensen, L.P., Boggero, J. & Bianci, M., 1990. Comparative leaf tissue analysis of potassium deficiency and a disorder resembling potassium deficiency in Thompson Seedless grapevines. Am. J. Enol. Vitic. 41, 77 - 83.

Conradie, W.J., 1980. Seasonal uptake of nutrients by Chenin blanc in sand culture: I. Nitrogen. S. Afr. J. Enol. Vitic. 1, 59 - 65.

Conradie, W.J., 1981a. Seasonal uptake of nutrients by Chenin blanc in sand culture: II. Phosphorus, potassium, calcium and magnesium. S. Afr. J. Enol. Vitic. 2, 7 - 13.

Conradie, W.J., 1981b. Nutrient consumption by Chenin blanc grown in sand culture and seasonal changes in the chemical composition of leaf blades and petioles. S. Afr. J. Enol. Vitic. 2, 15 - 18.

Conradie, W.J., 1986. Norms for leaf analyses of vines. Farming in South Africa: Viticulture and Oenology. E24.

Conradie, W.J., 1992. Die nut van blaarontledings by wingerd. Wynboer, Nov., 75 - 76.

Conradie, W.J., 1994. Wingerdbemesting. Handleiding van die werksessie oor wingerdbemesting. Nietvoorbij, 30 September, ARC Research Institute for Fruit, Vine and Wine, Private Bag X5026, Stellenbosch, 7600, R.S.A.

Conradie, W.J., 2005. Partitioning of mineral nutrients and timing of fertilizer applications for optimum efficiency. Proc. Soil Environ. Vine Mineral Nutr. Symp., L.P. Christensen, D.R. Smart (eds.), Am. Soc. En. Vitic. Davis, USA, pp.69 - 81.

Conradie, W.J. & de Wet, T., 1985. The effect of potassium fertilisation of grapevines on yield and quality. Proc. Potassium Symp. Dept. Agric. & Water Supply, Pretoria, 1 - 3 October, 181 – 183.

Conradie, W.J. & Saayman D., 1989. Effects of long-term nitrogen, phosphorus, and potassium fertilization on Chenin blanc vines. II. Leaf analyses and grape composition. Am. J. Enol. Vitic. 40, 91 - 98.

Conradie, W.J. & van Huyssteen, I., 1996. Nitrate content of grapevine petioles as a guideline for nitrogen fertilization. 76th General Assembly of the OIV, Cape Town, 10 - 18 November 1996.

Domingos, I., Silva, T., Correia, P.J., Pestana, M. and de Varennes, A., 2004. Effects of fertiliser practices on the growth and quality of two table grape cultivars: ‘Çardinal’ and ‘D. Maria’. Acta Hort. 652, 241 - 247.

Fregoni, M., 1984. Nutrient needs in wine production. In: Nutrient balances and fertiliser needs in temperate agriculture. Proc. 18th Coll. Int. Potash Institute. Malquori, A. (ed.), Gardone-Riviera, Italy. pp. 319 - 332.

Follet, R.H., Murphy, L.S. & Donahue, R.L., 1981. Fertilizers and soil amendments. Prentice-Hall Inc., London, UK.

Geraldson, G.M., 1979. Minimal calcium stress using the gradient mulch production system. Commun. Soil Sci. Plant Anal. 10, 163 - 169.

Hanger, B.C., 1979. The movement of calcium in plants. Commun. Soil Sci. Plant Anal. 10, 171 - 193.

Hunter, J.J., Volschenk, C.G. & Stevens, S., 2000. Effect of N and K fertilisation on organic acid accumulation and stability in grapes and must. Abstract: 2nd Int. Viticulture & Enology Congress, Cape Town, 8-10 November.

Iland, P.G., 1988. Grape berry ripening: the potassium story. Aust. Grapegrower & Winemaker 289, 22 - 24.

Isaac, R.A. & Johnson, W.C., 1998. Elemental determination by Inductively Coupled Plasma. Y.P. Kalra (Ed.). Handbook of reference methods for plant analysis, pp. 165 - 170. CRC Press, Boca Raton, USA.

Kirkby, E.A., 1979. Maximizing calcium uptake by plants. Commun. Soil Sci. Plant Anal. 10, 89 - 113.

Kirkby, E.A. & Pilbeam, D.J., 1984. Calcium as a plant nutrient. Plant, Cell & Environ. 7, 397 - 405.

Marcelle, R.D., 1990. Comparison of the mineral composition of leaf and fruit in apple and pear cultivars. Acta Hort. 274, 315 - 320.

Morris, J.R., Cawthon, D.L. & Fleming, J.W., 1980. Effects of high rates of potassium fertilization on raw product quality and changes in pH and acidity during storage of Concord grape juice. Am. J. Enol. Vitic. 31, 323 - 328.

Porro, D., Steffanini, M., Failla, O. & Stringari, G., 1995. Optimal leaf sampling time in diagnosis of grapevine nutritional status. Acta Hort. 383, 135 - 142.

Romero, I., García-Escudero, E. & Martin, I., 2010. Effects of leaf position on blade and petiole mineral nutrient concentration of Tempranillo grapevine (Vitis vinifera L.). Am. J. Enol. Vitic. 61, 544 - 550.

Sadowski, A., Scibisz, K., Tomala, K., Kozanecka, T. & Kepka, M., 1988. Negative effects of excessive nitrogen and potassium fertilization in a replanted apple orchard. Acta Hort. 233, 85 - 94.

SAS, 1990. SAS/STAT user's guide, version 8, first edition, volume 2. SAS Institute Inc., Campus drive, Cary NC 27513.

Shapiro, S.S. & Wilk, M.B., 1965. An analyses of variance test for normality (complete samples). Biometrika 52, 591 - 611.

Soil Classification Working Group, 1991. Soil classification: A taxonomic system for South Africa. Soil and Irrigation Research Institute, Dept. of Agric. Development, Pretoria, R.S.A.

Stirzaker, R., 2005. Managing irrigation with a wetting front detector. UK Irrigation 33, 22 - 24.

The Non-Affiliated Soil Analyses Work Committee, 1990. Handbook of standard soil testing methods for advisory purposes. Soil Sci. Soc. South Africa, P.O. Box 30030, Sunnyside, Pretoria.

Walker, R. & Winter, E., 2006. Vine carbohydrate dynamics and source-sink relationships. Workshop held by the Grape and Wine Research & Development Corporation, CSIRO, 31 January, Merbein, Australia.

CHAPTER IV

Accumulation of macro-nutrients (N, P, K, Ca and Mg) in berries by an early ripening table grape cultivar (*Vitis vinifera* L. cv. Prime Seedless) on a sandy soil

ABSTRACT

A four-year field trial was conducted on a sandy soil in the Paarl district of South Africa, using cv. Prime Seedless (*Vitis vinifera* L.) grapevines, grafted onto Ramsey, and trained to a gable trellis system. Nitrogen, potassium and calcium were applied, singular or in combination, at rates up to 300% the calculated annual nutritional requirement. The Control treatment received an annual application of 70 kg N/ha, 60 kg K/ha and 10 kg Ca/ha. Berry growth as well as average N, P, K, Ca and Mg concentration in the flesh and skin of all treatments was determined at various development stages for four growing seasons. Nutrient accumulation patterns per berry were also established.

Although rapid berry growth was maintained up to the first harvest, the decrease in nutrient concentrations was most rapid up to véraison, thereafter different patterns of change in berry nutrient concentration occurred between the nutrients. Accumulation of the nutrients, particularly K and Ca, occurred independent of one another. Calcium accumulation in the berry finished before véraison, while the other nutrients continued to accumulate, albeit at an increasingly slower rate towards first harvest for P, K and Mg.

For all the nutrients, the berry flesh contained the larger part of the total accumulated nutrients in the berry, although the skin concentration exceeded that of the flesh as the berry increased in size. Due to the role of the nutrient concentration, rather than total content, impact on berry quality, a better understanding of other dynamics that determine berry nutrient concentration is required. Furthermore, the rapid development of this early seedless variety, with berry size that far exceeds wine grapes, is accepted as an important factor influencing berry nutrient accumulation patterns to divert slightly from the generally established ones.

INTRODUCTION

Successful table grape production presumes fruit of good eating quality and post-harvest storage capacity. It is generally accepted that some aspects of grape quality, such as solid concentration, are positively correlated to fruit potassium (K) (Rogiers *et al.,* 2006). During storage, fruit quality is favoured by low nitrogen (N) and high calcium (Ca) levels (Marcelle, 1995; Bonomelli & Ruiz, 2010). Bunch rot is assumed to be enhanced when there is a higher soluble N fraction in the berries due to poor timing (after véraison) and excessive N fertilisation (Conradie, 1986). Ruiz *et al*. (2004) also found that the higher the N content of berries, the softer they were.

Grape berries are a large sink for K (Conradie 1981a). It is known that high juice K decreases free acids and increases overall pH (Morris *et al.,* 1982). Tartaric acid, a significantly stronger acid than malic acid (Rühl, 2000), precipitates with high K in salt form so that the free tartrate decreases. High K will therefore lead to reduced tartrate:malate ratios (Mpelasoka *et al*., 2003), probably affecting the taste of the berries significantly because of the much more crisp, fresh acid taste of tartaric acid (Rühl, 2000). Ripeness levels may be promoted because an acid titration would indicate less acidity. The colour quality and acidity of fresh grape juice were reduced by excessive K fertilisation (Morris *et al*., 1982). They, however, did not consider whether the excessive amounts of K affected the pH of intact cytoplasm.

In all fruits there is a decline in Ca influx during growth. This not only results from an increase in solute influx *via* the phloem during fruit ripening, but also from a decline in cell division rate and thus the formation of new binding sites for Ca, as well as an increase in volume/surface area, reducing the transpiration per unit weight of fruit (Kirkby & Pilbeam, 1984). Tagliavini *et al.* (2000) stated that the more tissues are subject to rapid expansion, like berry flesh, the lower is the Ca requirement. Polygalacturonase (PG), an enzyme that breaks down the pectin structure of cell walls during berry ripening and that is also used by *Botrytis cinerea* to gain access to cells, is inhibited by the presence of Ca (Poovaiah *et al*., 1988).

In the apple industry fruit quality was found to be dependent on its mineral composition. Fertilization therefore started to take into account the mineral composition of the fruit so that an optimal balance between different minerals, particularly potassium (K) and calcium (Ca) is now being sought (Marcelle, 1990).

In order to manipulate mineral content and balance in grapes, it is important to know the dynamics of nutrient accumulation in developing berries. Numerous studies have dealt with the nutrient accumulation patterns of grape berries, mainly with a focus on wine production (Conradie, 1980; Conradie, 1981a; Possner & Kliewer, 1985; Coombe, 1987; Donèche & Chardonnet, 1992; Schaller *et al*., 1992; Creasy *et al*., 1993; Chardonnet & Donèche, 1995; Ollat & Gaudillère, 1996; Cabanne & Donèche, 2003; Rogiers *et al*., 2006; Etchebarne *et al*., 2009). However, no information is available to confirm that nutrient accumulation patterns of very early maturing, seedless varieties with large berry size and therefore much smaller skin to flesh ratios than wine grapes, are similar to the models that have been proposed.

The objective of this study was to aid table grape nutrition practices as well as harvest timing and post-harvest quality prediction by investigating the nutrient accumulation patterns of Prime Seedless (*Vitis vinifera* L.), a very early seedless table grape variety that are produced with minimum berry diameter of 18mm. Furthermore, for fresh consumption, table grapes are harvested at lower total soluble solid concentrations than wine grapes. The study was therefore focused on nutrient accumulation up to early maturity, which is about 120 days after budbreak.

MATERIALS AND METHODS

Vineyard site, experimental design and treatments

A detailed description of the experiment vineyard, treatments and trial layout was given in Chapter II. The trial was conducted over four seasons (2006/07 to 2009/10) on Prime Seedless/Ramsey (*Vitis vinifera* L.) grapevines in a micro-irrigated commercial vineyard of De Hoop Farm in Paarl (33°45'S, 18°58'E), planted in 2002. Vines were grown in a Clovelly soil (Soil Classification Working Group, 1991) with a fine sandy texture containing less than 5% clay. Detail of the soil chemical composition was also provided in Chapter II. The grapevines were trained to a gable system, spaced 1.8 m x 3 m apart, head trained and cane pruned to eight buds. Standard cultural practices for the cultivar and region were followed as described in Anonymous (2007). It entailed shoot tipping and crop control after set, combined with removal of leaves that are in close proximity of the retained bunches. Bunch preparation entailed an application of 1 mg/L gibberellic acid (GA_3) at bloom for bunch thinning, shortening of bunches to 8 cm length at set, dipping bunches in 20 mg/L GA_3 when they were 8 to 10 mm in diameter, and again at 10-12 mm diameter, for berry enlargement and finally doing final hand-thinning of bunches just before véraison.

An experiment was laid out as a completely randomised block design where each treatment was replicated five times. Treatments consisted of combinations of different levels of soil applied nitrogen (N), potassium (K) and calcium (Ca), up to 300% of the annual nutritional requirement of the vineyard. An additional treatment, i.e. bunch applied Ca, was also included. Details of treatments are provided in Chapter II. Fertiliser was applied in six instalments throughout the growing season, two times prior to flowering, three times from set to véraison and once after harvest.

Instalment size was calculated from the total intended seasonal application of each nutrient, divided as a percentage of the seasonal requirement during each phenological period (Conradie,1980; Conradie, 1981a). Treatments (Control, Ca-Bunch, N, K, Ca and KCa as per Table 2 in Chapter II) were applied each year to the same plants. Since the percentage distribution at each application time corresponds to each nutrient's seasonal uptake pattern, the averages over all the treatments grouped together were calculated and are reported on. The effects of the treatments on berry nutrient content are discussed in the following chapter (Chapter V).

Measurements

Berry analyses

Berry samples were taken (cut at the pedicel base) for chemical analyses at various phenological stages (Table 1). Prime Seedless commonly show variance in berry development between bunches. Sampling times were therefore based on the development stage of the bulk of bunches, with the first harvest sample corresponding with the first cutting date of the producer. Chemical analyses were conducted on the berries during all four seasons. To address variation within bunches, sampling was done by removing three berries at the top, middle and bottom, respectively, of four randomly selected bunches per experimental vine, giving a sample of at least 48 berries. Berries were rinsed with distilled water, peeled and the skin and flesh separately frozen at -20^{o}C until analysis for N, P, cations and micro-nutrients. For this purpose, the fresh and dry mass was determined. The latter was obtained after oven-drying of two 10 g duplicate samples at 80^{o}C to constant mass. One sample was then used for total N content determination by total combustion on a Leco N-analyser (CNS-2000 Macro Elemental Analyzer; Leco Corp, St. Joseph, MI, USA), whereas the other sample was used to determine the mineral elements (K, Ca, Mg, Fe, Mn, Cu, Zn) as well as P and B by means of ICP-OES, after extraction with 0.5 M HCl (Isaac & Johnson, 1998).

Statistical procedures

Standard analyses of variance were performed for each sampling time and season, using Genstat 5 release 1.2 and SAS (SAS, 1990). Student's t-test was used to test for significant differences between treatment means and seasons. The Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965). Analyses of all the treatments were pooled for establishment of the seasonal nutrient accumulation pattern, calculating the average for each sampling date for each season, i.e. n = 85 samples for seasons 2006/07 to 2008/09 and n = 30 for 2009/10.

Table 1. Berry sampling times, indicated as days after anthesis (DAA), of a Prime Seedless/Ramsey (*Vitis vinifera* L.) micro-irrigated commercial vineyard in Paarl.

	Season			
	2006/07	**2007/08**	**2008/09**	**2009/10**
Set	-	9	-	
Pea-size berries[1]	20	20	21	21
	25	-	27	-
15 mm Berry size	40	34	35	37
	-	37	42	43
	-	45	-	-
Véraison	53	51	49	51
		-	55	-
	60	-	61	
First Harvest	71	70	69	72
10 days after First Harvest	-	80	76	-
	-	-	83	-
	-	-	90	-

1. Berry enlargement treatments, i.e. 20 mg/L gibberellic acid, were applied twice at 8 – 10 mm and 10 – 12 mm diameter.

RESULTS AND DISCUSSION

Berry growth

Berry growth is indicated as increase in fresh and dry mass, respectively, for seasons 2006/07 to 2009/10 in Figure 1. Fresh mass increased from pea berry size at a rapid rate, averaging 0.146 g/day over the four seasons and did not subside before first harvest. Berry dry mass increase showed a similar pattern, although the rapid increase was delayed until after 15 mm berry size (Table 2). This is ascribed to sugar accumulation (not measured), which commenced later than the berry fresh mass increase (Hrazdina *et al*., 1984) because

initial berry growth is mainly due to cell division while later growth is due to cell enlargement (Ollat *et al*., 2002).

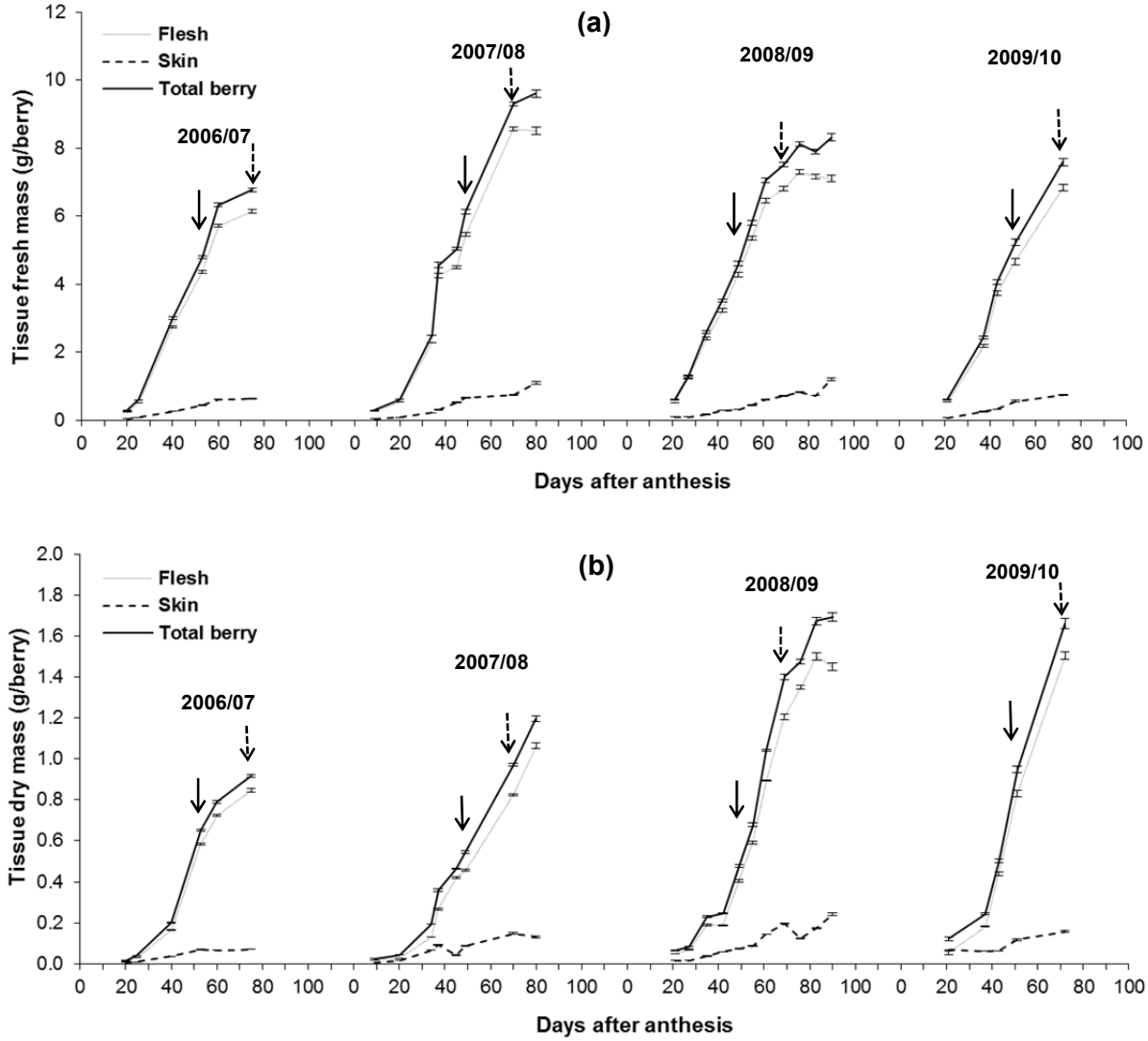

Figure 1. Berry fresh mass (a) and dry mass (b) during development and ripening of Prime Seedless/Ramsey berries in Paarl during seasons 2006/07 to 2009/10. Bars indicate the ± standard error of the means (p ≤ 0.05). Solid arrows indicate véraison, broken arrows indicate first harvest.

Table 2. Average fresh and dry mass accumulation rates (g/day) of Prime Seedless berries, cultivated in Paarl, between pea berry size and first harvest as calculated for four consecutive growing seasons.

Season	2006/07	2007/08	2008/09	2009/10	Average
Fresh mass (pea-size berries - first harvest)	0.130	0.174	0.144	0.137	0.146
Dry mass (pea-size berries - 15 mm berry size)	0.009	0.011	0.012	0.008	0.010
Dry mass (15 mm berry size - first harvest)	0.023	0.022	0.034	0.040	0.030
Dry mass (pea-size berries - first harvest)	0.018	0.019	0.028	0.030	0.024

Between seasons, berry fresh mass at harvest did not correspond with dry mass obtained, i.e. the fresh mass of berries at first harvest in 2008/09 and 2009/10 was lower (7.53g & 7.58 g) than in 2007/08 (9.30 g), while their dry mass was higher (1.401 g & 1.661 g *vs.* 0.971 g). This is partially ascribed to variance in total soluble solid concentration at first harvest (^{o}Brix), which was 14.8 ^{o}B, 14.1 ^{o}B, 15.2 ^{o}B and15.5 ^{o}B for the consecutive seasons.

The four season average flesh mass:skin mass ratio increased from pea-size berry stage up to harvest. Calculated for fresh mass, it increased from 6.2 to 10.1 and for dry mass from 3.0 to 7.6. The flesh mass:skin mass ratio therefore increased by 61% from pea-size berries to harvest when calculated from fresh mass, while tissue dry mass increased 145%. This is ascribed to accumulation of total soluble solids (including minerals) in the flesh, as also stated by Ollat *et al*. (2002).

The lag phase often observed just prior to véraison (Coombe, 1973; Ollat *et al*., 2002; Etchebarne *et al*., 2009) was not observed (Figure 1). It may be ascribed to the fact that Prime Seedless is a very early ripening variety, where berry growth does not decrease during the transition phase from pre- to post-véraison because of its brevity. The GA_3 applied as enlargement treatment at 8 to 12 mm berry size would further compact and boost this growth rate, most likely further masking the well-known double sigmoid curve. This vineyard showed a budbreak to first harvest period that varied between 114 days and 121 days for the experimental seasons.

Berry nitrogen

Berry flesh N concentration, of which 60 - 90 % of total N in grape berries is accounted for by amino acid fraction nitrogen (Kliewer, 1968; Tagliavini, 2000), decreased rapidly during all four seasons from set to shortly before véraison, thereafter the decrease slowed down (Figure 2a). Berry skin N concentration showed a similar trend. The initial rapid decrease in berry N concentration is ascribed to berry growth between set and véraison that exceeded N accumulation rates, probably due to cell growth demands. After véraison, the rate of decrease in N concentration slowed down. This is ascribed to the commencement of sugar accumulation which is also associated with arginine (Kliewer, 1968; Kliewer & Cook, 1974) and proline (Kliewer, 1968) accumulation in the berry. These are the two dominant amino acids, making up the bulk of total N in grape berries (Kliewer, 1968). Up to véraison, N concentration of the flesh is higher than that of the skin, but from the start of ripening skin N concentration exceeds the flesh N concentration (Figure 2a). This seems to indicate that N is partitioned mainly to the skin during ripening.

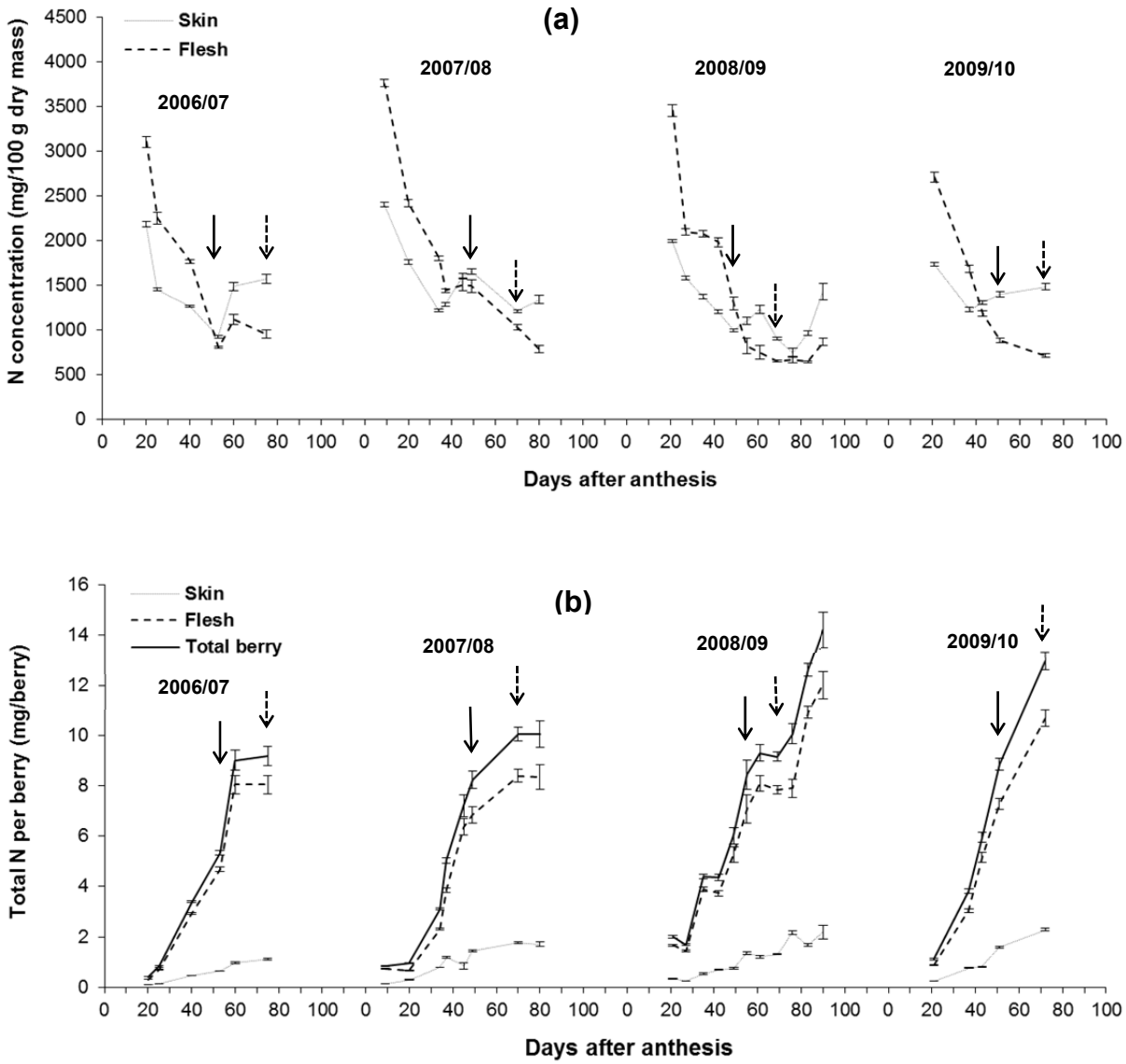

Figure 2. Concentration (a) and accumulation (b) of N in Prime Seedless berries throughout berry development, as determined for 2006/07 to 2009/10 seasons. Bars indicate the ± standard error of the means ($p \leq 0.05$). Solid arrows indicate véraison, broken arrows indicate first harvest.

Total berry N accumulation is also indicated in Figure 2b. Accumulation of N was rapid during the pre-véraison period, being associated with cell division and growth requiring N for chlorophyll, nucleotides, nucleic acids and proteins (Follet *et al*., 1981). After véraison, accumulation slowed down. In the 2008/09 season, the only season during which berry analyses was conducted at TSS that exceeded 16°B, rapid N accumulation, however, commenced again at later maturity. This is in accordance with Kliewer (1968), Kliewer & Cook (1974) and Stines *et al.* (2000) who found that proline concentration very rapidly increases after fruit maturity in various varieties. If the N compound is indeed proline, it indicates to some form of ageing or other stress (Davies & Robinson, 2000), with the implication that from a post-harvest storage point of view, N accumulation can be used as

indicator of optimal harvest time. This probably explains the increase in both flesh and skin N concentration during the last three sampling times of season 2008/09.

The bulk of N in the berry accumulates in the flesh, containing 88% of total berry N in 2006/07 at first harvest, 83% in 2007/08, 86% in 2008/09 and 82% in 2009/10.

Seasonal berry N accumulation up to the first harvest seems to follow a sigmoidal pattern (Figure 3). Data for late maturity (>16°B), however, are not sufficient to make definite conclusions.

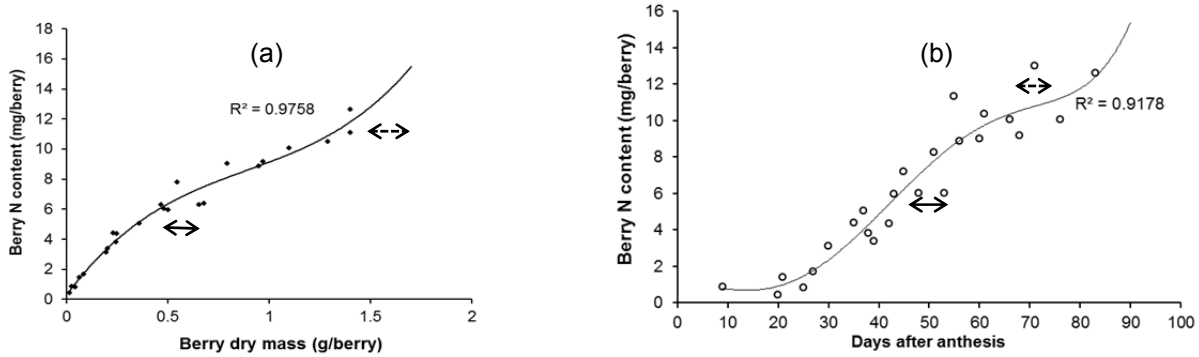

Figure 3. Prime Seedless berry dry mass correlated with berry N content (a) and seasonal accumulation pattern of N in Prime Seedless berries throughout berry development (b), using data of all four seasons (2006/07 to 2009/10). Values obtained as average of n = 85 for each data point. Solid arrows indicate period of véraison, broken arrows indicate first harvest.

Berry phosphorus

Similar to N, berry flesh and skin P concentration decreased rapidly during all four seasons from set to shortly before véraison, after which the decrease slowed down (Figure 4a). The initial rapid decrease in berry P concentration is ascribed to berry growth between set and véraison that exceeds P accumulation rates, i.e. cell expansion. Although berry growth did not stop, P skin concentration stabilised after véraison and, likewise, flesh P concentration for two of the four seasons (2006/07 & 2008/09). Since P concentration is expressed in mg P per 100 g dry mass, it is not expected to decrease dramatically after véraison because most of berry growth is due to water and sugar accumulation in the vacuoles (Ollat *et al*., 2002).

Initially skin P concentration was lower than berry flesh concentration, but the concentrations coincided after véraison as the berries matured (Figure 2a). This is also ascribed to cell enlargement of the flesh, when vacuoles expand during ripening on account of water, sugar and K^+ accumulation (Ollat *et al*., 2002). Since most of the P is found in proteins in the cytoplasm (DNA, RNA, ATP, etc.) (Follet *et al.*, 1981), which break down with berry flesh cell

expansion, while the cytoplasm of the skin cells remains more intact (Chardonnet & Donèche, 1995), skin P concentration seems to be better maintained.

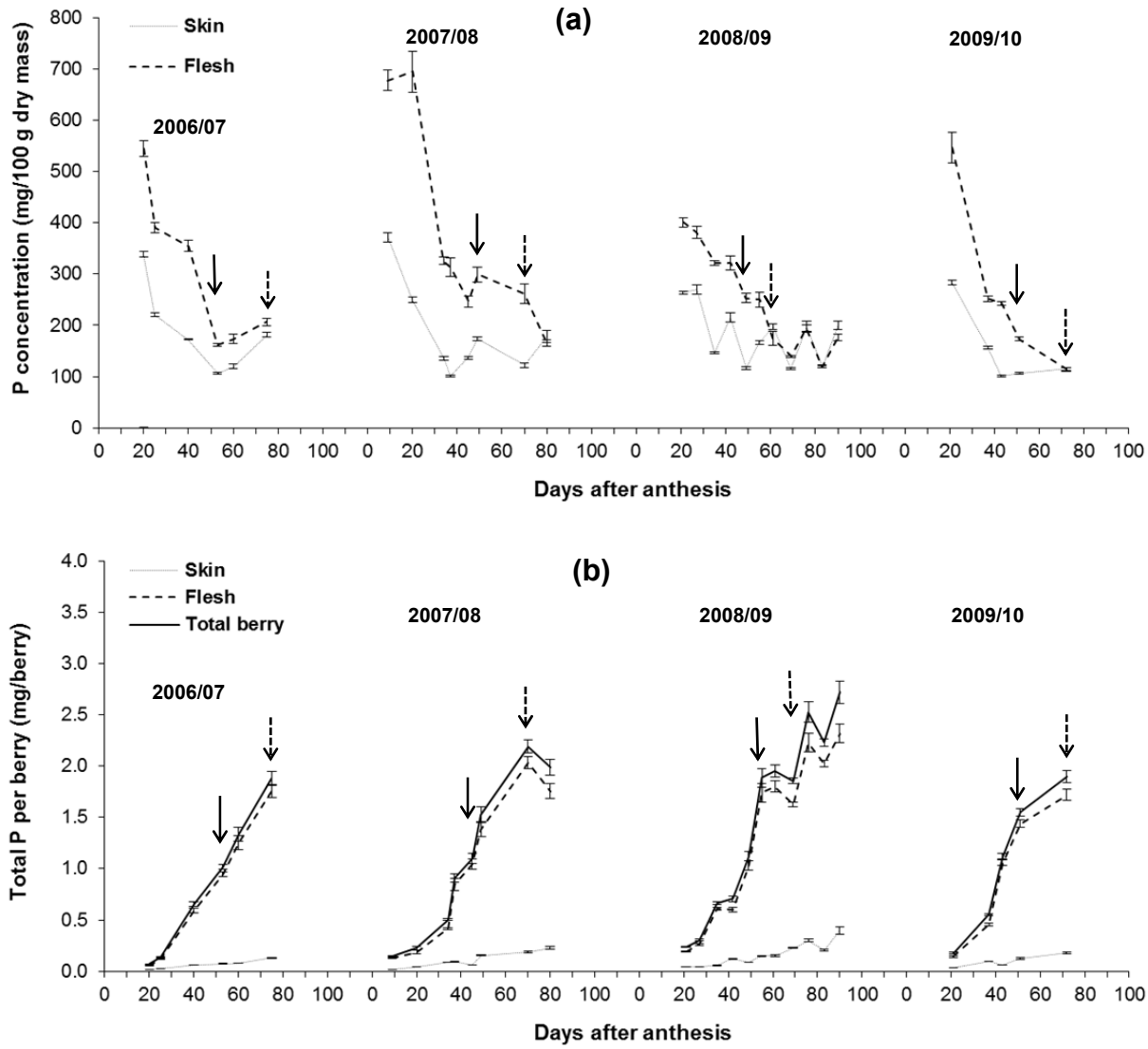

Figure 4. Concentration (a) and accumulation (b) of P in Prime Seedless berries throughout berry development, as determined for 2006/07 to 2009/10 seasons. Bars indicate the ± standard error of the means ($p \leq 0.05$). Solid arrows indicate véraison, broken arrows indicate first harvest.

Berry P accumulation was most rapid up to véraison, after which it increased at a slower rate, except 2006/07 (Figure 4b). Using all data obtained in the four seasons, the general trend in berry P accumulation also support the notion that cell growth of the flesh after véraison occurs mainly due to vacuole expansion driven by water and solute (sugar and K^+) accumulation (Ollat *et al*., 2002), explaining the decrease in P accumulation rate in berry flesh during ripening and when the berry dry mass accumulation subsides (Figure 5).

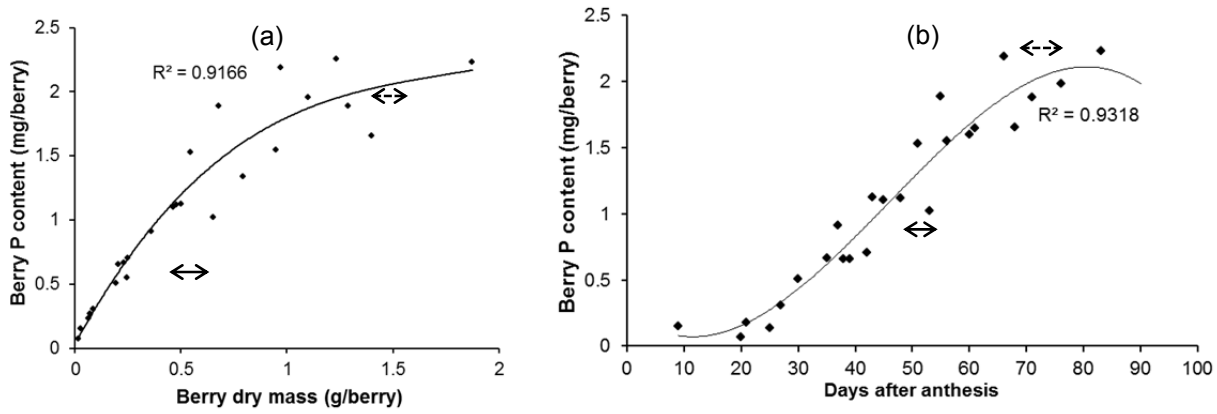

Figure 5. Prime Seedless berry dry mass correlated with berry P content (a) and seasonal accumulation pattern of P in Prime Seedless berries throughout berry development (b), using data of all four seasons (2006/07 to 2009/10). Values obtained as average of n = 85 for each data point. Solid arrows indicate period of véraison, broken arrows indicate first harvest.

Berry potassium

Although a general decrease in K concentration of berry flesh is observed, varying changes in the patterns of K concentration was obtained over the four seasons (Figure 6a). Berry skin K concentration showed an increase from 15 mm berry size onwards, but also with varying patterns between the seasons. The seasonal variance in K concentrations correspond with previous work (Etchebarne *et al*., 2009) where a marked difference in post-véraison K accumulation in grape berries was found between years and also on account of vine water status (not measured in this study).

Rogiers *et al.* (2006), who did not distinguish between berry tissues, found that berry K concentration, expressed as mg per kg fresh mass, increased from set throughout berry development. The data obtained in this experiment shows increased skin K concentration from 15 mm berry size onwards. Potassium concentration in both tissues however decreased during the early stages of berry development. Given the fact that the cv. Shiraz was used by Rogiers *et al.* (2006), of which berry size never exceeded 1.8 g fesh mass, compared to the 8 - 10 g obtained for Prime Seedless in this experiment, the initial decrease in K concentration during early stages of rapid berry growth in this study is not unexpected. Likewise Mpelasoka *et al.* (2003) are of the opinion that berry K concentration need not increase, especially in conditions where berry growth and berry K accumulation are maintained at similar rates. This implies that factors such as cultivar (berry size), crop load, and climatic conditions that determine berry growth and cultural practices that affect rate of berry growth and/or K accumulation in the berry will affect berry K concentration. It may also be stressed again that Prime Seedless is a very early ripening variety and berry growth does not decrease during the transition phase from pre- to post-véraison. Furthermore, the enlargement GA_3 treatments at 8 to 12 mm berry size boost berry growth rate, most likely

further masking the well-known double sigmoid curve. Despite this, there is a very definite point of change in berry flesh K concentration dynamics before or around véraison, probably indicating a change in it ripening physiology.

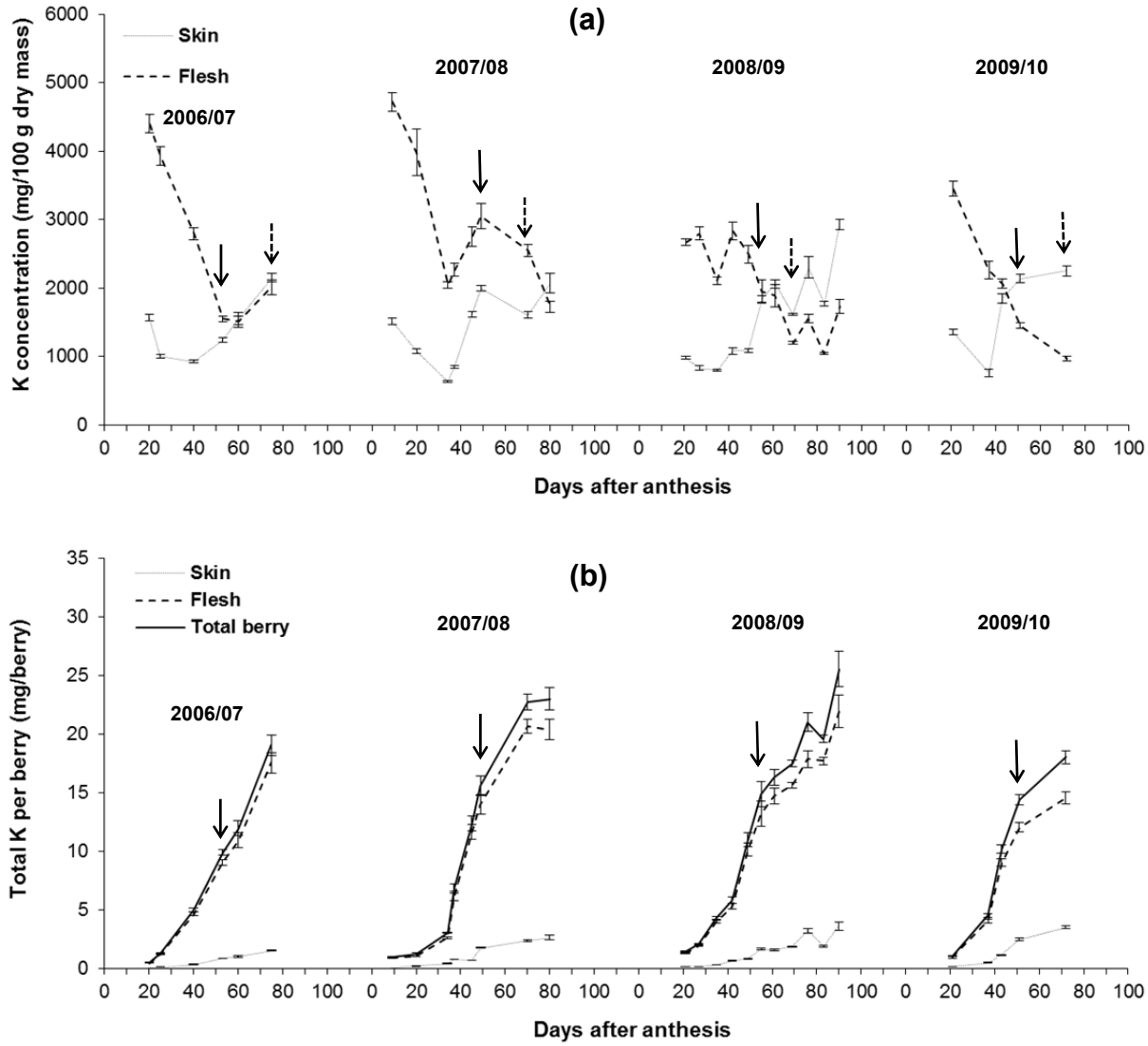

Figure 6. Concentration (a) and accumulation (b) of K in Prime Seedless berries throughout berry development, as determined for 2006/07 to 2009/10 seasons. Bars indicate the ± standard error of the means ($p \leq 0.05$). Solid arrows indicate véraison, broken arrows indicate first harvest.

During early berry development, K concentration in berry flesh was higher than in skins. However, increased skin K concentration exceeded flesh K concentration at different stages between the seasons and in different magnitudes (Figure 6a). According to Iland (1988), Coombe (1992) and Ollat *et al.* (2002) K concentration in grape berry skin cells is higher than in the flesh. Storey (1987) found the K concentration of mature Tarrango and Shiraz grape skins to be four to five times higher than that of the pulp, while Rogiers *et al.* (2006) indicated it to be only 50% higher for Shiraz. The latter might explain why data in this experiment show

differences in K concentration at first harvest between the tissues, i.e. the first two seasons were at 14.8°B and 14.1°B, respectivey, compared to 15.2°B and 15.5°B for 2008/09 and 2009/10. Storey (1987), however, found that skin K concentration was higher for smaller berries compared to larger berries.

Total K content in the berries continued to increase throughout the season (Figure 6b), confirming previous work that K is the principal cation accumulated by the berry over the entire growth period (Conradie, 1981b; Rogiers *et al*., 2006; Etchebarne *et al*., 2009). The most rapid rate of accumulation was in the pre-véraison period, after which it slowed down during ripening, except for the 2006/07 season. Given the fact that berry dry mass accumulation was maintained after véraison at the same rate than pre-véraison for all the seasons, except for 2006/07 (Figure 1b), this work seems to indicate that dry mass accumulation was not associated with K accumulation. This is contrary to results obtained by both Rogiers *et al*. (2006) and Etchebarne *et al*. (2009) who found that rate of K accumulation of wine grape berries was the highest after véraison and that a strong relationship between K and berry dry mass accumulation exists. The difference results might be due to the fact that K translocation and partitioning in the vine is affected by plant water status (Esteban *et al*., 1999; Etchebarne *et al*., 2009; Etchebarne *et al*., 2010), presence of seed (Mpelasoka *et al*., 2003; Rogiers *et al*., 2006; Etchebarne *et al*., 2009); rate of berry growth (Mpelasoka *et al*., 2003) with final size affecting the skin:flesh ratio (Mpelasoka *et al*., 2003; Rogiers *et al*., 2006).

Compared to various wine grape cultivars, for which between 32% and 50% of K accumulated in the berry is directed towards the skin (Iland, 1988; Rogiers *et al*., 2006), the fraction of K in the skin of Prime Seedless grapes was found to be much less (Table 4). This is ascribed to the higher ratio of pulp to skin obtained for the larger berries typical of table grape varieties.

Table 4. Proportion (%) of total K accumulated in the berry that is located in the skin of Prime Seedless cultivated in Paarl.

	Proportion (%) of total K accumulated in the berry			
Season	**2006/07**	**2007/08**	**2008/09**	**2009/10**
Pea-size berry	15 ± 0.04	14 ± 0.47	12 ± 0.46	13 ± 0.46
Véraison	9 ± 0.23	11 ± 0.31	8 ± 0.26	17 ± 0.76
First harvest	8 ± 0.23	10 ± 0.83	11 ± 0.24	19 ± 0.67

Up to véraison, the berries accumulated 52%, 67%, 63% and 80% of total K measured at first harvest during the consecutive seasons, which respectively were at 14.8°B, 14.1°B,

15.5°B and 15.8°B. According to Etchebarne *et al.* (2009), approximately 50% of total K measured for Grenache noir at maturity is accumulated before véraison. The high percentage of accumulation at véraison for Prime Seedless can be ascribed to it being harvested at a less mature stage, given the fact that K continue to accumulate during ripening. However, in this trial total K accumulated in Prime Seedless by first harvest was 19.0 mg/berry, 22.7 mg/berry, 17.5 mg/berry and 18.0 mg/berry for each year, which demonstrate that K content is not necessarily related to TSS, although there is a strong correlation between berry dry mass accumulation (r^2 = 0.95, p ≤ 0.01) and berry K content as shown in Figure 7.

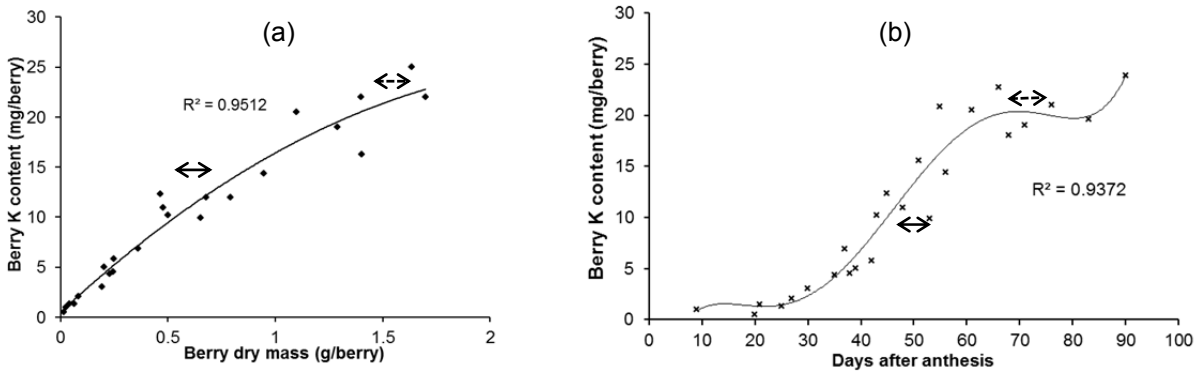

Figure 7. Prime Seedless berry dry mass correlated with berry K content (a) and seasonal accumulation pattern of K in Prime Seedless berries throughout berry development (b), using data of all four seasons (2006/07 to 2009/10). Values obtained as average of n = 85 for each data point. Solid arrows indicate period of véraison, broken arrows indicate first harvest.

Furthermore, correlation of berry dry mass with berry K content also shows that K accumulation was fastest during the pre-véraison period and smaller berry sizes, with its rate decreasing progressively from véraison onwards as berry size increased (Figure 7a). This is also illustrated by K accumulation over time as indicated in Figure 7b. In 2006/07 the pre-véraison rate of K accumulation was 0.28 mg/berry/day, 0.50 mg/berry/day in 2007/08, 0.34 mg/berry/day in 2008/09 and 0.44 mg/berry/day in 2009/10. This is about 10 times faster than the 0.03 – 0.04 mg/berry/day obtained by Etchebarne *et al*. (2009) for var. Grenache noir.

Berry calcium

Calcium concentration showed a decreasing pattern throughout berry development (Figure 8a). Donèche & Chardonnet (1992) also found that Ca concentration in Cabernet Sauvignon decreased throughout berry development, while in Shiraz berries Rogiers *et al.* (2006) observed a decline in concentration only from after véraison. The rate of decrease seems to be related to progressively reduced influx rates assosciated with berry growth since

it reduced most rapidly during the pre-véraison period for Prime Seedless, which coincided with rapid berry growth (Figure 1). In the case of wine grapes, berry growth predominently occur after véraison, as was the case with the Shiraz berries studied by Rogiers *et al.* (2006). Furtermore, decrease in xylem flow after véraison reduces Ca movement into the berry (Creasy *et al*., 1993).

In three (2007/08, 2008/09 and 2009/10) of the four seasons a similar pattern of Ca accumulation in the berries was found to that reported by various authors (Conradie, 1981b; Creasy *et al*., 1993; Rogiers *et al.*, 2006; Bonomelli & Ruiz, 2010), i.e. Ca uptake by the berry terminates, or reduces dramatically (Figure 8b), after véraison. In 2008/09, when sampling was done up to three weeks after first harvest, however, Ca accumulation resumed after first harvest. Cabanne & Donèche (2003); Etchebarne *et al*. (2009) & Etchebarne *et al*. (2010) stated that Ca that continues to accumulate after véraison points to maintenance of partial functioning of the berry xylem in the post-véraison period. Resumption of Ca uptake in this study however only occurred about three weeks after véraison (Figure 8), which points to a definite interruption of xylem flow, albeit temporary, and is probably connected to rapid berry growth as postulated by Creasy *et al.* (1993).

As found by Possner & Kliewer (1985), Ca is accumulated at its most rapid rate between pea-size berries and 15 mm berry size. Calculated as an average for all four seasons, including 2008/09 when ca uptake resumed after first harvest, there accumulated 0.52 mg Ca (69 % of total at first harvest) per berry up to 15 mm berry size, and at véraison 0.64 mg Ca (85 % of total at first harvest). This is in accordance with the fact that Ca is phloem immobile, which means that it cannot be transported into the berry as the transpirational water loss of the berry starts to decline (Saxton, 2002).

Possner & Kliewer (1985) found that the berry skin contains the highest Ca content during the whole of berry development. For Prime Seedless in this study, however, the flesh contained the most Ca throughout development (Figure 8b). This may be ascribed to the berries being much larger than wine grape berries, with much lower skin mass in comparison to flesh mass (Figure 1).

Various authors found that Ca continues to accumulate throughout the ripening phase (Rogiers *et al*., 2001; Cabanne & Donèche, 2003; Etchebarne *et al*., 2009). Since the resuming of Ca accumulation after the first harvest (from 69 DAA at 15.5^{o}B) was found only during the 2008/09 season in this study, it is not possible to conclude whether this is typical

of Ca accumulation. Evolution of Ca content of berries is known to vary from year to year due to edaphic and climatic factors (Cabanne & Donèche, 2003; Etchebarne *et al*., 2009).

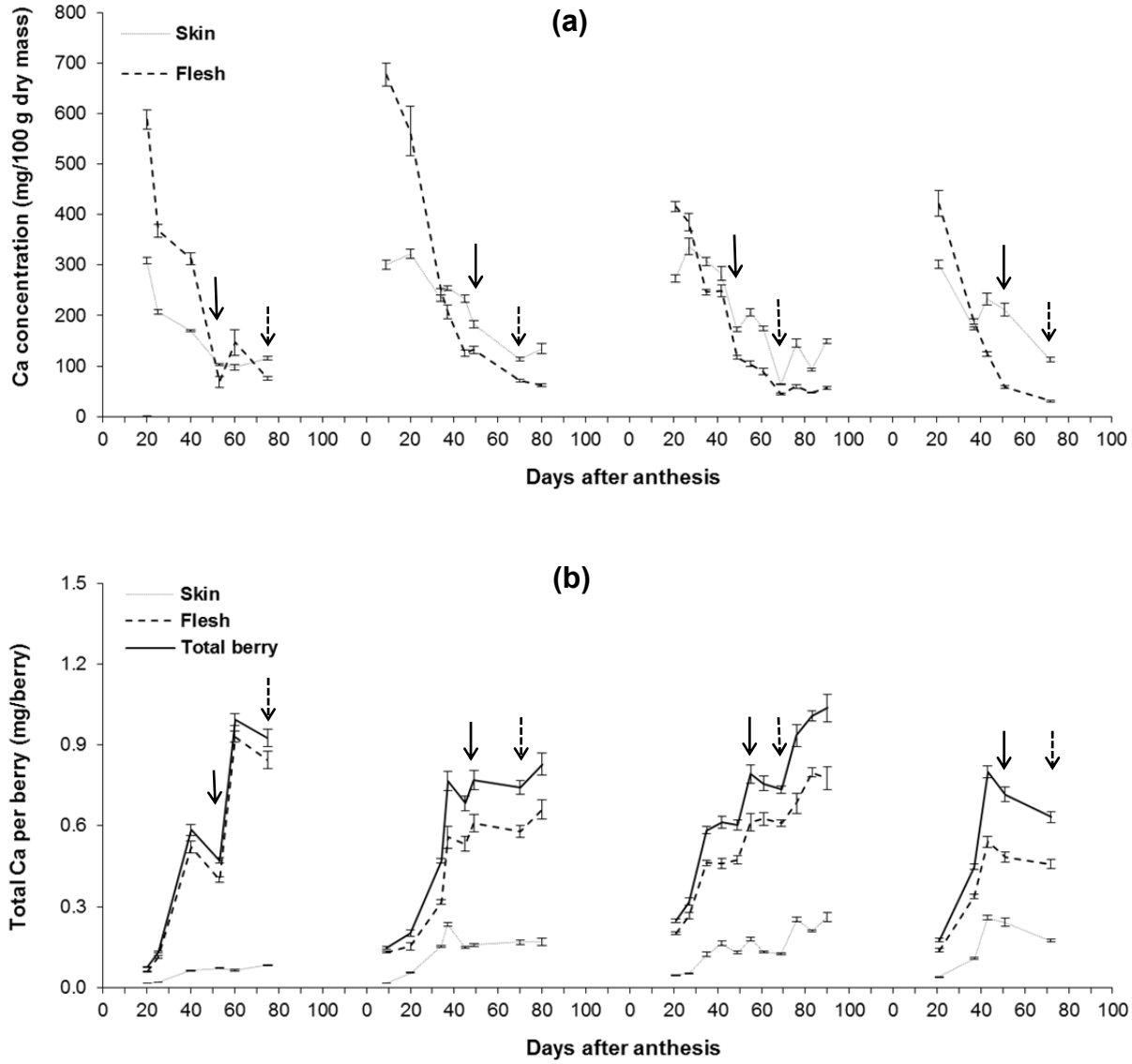

Figure 8. Concentration (a) and accumulation (b) of Ca in Prime Seedless berries throughout berry development, as determined for 2006/07 to 2009/10 seasons. Bars indicate the ± standard error of the means (p ≤ 0.05). Solid arrows indicate véraison, broken arrows indicate first harvest.

In support of the data discussed above, and in accordance with previous research (Creasy *et al*., 1993; Cabanne & Donèche, 2003; Rogiers *et al.*, 2006; Bonomelli & Ruiz, 2010), correlation of berry dry mass with berry Ca content shows that Ca accumulation was most rapid during the pre-véraison period of vegetative growth, with the rate decreasing dramatically from véraison onwards (Figure 9a). In 2006/07, 2007/08 and 2009/10, Ca accumulation peaked before véraison with 100% of total Ca accumulated in the berry before ripening starts. In 2008/09 it peaked at véraison. The combined data of all four seasons

illustrate that all the Ca in the berry by first harvest already accumulated at véraison (Figure 9b). Etchebarne *et al*. (2009) found that 83% of total Ca was present in berries of the cv. Grenache noir by véraison, which is similar to the accumulation obtained in this study when the resumed accumulation in 2008/09 in included in the calculation of the final berry Ca content.

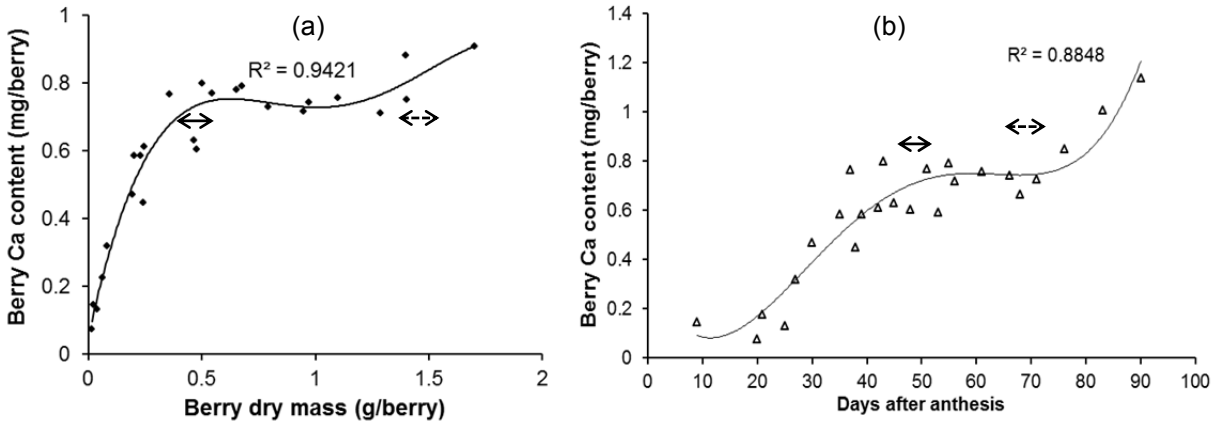

Figure 9. Prime Seedless berry dry mass correlated with berry Ca content (a) and seasonal accumulation pattern of Ca in Prime Seedless berries throughout berry development (b), using data of all four seasons (2006/07 to 2009/10). Values obtained as average of n = 85 for each data point. Solid arrows indicate period of véraison, broken arrows indicate first harvest.

Cabanne & Donèche (2001) found that mature Sauvignon blanc and Sémillon berries contained 69% and 60%, respectively, of total berry Ca in the skin. In later work, Cabanne & Donèche (2003) ascribed the high percentage of total berry Ca that occurs in the skin to Ca migration from the flesh to the skin. Data obtained in this trial for Prime Seedless over four experimental seasons, however, showed that the skin contained much lower fractions of the total berry Ca, ranging between 9% and 27% only.

Berry magnesium

The pattern of change in berry Mg concentration was similar to that of K, i.e. Mg concentration of berry flesh decreased throughout the season, while skin Mg concentration showed an increase from as early as 15 mm berry size onwards (Figure 10a). Furthermore, similar to K, skin Mg concentration exceeded flesh Mg concentration, albeit at different stages of berry development between seasons (Figure 10a).

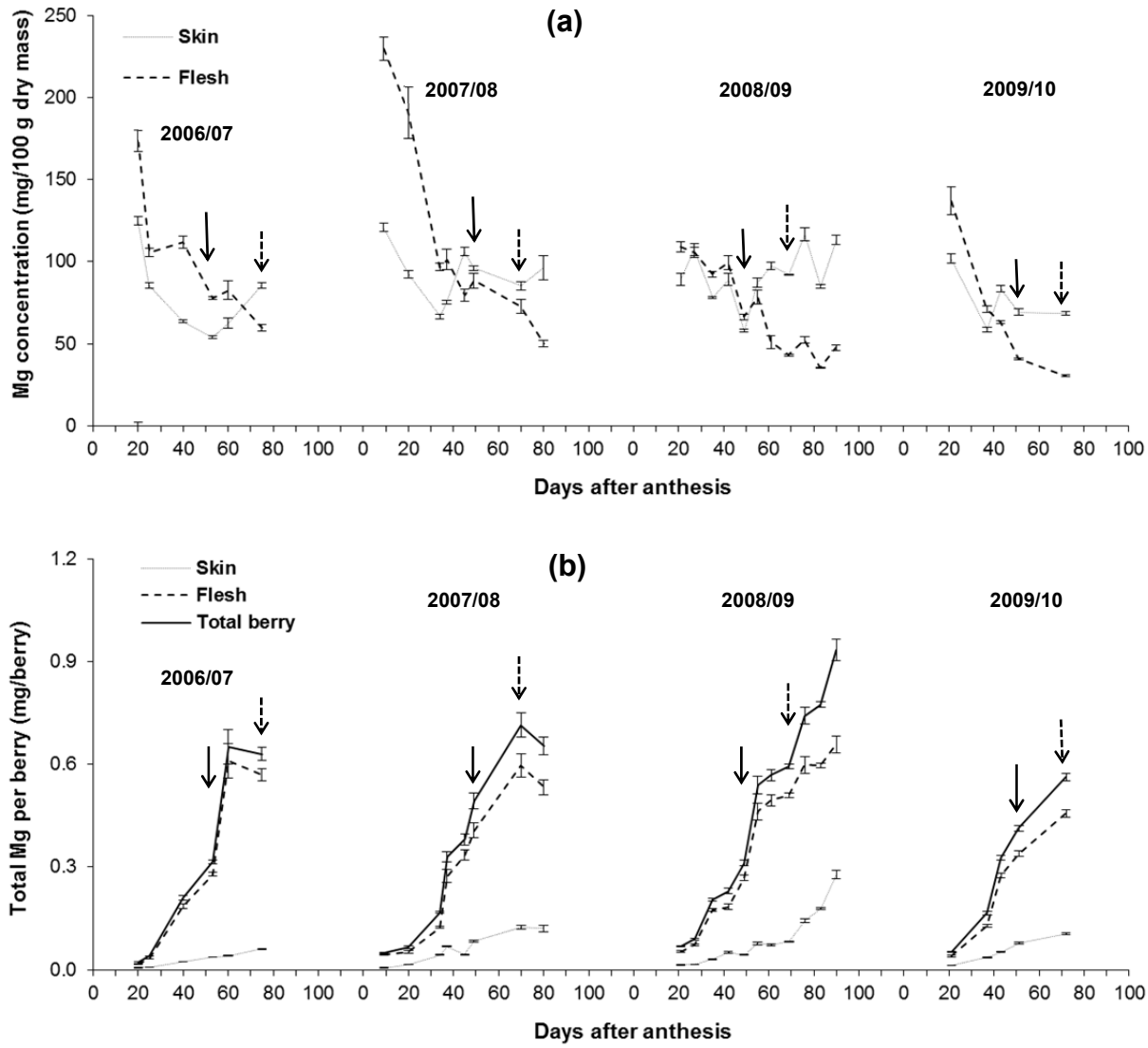

Figure 10. Concentration (a) and accumulation (b) of Mg in Prime Seedless berries throughout berry development, as determined for 2006/07 to 2009/10 seasons. Bars indicate the ± standard error of the means ($p \leq 0.05$). Solid arrows indicate véraison, broken arrows indicate first harvest.

Although Rogiers *et al.* (2006) grouped Mg with K as elements that are highly mobile in the phloem, they found that berry Mg concentration, expressed as mg per kg fresh berry tissue, decreased from véraison onwards. This was in contrast to that found for K, which showed a renewed rate of concentration increase after véraison. Data obtained in this experiment, however, seem to indicate that berry Mg concentration stabilised from véraison onwards, since the rate of decrease in Mg concentration in the flesh subsided, while skin Mg concentration increased.

Like Rogiers *et al.* (2006), Mg accumulation in the berry was found to occur throughout berry development (Figure 10b) and in a similar pattern than that of K. When correlated to berry growth, the rate of accumulation also subsided after véraison (Figure 11a), although over

time it seems to be more linear (Figure 11b). In contrast, Rogiers *et al*. (2006) found that rate of Mg accumulation of Shiraz berries was highest after véraison. Total K accumulated in the Prime Seedless berries was 30 times more than the Mg that accumulated. This ratio is similar in magnitude to what Rogiers *et al.* (2006) reported for Shiraz, although the total Mg accumulated per berry in this study ranged between 0.57 and 0.93 mg, whereas in the Shiraz berries it reached only 0.18 mg per berry.

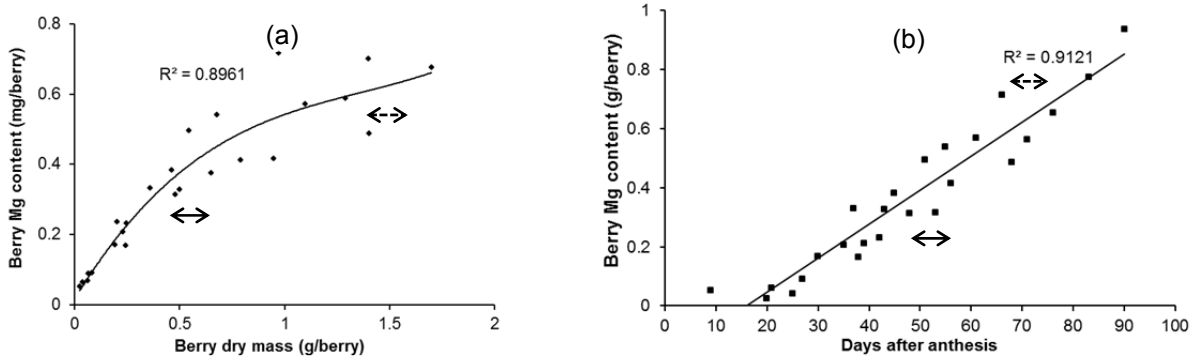

Figure 11. Prime Seedless berry dry mass correlated with berry Mg content (a) and seasonal accumulation pattern of Mg in Prime Seedless berries throughout berry development (b), using data of all four seasons (2006/07 to 2009/10). Values obtained as average of n = 85 for each data point. Solid arrows indicate period of véraison, broken arrows indicate first harvest.

CONCLUSIONS

Depending on the development stage, the dynamics of berry growth also impact on berry nutrient concentration. Early rapid berry growth, predominantly due to cell division and cell growth, is associated with the most rapid decreases in N, P and Ca concentration. Due to mobility of K and Mg in the plant that exceeds other nutrients, the decrease in concentration of these two mineral elements was not as pronounced as that of the others.

Nutrient accumulation was most rapid during the pre-véraison period, but only Ca showed a definite termination during the early ripening period. The continued inflow of N, P, K and Mg, albeit at slower rates immediately after véraison, should be taken into consideration when fertilisation is applied.

Potassium and Ca seemed to have uptake patterns which are strongly connected to véraison and the changes in berry physiology, with corresponding switch from both xylem and phloem influx to only phloem mobile products and nutrients entering the berry. This research, however, indicated that for a very early variety like Prime Seedless, nutrient accumulation

dynamics can already start to change during the pre-véraison period in some seasons due to different edaphic and climatic conditions.

As a table grape, total accumulation of each nutrient in Prime Seedless berries also far exceeded that of most cultivars studied this far. A particular difference is that the flesh:skin ratio of these berries is much higher, leading to higher levels of nutrient accumulation in the flesh.

Nutrient concentration is expected to impact berry quality more than total berry nutrient content. Berry nutrient accumulation patterns, however, did not correspond to their concentration changes due to dilution effects of water influx, sugar accumulation and cell expansion. This can be ascribed to seasonal differences which lead to variance in berry growth and size, as well as TSS accumulation. In the next chapter the effect of high rates of N, K and Ca fertilisation on berry nutrient concentration and accumulation is discussed. Future research on table grapes would need to develop a better understanding of the other factors and dynamics that determine berry nutrient concentration and accumulation.

LITERATURE CITED

Anonymous, 2007. Guidelines for preparing export table grapes. Capespan Exports (Pty) Ltd., Bellville.

Bonomelli, C. & Ruiz, R., 2010. Effects of foliar and soil calcium application on yield and quality of table grape cv. “Thompson Seedless”. J. Plant Nutr. 33, 299 - 314.

Cabanne C. & Donèche, B., 2001. Changes in polygalacturonase activity and calcium content during ripening of grape berries. Am. J. Enol. Vitic. 52, 331 - 335.

Cabanne C. & Donèche, B., 2003. Calcium accumulation and redistribution during the development of grape berry. Vitis 1, 19 - 21.

Chardonnet, C. & Donèche, B., 1995. Relation entre la teneur en calcium et la résistance à la digestion enzymatique du tissu pelliculaire au cours de la maturation du raison. Vitis 34, 95 - 98.

Coombe, B.G., 1973. The regulation of set and development of the grape berry. Acta Hort. 34, 261 - 271.

Coombe, B.G., 1987. Distribution of solids within the developing grape berry in relation to its morphology. Am. J. Enol. Vitic. 38, 120 - 127.

Coombe, B.G., 1992. Research on development and ripening of the grape berry. Am. J. Enol. Vitic. 43, 101 - 110.

Conradie, W.J., 1980. Seasonal uptake of nutrients by Chenin blanc in sand culture: I. Nitrogen. S. Afr. J. Enol. Vitic. 1, 59 - 65.

Conradie, W.J., 1981a. Seasonal uptake of nutrients by Chenin blanc in sand culture: II. Phosphorus, potassium, calcium and magnesium. S. Afr. J. Enol. Vitic. 2, 7 - 13.

Conradie, W.J., 1981b. Nutrient consumption by Chenin blanc grown in sand culture and seasonal changes in the chemical composition of leaf blades and petioles. S. Afr. J. Enol. Vitic. 2, 15 - 18.

Conradie, W.J., 1986. Nitrogen nutrition of the grape-vine (*Vitis vinifera* L.), PhD Thesis, Univ. of Stellenbosch.

Creasy, G.L., Price, S.F. & Lombard, P.B., 1993. Evidence of xylem discontinuity in Pinot noir and Merlot grapes: Dye uptake and mineral composition during berry maturation. Am. J. Enol. Vitic. 44, 187 - 192.

Davies, C. & Robinson, S.P., 2000. Differential screening indicates a dramatic change in mRNA profiles during grape berry ripening, cloning and characterization of cDNAs encoding putative cell wall and stress response proteins. Pl. Phys. 122, 803 – 812.

Donèche, B. & Chardonnet, C., 1992. Evolution et localisation des principaux cations au cours du développement du raisin. Vitis 31, 175 - 181.

Esteban, A., Villanueva, J. & Lissarrague, J.R., 1999. Effect of irrigation on changes in berry composition of Tempranillo during maturation. Sugars, organic acids, and mineral elements. Am. J. Enol. Vitic. 50, 418 - 434.

Etchebarne, F., Ojeda, H. & Deloire, A., 2009. Influence of water status on mineral composition of berries in ‘Grenache Noir’ (*Vitis vinifera* L.). Vitis 48, 63 - 68.

Etchebarne, F., Ojeda, H. & Hunter, J.J., 2010. Leaf:fruit ratio and vine water status effects on Grenache noir (*Vitis vinifera* L.) berry composition: water, sugar, organic acids and cations. S. Afr. J. Enol. Vitic. 31, 106 - 115.

Follet, R.H., Murphy, L.S. & Donahue, R.L., 1981. Fertilizers and soil amendments. Prentice-Hall Inc., London, UK.

Hrazdina, G., Parsons, G.F. & Mattick, L.R., 1984. Physiological and biochemical events during development and maturation of grape berries. Am. J. Enol. Vitic. 35, 220 - 227.

Iland, P.G., 1988. Grape berry ripening: the potassium story. Aust. Grapegrower & Winemaker 289, 22 - 24.

Isaac, R.A. & Johnson, W.C., 1998. Elemental determination by Inductively Coupled Plasma. Handbook of reference methods for plant analysis. Y.P. Kalra (ed.), CRC Press, Boca Raton, USA, pp 165-170.

Kirkby, E.A. & Pilbeam, D.J., 1984. Calcium as a plant nutrient. Plant, Cell & Environ. 7, 397 - 405.

Kliewer, W.M., 1968. Changes in the concentration of free amino acids in grape berries during maturation. Am. J. Enol. Vitic. 19, 166 - 174.

Kliewer, W.M. & Cook, J.A., 1974. Arginine levels in grape canes and fruits as indicators of nitrogen status of vineyards. Am. J. Enol. Vitic. 25, 111 - 118.

Marcelle, R.D., 1990. Predicting storage quality from preharvest fruit mineral analyses: A review. Acta Hort. 274, 305 - 313.

Marcelle, R.D., 1995. Mineral nutrition and fruit quality. Acta Hort. 383, 219 - 226.

Morris, J.R., Sims, C.A. & Cawthon, D.L., 1982. Excessive potassium fertilisation destroys grape juice quality. ARstHortSoc. 103, 106 - 108.

Mpelasoka, B.S., Schachtman, D.S., Treeby, M.T. & Thomas, M.R., 2003. A review of potassium nutrition in grapevines with special emphasis on berry accumulation. Aust. J. Grape and Wine Res. 9, 154 - 168.

Ollat, N., Diakou-Verdin, P., Carde, J.-P., Barrieu, F., Gaudillère, J.-P. & Moing, A., 2002. Revue bibliographique: Développement de la baie de raison. J. Int. Sci. Vigne Vin 36, 109 - 131.

Ollat, N. & Gaudillère, J.P., 1996. Investigation of assimilate import mechanisms in berries of *Vitis vinifera* var. Cabernet Sauvignon. Acta Hort. 427, 141 - 149.

Poovaiah, B.W., Glenn, G.M. & Reddy, A.S.N., 1988. Calcium and fruit softening: physiology and biochemistry. Hort. Reviews 10, 107 - 152.

Possner, D.R.E & Kliewer, W.M., 1985. The localisation of acids, sugars, potassium and calcium in developing grape berries. Vitis 24, 229 - 240.

Rogiers, S.Y., Greer, D.H., Hatfield, J.M., Orchard, B.A. & Keller, M., 2006. Mineral sinks within ripening grape berries (*Vitis vinifera* L.). Vitis 45, 115 - 123.

Rühl, E.H., 2000. Effect of rootstocks and K^+ supply on pH and acidity of grape juice. Acta Hort. 512, 31 - 37.

Ruiz, R., Moyano, S. & Navia, T., 2004. Accumulation of nitrogen compounds as related to the “soft berry” problem in table grapes. Agricultura Téchnica Chile 64, 426 - 430.

SAS, 1990. SAS/STAT user’s guide, version 8, first edition, volume 2. SAS Institute Inc., Campus drive, Cary NC 27513.

Saxton, V., 2002. Calcium in viticulture – unraveling the mystique of French terroir. Wine Ind. J. 17, 28 - 33.

Schaller, K., Löhnertz, O. & Chikkasubbanna, V., 1992. Calcium absorption by the grape berries of different cultivars during growth and development. Vit. Enol. Sci. 47, 62 - 65.

Shapiro, S.S. & Wilk, M.B., 1965. An analyses of variance test for normality (complete samples). Biometrika 52, 591 - 611.

Soil Classification Working Group, 1991. Soil classification: A taxonomic system for South Africa. Soil and Irrigation Research Institute, Dept. of Agricultural Development, Pretoria.

Stines, A.P., Grubb, J., Gockowiak, H. & Henschke, P.A. 2000. Proline and arginine accumulation in developing berries of *Vitis vinifera* L. in Australian vineyards: Influence of vine cultivar, berry maturity and tissue type. Aust. J. Grape Wine Res. 6, 150 - 158.

Storey, R., 1987. Potassium localization in the grape berry pericarp by enery-dispersive X-ray microanalyses. Am. J. Enol. Vitic. 38, 301 - 309.

Tagliavini, M., Zavalloni, C., Romolà, A.D., Quartieri, M., Malaguti, D., Mazzanti, F., Millard, P. & Marangoni, B., 2000. Mineral nutrient partitioning to fruits of deciduous trees. Acta Hort. 512, 131 - 140.

CHAPTER V

Excessive N, K and Ca fertilisation effects on ripening, berry nutrient content and post-harvest quality of an early ripening table grape cultivar (*Vitis vinifera* L. cv. Prime Seedless), grafted onto Ramsey on a sandy soil

ABSTRACT

A four-year field trial was conducted on a sandy soil in the Paarl district of South Africa, using cv. Prime Seedless (*Vitis vinifera* L.) grapevines, grafted onto Ramsey, and trained onto a gable trellis system. Nitrogen, potassium and calcium were applied, singular or in combination, at rates equal to 300% the calculated annual nutritional requirement. The Control treatment received an annual application of 70 kg N/ha, 60 kg K/ha and 10 kg Ca/ha. The effect of fertilisation on berry fresh mass, total soluble solids, titratable acidity, as well as N, P, K, Ca and Mg concentration and content in the flesh and skin was determined for four growing seasons. Larger berries were obtained for treatments that received excessive applications of N, but not for those with excessive K applications. The first mentioned is ascribed to better response obtained from GA_3 applications to N fertilised vines. Nitrogen and K levels in the berries were increased by N and K fertilisation treatments, respectively, while K fertilisation reduced berry N. Neither soil nor bunch applied Ca had any effect on berry Ca concentration or content. Occurrence of decay was not affected by either N, K, Ca fertilisation or bunch applied Ca. Indications of increased internal browning as a result of excessive K applications were obtained. The value of soil or bunch applied Ca applications to increase berry Ca levels, reduce decay and prevent berry browning, is questioned by this research. Furthermore, the role of berry N in susceptibility to decay as well as berry K on the occurrence of berry browning, needs to be validated.

INTRODUCTION

It is generally accepted that some aspects of fruit quality, such as solid concentration, are positively correlated to fruit K (Rogiers *et al.,* 2006), while during storage fruit quality is favoured by low N and high Ca levels (Marcelle, 1995; Bonomelli & Ruiz, 2010). This is why K and Ca fertilisation have become standard practices in South Africa for table grapes, even if the soil K and Ca are sufficient.

Nitrogen in grapes originates directly from the uptake of nitrate from the soil solution or indirectly from mobilization of storage compounds (Conradie, 1980). Löhnertz (2000) found a strong correlation between nitrate supply of the soil solution and the amino acid concentration or arginine content of grapes. Many researchers have reported increases in the concentration of various nitrogenous compounds with application of N fertilizer (Kliewer, 1977; Conradie, 1986; Löhnertz *et al*., 2000; Frank *et al*., 2005; Choné *et al*., 2006; Mundy & Beresford, 2007). However, N obtained from organic matter, cultural practices, climatic factors, and the ability of the plant to take up N from the soil, may override the effectiveness of N fertilizer (Conradie, 2005). According to Mundy & Beresford (2007) berries with low yeast assimilable nitrogen (YAN) seem to have a lower incidence of *Botrytis* bunch rot. Ruiz *et al*. (2004) found a positive correlation between N content of berry skin and pulp of soft Thompson Seedless berries and arginine as well as putrescine levels in berries. The higher the contents, the softer were the berries.

Potassium is the principal osmotically active cation in the berry's phloem and appears to contribute to phloem flow and loading of soluble sugars, thus helping to establish an osmotic gradient between leaves (source) and the berries (sink) (Rogiers *et al*., 2006; Etchebarne *et al.*, 2009). According to Conradie (1981b) and Iland (1988), potassium concentration in petioles decreases as the season progresses, while it increases in the fruit. Furthermore, due to a link between leaf photosynthetic activity and K transport, any conditions that reduce leaf photosynthetic activity could contribute to increased K levels in the phloem and subsequently in the berries (Iland, 1988). Rogiers *et al*. (2006) also indicated that due to a strong correlation found between K accumulation and berry fresh mass, K plays a key role in cell expansion, and therefore berry growth. Last mentioned was supported by the strong relationship between berry K content and both sugar and dry mass accumulation.

The addition of K fertiliser reduced the N content of grape juice (Conradie & De Wet, 1985). They also found that K appeared to increase resistance against *Botrytis* rot and ascribed it to the fact that K suppressed the uptake of N. In apples, it was found that K and Mg are lower in

concentration in both the pedicels and flesh of healthy fruit as compared with fruit showing bitter pit (Terblanche *et al.*, 1979).

There are many studies about the effects of Ca applications of fruit quality, especially for apples (Terblanche *et al.*, 1979; Drake & Spayd, 1983; Wójcik *et al*., 2010; Schlegel & Schönherr, 2002; Casero *et al.,* 2010). These include different ways of delivery (soil vs. foliar), different chemical forms and timing. The results are, however, contradictory and very limited for table grapes. Christensen & Boggero (1985) found that the incidence or severity of waterberry in Thompson Seedless could not be related to Ca or Mg levels in the rachis tissue or to elevated K:(Ca+Mg) ratios. Bonomelli & Ruiz (2010) found that both soil and foliar applied $CaCl_2$ did not affect berry Ca content nor berry quality.

The objectives of this research were to study the effects of high application rates of N, K and Ca, as well as bunch applied Ca, on berry nutrient composition and quality of the early ripening table grape cultivar Prime Seedless.

MATERIALS AND METHODS

Vineyard site, experimental design and treatments

A detailed description of the experiment vineyard, treatments and trial layout was given in Chapter II. The trial was conducted over four seasons (2006/07 to 2009/10) on Prime Seedless/Ramsey (*Vitis vinifera* L.) grapevines in a micro-irrigated commercial vineyard of De Hoop Farm in Paarl (33°45'S, 18°58'E), planted in 2002. Vines were grown in a Clovelly soil (Soil Classification Working Group, 1991) with a fine sandy texture containing less than 5% clay, optimal pH (pH_{KCl} = ±5.6), low K (<45 mg/kg), low Mg (<0.3 cmol/kg) and low organic C content (<0.4%) (see Table 1 in Chapter II).

The grapevines were trained to a gable system, spaced 1.8 m x 3 m apart, head trained and cane pruned to eight buds. Standard cultural practices for the cultivar and region were followed as described in Anonymous (2007). It entailed shoot tipping and crop control after set, combined with removal of leaves that are in close proximity of the retained bunches. On this planting width, the number of bunches per vine were reduced to 24, while each bunch was trimmed to retain 90-100 berries. Bunch preparation furthermore entailed an application of 1 mg/L gibberellic acid (GA_3) at bloom for bunch thinning, shortening of bunches to 8 cm length at set, dipping bunches in 20 mg/L GA_3 when they were 8 to 10 mm in diameter, and again at 10-12 mm diameter, for berry enlargement and finally doing final hand-thinning of bunches just before véraison.

The experiment was laid out as a completely randomised block design where each treatment was replicated five times. The treatments consisted of combinations of different levels of soil applied nitrogen (N), potassium (K) and calcium (Ca), up to 300% of the annual nutritional requirement of the vineyard (Table 1). An additional treatment, i.e. bunch applied Ca (Table 1), was also included. Fertiliser was applied in six instalments throughout the growing season, two times prior to flowering, three times from set to véraison and once after harvest. The control treatment received fertilisation as required for commercial production and applied by the producer, i.e. 70 kg N/ha/year & 10 kg Ca/ha/year, both split in two instalments before set and post-harvest and 60 kg K/ha/year applied after set.

Table 1. Fertilisation treatments applied to a Prime Seedless/Ramsey (*Vitis vinifera* L.) micro-irrigated commercial vineyard in Paarl.

Treatment	Total annual nutrient application (kg/ha)		
	N	K	Ca
Control	70	60	10
Ca-Bunch[1]	70	60	10
N[2]	210	60	10
K[3]	70	180	10
Ca[4]	70	60	150
KCa[5]	70	180	150
NCa[6]	210	60	150

[1] A mixture of 8L/ha Stopit plus 5 L/ha Caltrac, applied directly to bunches every two weeks from berry set to vèraison (three applications). A total of 10 kg Ca/ha was therefore applied.
[2] LAN (28%) was used as nitrogen source.
[3] KCl was used as K source.
[4] Gypsum ($CaSO_4$) was used as Ca source for the 2006/07, 2007/08 and 2008/09 seasons. In the 2009/10 season, $CaCl_2$ was used.
[5] A combination of KCl and $CaSO_4$ was used as K and Ca sources in the 2006/07, 2007/08 and 2008/09 seasons, while $CaCl_2$ was used instead of $CaSO_4$ in the 2009/10 season.
[6] A combination of $CaNO_3$, LAN and $CaSO_4$ was used as N and Ca source in the 2006/07, 2007/08 and 2008/09 seasons, while $CaCl_2$ was used instead of $CaSO_4$ in the 2009/10 season.

Instalment size was calculated from the total intended seasonal application of each nutrient, divided as a percentage of the seasonal requirement during each phenological period (Conradie,1980; Conradie, 1981a). Treatments (Control, N, K, Ca, KCa, NCa) were applied each year to the same plants with respect to the first three years, while NCa was not applied in the final (fourth) year.

Analyses

Soil samples for chemical analyses were taken in June 2007, 2008 and 2010 from the 0-30 cm layer with an auger, combining soil from between the two central experimental vines of both experimental vine rows in each plot. The soil was analysed for pH (1.0 M KCl), P (Bray II) and total extractable cations, namely K, Ca, Mg and Na (extracted at pH = 7 with

0.2 M ammonium acetate) and organic matter as described in Chapter II. Soil water from wetting front detectors was analysed for NH_4^+, NO_3^-, K^+ and Ca^{2+} as described in Chapter II.

Leaf petiole samples were taken at various phenological stages (see Chapter II) for chemical analyses during the four seasons. Berries were sampled (cut at the pedicel base) for titratable acidity (TA) and total soluble sugars (TSS), as well as chemical analyses, at various phenological stages during the four seasons. Three berries were removed, respectively at the top, middle and bottom of four randomly selected bunches per experimental vine, giving a sample of at least 48 berries. Titratable acidity, expressed as g/L (tartaric acid), was determined by 0.1N NaOH titration (Crison Compact Titrator). Total soluble solid content, expressed as ^{o}Brix, was measured using a temperature compensated refractometer (Atago model ATC-1). Berry chemical analyses were done as discussed in Chapter III. The sampling times for which the chemical analyses are reported in this chapter are listed in Table 2 with TSS at first harvest that was 14.8^{o}B, 15.6^{o}B, 15.5^{o}B and 15.8^{o}B for the consecutive seasons.

Table 2. Berry sampling times, indicated as days after anthesis (DAA), of a Prime Seedless/Ramsey (*Vitis vinifera* L.) micro-irrigated commercial vineyard in Paarl.

Berry development stage	Season			
	2006/07	2007/08	2008/09	2009/10
Pea-size berries	20	20	21	21
15 mm berry size	40	34	35	37
Véraison	53	49	49	51
First harvest	75	70	69	72

Berry quality

For post-harvest quality evaluation, two bunches per experimental vine were picked and field packed in a 4.5 kg corrugated carton at first harvest (2006/07, 2007/08 and 2008/09), second harvest (20087/08) and third harvest (2008/09 and 2009/10). Individual bunches were placed in plastic carry bags and the carton content was enclosed in a 54 x 2 mm perforated LDPE liner. In each of the liners, an Uvasys® SO_2 generator sheet was positioned on top of the grapes, with a MAM sheet enclosed between the SO_2 sheet and the grapes. The liners were then closed and sealed. The grapes were transported to the Infruitec-Nietvoorbij Research Institute for Fruit, Vine & Wine where it was cooled to -0.5^{o}C and stored for 5 weeks, thereafter it was kept for an additional 10 days at 7^{o}C. After cold storage the extent of loose and split berries, decay, internal-, external- and total browning, soft tissue decay and waterberry was determined and expressed as percentage of total weight of grapes in the carton.

Sensory evaluation of the grapes was done at first harvest in 2006/07 and 2007/08 by a panel of thirty persons using a Hedonic scale (where 1 = extremely dislike, 5 = neither like nor dislike and 9 = extremely like). Evaluated parameters were: 1) general impression of the grapes, 2) berry colour, 3) taste, 4) berry firmness and 5) skin consistency.

Statistical procedures

Standard analyses of variance were performed for each season and over all seasons, using Genstat 5 release 1.2 and SAS (SAS, 1990). Student's t-test was used to test for significant differences between treatment means and seasons. The Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965).

RESULTS AND DISCUSSION

Soil and soil water analyses

Berry nutrient composition cannot be discussed in isolation of soil analyses. The effect of fertilisation treatments on soil chemical composition and nutrient availability should be taken into account. The impact of the fertilisation treatments on soil chemical as well as soil water extract composition was discussed in detail in Chapter II. Treatments containing K significantly increased the soil K content, while Ca fertilisation did not have a significant effect on the soil Ca concentration. The N concentration of soil water extracts of treatments N and NCa reflected higher N availability (Chapter II). High rates of N applications (treatments N & NCa) stimulated leaching-losses of K and Ca from the soil. Furthermore, the Mg content of the soil was also reduced significantly for all the treatments. These shifts, however were not clearly reflected in the soil water extract composition. Soil pH of treatment N and NCa decreased to levels detrimental to vine root growth and plant performance, i.e. $pH_{KCl} < 5.6$.

Berry size, total soluble solids (sugar) and titratable acidity

The effect of excessive N, K and Ca fertilisation on berry growth is shown in Table 3. Only in 2006/07 a significant larger berry size than the control was obtained; in this case for treatment N. However, for five of the nine sampling times over the seasons, berries of N containing treatments (N or NCa) showed the largest berry size. This is ascribed to an earlier response in vegetative growth obtained, albeit slightly, for N containing treatments in especially 2006/07 (Chapter II), giving a slightly better response to GA_3 treatments applied shortly after set (Anonymous, 2007).

Table 3. Berry fresh mass (g) as affected by excessive nitrogen, potassium and calcium fertilisation of Prime Seedless grown in Paarl.

Treatment	Berry fresh mass (g)								
	2006/07	2007/08		2008/09				2009/10	
	75 DAA[1]	70 DAA	80 DAA	69 DAA	76 DAA	83 DAA	90 DAA	72 DAA	79 DAA
Control	6.68b	9.22	8.79ab	7.25ab	7.73ab	7.74ab	7.40	7.84	8.49
Ca(Bunch)	6.72b	9.21	8.91ab	7.30ab	7.50b	7.34b	7.92	7.83	7.98
N	7.42a	9.26	9.87a	7.84ab	8.49a	8.42a	7.97	7.75	8.23
K	6.84ab	9.28	8.50b	7.70ab	8.03ab	7.70ab	8.18	7.44	8.28
Ca	7.02ab	9.37	8.82ab	6.97b	8.03ab	7.34b	7.76	7.25	8.03
KCa	6.37b	9.57	8.45b	7.67ab	8.18ab	7.83ab	8.56	7.39	8.32
NCa	6.60b	9.13	8.56b	8.04a	8.32ab	8.27a	8.52	-	-
LSD ($p \leq 0.05$)	**0.68**	**NS[2]**	**1.17**	**0.80**	**0.84**	**0.80**	**NS**	**NS**	**NS**

[1] DAA = days after anthesis
[2] NS = not significant

The results furthermore did not correspond to that obtained by Conradie & De Wet (1985) and Rogiers *et al*. (2006) who indicated that a strong correlation exists between K accumulation and berry fresh mass on wine grape varieties. The difference in results may be ascribed to the effect of plant growth regulators (GA_3), utilised to obtain berry size in the production of seedless table grapes. This probably negated potential effects of K on berry size, since berry growth was strongly enhanced in these conditions by active vegetative growth, as obtained by N fertilisation.

Table 4 shows that the effect of the treatments on berry TSS was not constant. Both the control and treatment K, however, showed a tendency to have the highest TSS. The slightly positive effect of K fertilisation on berry sugar content is in accordance with Conradie & De Wet (1985) who found that K fertilisation up to 90 kg/ha slightly increased sugar content compared to the control. Rogiers *et al.* (2006) stated that K, being the main osmotically active cation in the phloem sap and grape berries, contributes to phloem sap flow (sugar import) by helping to establish an osmotic potential gradient between the leaves (source) and the berries (sink). This was supported by a strong relationship found between berry K content and both sugar and dry mass accumulation. On the other hand, Morris *et al.* (1980) found that even with K applications of 450 kg/ha, TSS was not affected in Concord grape juice. Nitrogen fertilisation had no definite effect on TSS of the berries (Table 4). This is contrary to results obtained by Christensen *et al*. (1994), Spayd *et al.* (1994) and Keller *et al.* (1998), who found that N fertilization delayed fruit maturity of various wine grape cultivars, as indicated by lower TSS concentrations, as levels of N fertilisation increased. The lack of response in vegetative growth obtained in this trial for high rates of N applications (Chapter II) might therefore explain why N did not affect berry TSS accumulation.

Titratable acidity decreased between consecutive sampling times for both of the seasons in which it was determined (Table 5). As found by Conradie & De Wet (1985), treatment K showed the highest TA content for the first and second sampling time during 2009/10 and also, together with KCa, for the third sampling time. It was, however, not significantly higher than the control. In Concord grapes Morris *et al.* (1980), however, obtained reduced TA in response to excessive K applications. No consistent effect from either N or Ca applications on TA was obtained. Keller *et al.* (1998) found that abundant N reduces TA levels of Cabernet sauvignon by the beginning of ripening. On the other hand, Bell *et al.* (1979) found that berry total acidity increased significantly with increasing nitrogen fertilization. They ascribed it to increased acidity obtained on account of denser canopy that accompanies nitrogen fertilization, resulting in more shading of fruit and lower fruit temperature than on unfertilized vines. On the other hand, excessive vigour that might lead to suboptimal condi-

Table 4. Berry total soluble solids (°Brix) as affected by excessive nitrogen, potassium and calcium fertilisation of Prime Seedless grown in Paarl.

Treatment	Berry total soluble solids (°Brix)								
	2006/07	2007/08		2008/09			2009/10		
	75 DAA[1]	70 DAA	80 DAA	76 DAA	83 DAA	90 DAA	72 DAA	79 DAA	86DAA
Control	15.3a	14.1ab	16.3a	18.6a	19.1	19.7	16.4	16.6	18.1ab
(Control-Bunch)	14.3ab	14.1ab	15.7ab	17.1ab	17.7	18.5	15.9	17.2	18.4ab
N	14.3ab	13.7b	15.6ab	16.3ab	18.0	18.3	15.8	17.2	18.0ab
K	15.2a	14.6a	15.8ab	16.3ab	19.2	19.5	15.9	17.5	19.3a
Ca	15.0a	14.2ab	15.5ab	17.2ab	18.3	19.5	16.6	17.3	18.6ab
KCa	15.2a	14.1ab	15.6ab	16.0b	17.0	19.4	14.2	17.5	15.9b
NCa	13.5b	13.4b	14.6b	17.2ab	17.8	18.8			
LSD (p ≤ 0.05)	1.1	0.8	1.2	2.5	NS	NS	NS[2]	NS	2.7

[1] DAA = days after anthesis
[2] NS = not significant

tions for photosynthesis, stimulate translocation of K to the berries resulting in reduced titratable acidity (Iland, 1988). The lack of response in vegetative growth, resulting in bunches that were well-exposed to sunlight (not measured), that was obtained for Prime Seedless in this trial (Chapter II) might therefore explain the reason why excessive N fertilisation did not result in increased acidity.

Table 5. Berry titratable acidity (g/L) as affected by excessive nitrogen, potassium and calcium fertilisation of Prime Seedless grown in Paarl.

Treatment	Berry titratable acidity (g/L)				
	2007/08		2009/10		
	70 DAA[1]	80 DAA	72 DAA	79 DAA	86DAA
Control	3.51	3.05a	3.58ab	3.00ab	2.98abc
(Control-Bunch)	3.51	2.94ab	3.36abc	2.90ab	2.90bc
N	3.81	2.71ab	3.34abc	2.72b	2.78cd
K	3.67	2.69ab	3.82a	3.28a	3.10ab
Ca	3.48	3.00ab	3.12bc	2.78ab	2.62d
KCa	3.72	3.02ab	3.06c	2.98ab	3.16a
NCa	3.60	2.59c			
LSD ($p \leq 0.05$)	NS[2]	0.36	0.48	0.55	0.25

[1] DAA = days after anthesis
[2] NS = not significant

Berry nitrogen

Nitrogen concentration and content in the berries were increased for most of the sampling times by treatments that contain N (N & NCa) although not always significantly (Table 6). Likewise, Löhnertz *et al*. (2000) found a correlation between soil nitrate supply and arginine content of Riesling berries, but not with proline. Treatments K and KCa furthermore reduced berry N concentration and content compared to treatment N and, in some cases, also compared to Control. This is ascribed to the reducing effect that K has on N uptake, as discussed in Chapter III, and also found by Conradie & De Wet (1985) where N content of must of Chenin blanc was reduced when fertilised with 90kg K per ha. In the previous chapters a lack of vegetative growth responses and petiole N content increases on account of excessive N fertilisation was ascribed to the impact that high rates of N fertilisation had on soil pH and cation content. However, the increased berry N content obtained points to the mobility of N in the vine and that it is readily translocated to the berries, as also indicated by Wermelinger (1991).

Table 6. Effect of excessive nitrogen, potassium and calcium fertilisation on nitrogen concentration and total nitrogen content of Prime Seedless berries, cultivated in Paarl.

Season	Treatment	Véraison					First harvest				
		Concentration (mg/100g dry mass)		Total content (mg/berry)			Concentration (mg/100 g dry mass)		Total content (mg/berry)		
		Skin	Flesh	Skin	Flesh	Total	Skin	Flesh	Skin	Flesh	Total
2006/07	Control	1019a	729d	0.65ab	3.93bcd	4.58c	1876a	985ab	1.33	8.2abc	9.5abc
	Ca-Bunch	894b	734d	0.65ab	4.47bc	5.12bc	1916a	1278a	1.35	10.6ab	12.0abc
	N	1054a	829bc	0.70a	4.60bc	5.30b	1681ab	1240ab	1.31	11.5a	12.8a
	K	1046a	647e	0.70a	3.66d	4.37c	1852a	1274a	1.32	10.8ab	12.2ab
	Ca	873b	776c	0.59b	4.41b	5.00bc	1289b	820ab	0.95	7.2bc	8.2bc
	KCa	820b	871b	0.57b	5.13b	5.71b	1413ab	776b	0.95	6.2c	7.2c
	NCa	1003a	1056 a	0.72a	6.43a	7.15a	1599ab	1003ab	1.12	8.3abc	9.4abc
	LSD (p ≤ 0.05)	**113**	**80**	**0.11**	**0.75**	**0.83**	**538**	**475**	**NS[2]**	**3.9**	**4.1**
2007/08	Control	1451b	1626	1.29	7.34	8.64	1035c	852b	1.53bc	6.9bc	8.4bc
	Ca-Bunch	1449b	1659	1.33	7.11	7.69	1093bc	687b	1.80ab	5.5c	6.2c
	N	1855a	1809	1.67	8.41	10.07	1298a	1184a	1.87ab	9.7a	11.6a
	K	1756ab	1778	1.58	8.39	9.97	1200abc	837b	1.43c	6.9bc	8.4bc
	Ca	1591ab	1381	1.37	6.39	7.76	1241ab	955ab	2.10a	7.8ab	9.9ab
	KCa	1620ab	1259	1.36	5.66	7.03	1193abc	960ab	1.59ab	8.2a	9.8ab
	NCa	1844ab	1913	1.48	8.92	10.38	1270ab	1163a	2.01a	9.3a	11.3a
	LSD (p ≤ 0.05)	**306**	**NS**	**NS**	**NS**	**NS**	**178**	**293**	**0.52**	**2.3**	**2.6**
2008/09	Control	920	888c	0.59b	3.62b	4.21c	672bc	677bc	1.30ab	7.9	9.2
	Ca-Bunch	1015	2096a	0.96a	8.59a	9.55a	590c	620bcd	1.14ab	7.2	8.4
	N	1034	1695a	0.85a	7.14a	7.99ab	726ab	714b	1.42a	9.0	10.4
	K	918	954c	0.73ab	3.78b	4.51c	558d	549d	1.11b	6.7	7.8
	Ca	1030	1364bc	0.75ab	5.09b	5.84bc	675bc	586cd	1.30ab	6.5	7.8
	KCa	922	1034bc	0.73ab	3.88b	4.61bc	562d	559cd	1.15ab	6.8	8.0
	NCa	1060	1325bc	0.80ab	5.73ab	6.53abc	778a	834a	1.41a	10.9	12.3
	LSD (p ≤ 0.05)	**NS**	**731**	**0.24**	**3.35**	**3.44**	**88**	**113**	**0.29**	**1.5**	**1.7**
2009/10[1]	Control	1417ab	894	1.57	7.36	8.93	1369b	677	2.27b	10.5	12.8ab
	Ca-Bunch	1370ab	846	1.66	6.72	8.38	1423ab	693	2.38ab	10.8	13.2ab
	N	1515a	959	1.63	7.87	9.51	1560ab	780	2.67a	11.9	14.6a
	K	1405ab	942	1.44	7.65	9.09	1413ab	714	2.07b	10.5	12.5ab
	Ca	1403ab	812	1.54	6.91	8.14	1643a	731	2.10b	10.5	12.6ab
	KCa	1238b	825	1.71	7.19	9.07	1450ab	668	2.18b	9.7	11.8b
	LSD (p ≤ 0.05)	**221**	**NS**	**NS**	**NS**	**NS**	**238**	**NS**	**0.37**	**NS**	**2.6**

[1] Treatment NCa was not applied or sampled during this growing season.
[2] NS = not significant.

Berry potassium

Potassium fertilisation significantly increased berry K concentration and content, both at véraison and harvest, in all four seasons (Table 7). Conradie & de Wet (1985) also found that application of K fertiliser to a soil with 4% K saturation led to a marked increase in K concentration of Chenin blanc grapes. According to Etchebarne *et al*. (2009) berries of irrigated Grenache noir vines contained more K at harvest than non-irrigated vines, but total berry K never exceeded 5.6 mg/berry. In this trial berry K content ranged from 11.33 mg/berry to 31.20 mg/berry at first harvest (Table 7).

Contrary to what was found for petioles (Chapter III), no definite trend that high rates of N or Ca fertilisation reduce berry K below that of the Control was found. This is ascribed to K translocation that occurs readily in the vine, especially towards the berries, even in conditions of reduced K nutritional status (Mpelasoka *et al*., 2003).

The generally accepted large increases in berry K after véraison (Mpelasoka *et al*., 2003) was also observed, especially in 2006/07 (Table 8). Inconsistent trends in K increase from véraison to first harvest were however obtained for the treatments and between seasons. The treatment with the highest K concentration or content at véraison therefore did not necessarily have the highest value at harvest. This indicates that various factors other than only K availability affects K translocation to the berries, e.g. irrigation, canopy conditions and factors that affect leaf photosynthetic activity (Iland, 1988).

Except for 2006/07, the percentage increase in skin K content was generally higher than the flesh (Table 8), in accordance with Etchebarne *et al.* (2009). Consistently higher K concentrations in the skins were however not obtained over all the seasons or between treatments. This is contrary to Storey (1987), Iland (1988), Mpelasoka *et al.* (2003) and Rogiers *et al.* (2006) who stated that K concentration in the berry skins is higher than in flesh. The difference in results is ascribed to the effect of large berry size on skin K. Compared to varieties where the flesh:skin ratio is much smaller, the skin of large berries is probably less of a sink for K. The difference in results is ascribed to the effect of large berry size on skin K. Compared to varieties where the flesh:skin ratio is much smaller, the skin of large berries is probably less of a sink for K.

Table 7. Effect of excessive nitrogen, potassium and calcium fertilisation on potassium concentration and total potassium content of Prime Seedless berries, cultivated in Paarl.

Season	Treatment	Véraison					First harvest				
		Concentration (mg/100g dry mass)		Total content (mg/berry)			Concentration (mg/100 g dry mass)		Total content (mg/berry)		
		Skin	Flesh	Skin	Flesh	Total	Skin	Flesh	Skin	Flesh	Total
2006/07	Control	1627b	1188c	1.05b	6.35c	7.40c	2297a	2024b	1.61a	16.95b	18.56b
	Ca-Bunch	1075c	1674b	0.78c	10.19a	10.97ab	2398a	2570ab	1.69a	21.32ab	23.02ab
	N	1128c	1563c	0.75c	8.73b	9.48bc	1942ab	2140b	1.52ab	19.84b	21.36b
	K	1968a	1165c	1.33a	6.61bc	7.94c	2139ab	3477a	1.52ab	29.67a	31.20a
	Ca	1182c	1287c	0.79c	7.25bc	8.05c	2139ab	1772b	1.56ab	15.61b	17.17b
	KCa	1184c	2089a	0.82c	12.30a	13.12a	2306a	1645b	1.54ab	13.16b	14.71b
	NCa	1031c	1387bc	0.74c	8.31bc	9.05bc	1638b	1748b	1.14b	14.43b	15.57b
	LSD (p ≤ 0.05)	**242**	**366**	**0.18**	**2.31**	**2.34**	**590**	**1124**	**0.45**	**9.38**	**9.50**
2007/08	Control	1868b	3436	1.68b	15.40	17.08	1280c	2103	1.90	16.99b	18.89b
	Ca-Bunch	2070b	3151	1.87a	13.62	13.72	1477bc	2186	2.51	17.66ab	16.64b
	N	1907b	3145	1.68b	14.64	16.33	1556abc	2250	2.25	18.39ab	20.64ab
	K	2543a	4141	2.31a	19.55	21.86	1794ab	2156	2.13	18.13ab	20.25ab
	Ca	1860b	3336	1.62b	15.56	17.18	1608abc	2253	2.70	18.49ab	21.19ab
	KCa	2043b	3120	1.71b	14.07	15.79	2041a	2808	2.71	24.00a	26.70a
	NCa	1683b	3382	1.35b	15.76	17.06	1473bc	2462	2.34	19.53ab	21.88ab
	LSD (p ≤ 0.05)	**413**	**NS**	**0.55**	**NS**	**NS**	**492**	**NS**	**NS**	**6.54**	**7.18**
2008/09	Control	1141ab	1776b	0.74ab	7.27b	8.01b	670d	1125a	1.29c	13.00a	14.29a
	Ca-Bunch	1116ab	3564a	1.06a	14.17a	15.23a	940bc	1196a	1.82ab	14.03a	15.85a
	N	919bc	2823ab	0.75ab	11.89ab	12.64ab	874c	1172a	1.71b	14.69a	16.40a
	K	1213a	2296ab	0.99ab	9.12ab	10.12ab	1059a	1070a	2.12a	13.16a	15.29a
	Ca	998abc	2508ab	0.74ab	9.55ab	10.28ab	998ab	857b	1.92ab	9.40b	11.33b
	KCa	1221a	2465ab	0.99ab	9.33ab	10.32ab	1057a	1056ab	2.15a	12.88a	15.03a
	NCa	843c	1814b	0.65b	7.68b	8.33b	887c	994ab	1.61bc	12.92a	14.54a
	LSD (p ≤ 0.05)	**249**	**1423**	**0.32**	**6.34**	**6.48**	**82**	**210**	**0.39**	**2.55**	**2.69**
2009/10[1]	Control	2109b	1486	2.34	12.24	14.58	2006b	985ab	3.33bc	15.25ab	18.58ab
	Ca-Bunch	2097b	1395	2.55	11.18	13.73	2030b	839b	3.39bc	12.99ab	16.38ab
	N	2040b	1442	2.27	11.78	14.05	2015b	791c	3.47abc	12.19b	15.66b
	K	2549a	1596	2.63	13.06	15.69	2618a	1058ab	3.87ab	15.48ab	19.35ab
	Ca	1882b	1349	2.09	11.39	13.06	2250b	1077ab	2.88c	15.58ab	18.46ab
	KCa	2096b	1441	2.91	12.58	15.45	2748a	1122a	4.14a	16.22a	20.36a
	LSD (p ≤ 0.05)	**396**	**NS**	**NS**	**NS**	**NS**	**324**	**240**	**0.68**	**3.94**	**4.09**

[1] Treatment NCa was not applied or sampled during this growing season.
[2] NS = not significant.

Table 8. Percentage increase in Prime Seedless total berry K content from véraison to first harvest, as affected by excessive nitrogen, potassium and calcium fertilisation.

Season	Treatment	Increase in K content from véraison to first harvest (%)		
		Skin	Flesh	Total berry
2006/07	Control	53	166	151
	Ca-Bunch	117	109	109
	N	126	127	125
	K	114	348	292
	Ca	97	115	113
	KCa	87	7	12
	NCa	54	74	72
2007/08	Control	13	10	11
	Ca-Bunch	34	30	29
	N	34	26	26
	K	0	0	0
	Ca	67	19	23
	KCa	58	71	69
	NCa	73	24	29
2008/09	Control	74	79	78
	Ca-Bunch	72	0	4
	N	128	24	30
	K	114	44	51
	Ca	159	0	10
	KCa	117	38	46
	NCa	148	68	75
2009/10	Control	42	25	27
	Ca-Bunch	33	16	19
	N	152	3	11
	K	47	19	23
	Ca	38	37	41
	KCa	42	29	32

Berry calcium

No indication that soil applied Ca or bunch applied Ca has a consistently positive effect on berry Ca concentration or content could be obtained. Only in the 2008/09 season berry Ca concentration and Ca content of berry flesh were significantly increased, leading to significantly higher berry Ca at both véraison and first harvest (Table 9). From these results it can be concluded that the rapid rates of berry growth, that continued through véraison (Chapter IV), may have led to the decreasing Ca concentrations observed in Table 9 from véraison to harvest (discussed for the whole period of berry development in Chapter IV). The extent of this decrease in berry concentration therefore negates the effect of soil Ca treatments. Furthermore, berry Ca accumulation terminates around véraison (Chapter IV), with no Ca flowing into the berry during ripening (Creasy *et al.* 1993).

Table 9. Effect of excessive nitrogen, potassium and calcium fertilisation on calcium concentration and total calcium content of Prime Seedless berries, cultivated in Paarl.

Season	Treatment	Véraison					First harvest				
		Concentration (mg/100g dry mass)		Total content (mg/berry)			Concentration (mg/100 g dry mass)		Total content (mg/berry)		
		Skin	Flesh	Skin	Flesh	Total	Skin	Flesh	Skin	Flesh	Total
2006/07	Control	142a	55.8c	0.092a	0.298c	0.390c	113b	72bc	0.090ab	0.608bc	0.716bc
	Ca-Bunch	106bc	75.4ab	0.076ab	0.458ab	0.534ab	127ab	106ab	0.113a	0.880ab	0.698bc
	N	113bc	77.8a	0.074ab	0.428ab	0.502ab	160a	88abc	0.088ab	0.811ab	0.993ab
	K	128ab	61.4bc	0.086ab	0.340bc	0.427bc	113b	118a	0.093ab	1.014a	0.899ab
	Ca	104b	61.4bc	0.070b	0.348bc	0.418bc	130ab	73bc	0.084ab	0.633bc	1.107a
	KCa	100c	88.4a	0.069b	0.523a	0.593a	113b	56c	0.075b	0.446c	0.521c
	NCa	115bc	66.6bc	0.084ab	0.400bc	0.483abc	131ab	74bc	0.092ab	0.611bc	0.703bc
	LSD (p ≤ 0.05)	**24**	**19.8**	**0.018**	**0.117**	**0.126**	**37**	**39**	**0.029**	**0.326**	**0.339**
2007/08	Control	255a	138	0.211a	0.625	0.836	94	58.6	0.137	0.519	0.613
	Ca-Bunch	180ab	121	0.163ab	0.521	0.680	131	60.5	0.222	0.475	0.612
	N	178ab	161	0.160ab	0.755	0.915	125	71.0	0.183	0.487	0.765
	K	184ab	146	0.164ab	0.694	0.858	112	51.0	0.133	0.582	0.560
	Ca	171ab	140	0.149ab	0.652	0.801	114	63.6	0.193	0.427	0.713
	KCa	154b	118	0.129b	0.537	0.666	130	67.4	0.176	0.578	0.754
	NCa	175ab	162	0.140b	0.751	0.889	110	75.8	0.175	0.602	0.776
	LSD (p ≤ 0.05)	**88**	**NS**	**0.069**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
2008/09	Control	178	106bc	0.114	0.427b	0.541b	51.4c	48.0ab	0.099c	0.555b	0.654b
	Ca-Bunch	159	172a	0.148	0.674a	0.822a	68.4abc	56.8a	0.132ab	0.668a	0.799a
	N	153	112	0.125	0.482b	0.607b	59.2bc	53.8a	0.114bc	0.672a	0.785a
	K	171	99c	0.136	0.395b	0.531b	57.0c	40.2bc	0.115bc	0.494b	0.609b
	Ca	193	141ab	0.139	0.531ab	0.669ab	62.6abc	41.0bc	0.120ab	0.451b	0.571b
	KCa	170	95c	0.133	0.361b	0.494b	73.8a	37.6c	0.148a	0.460b	0.608b
	NCa	195	121b	0.145	0.509ab	0.654ab	70.6ab	53.0a	0.128ab	0.686a	0.814a
	LSD (p ≤ 0.05)	**NS**	**40**	**NS**	**0.185**	**0.209**	**12.7**	**8.9**	**0.030**	**0.107**	**0.124**
2009/10[1]	Control	244	56.4	0.258a	0.461	0.720	109b	30.0ab	0.180ab	0.466ab	0.646ab
	Ca-Bunch	214	59.0	0.262a	0.464	0.726	117ab	30.0ab	0.195a	0.461ab	0.657ab
	N	188	56.4	0.206a	0.462	0.668	105b	29.4ab	0.179ab	0.452ab	0.631ab
	K	194	59.4	0.195b	0.488	0.683	107ab	30.5ab	0.157bc	0.449ab	0.606ab
	Ca	212	59.8	0.235a	0.508	0.696	139a	35.6a	0.177ab	0.517a	0.695a
	KCa	226	59.0	0.309a	0.521	0.829	96b	26.5b	0.143c	0.388b	0.531b
	LSD (p ≤ 0.05)	**NS**	**NS**	**0.105**	**NS**	**NS**	**25**	**7.5**	**0.031**	**0.127**	**0.149**

[1] Treatment NCa was not applied or sampled during this growing season.
[2] NS = not significant.

The lack of response obtained to bunch applied Ca (Ca-Bunch), on the other hand, is ascribed to the fact that Ca^{2+} is not effectively taken up by the berry. The annual amount of Ca that can be applied to the crop by this means is not too little since the total Ca in the crop at harvest ranges from 1.5 kg to 4.0 kg per ha, while about 8 kg Ca per ha is applied to the crop with every spray.

Wojcik (2001) found that Ca applied directly to the fruit can have low penetration, depending on the epidermis characteristics, and the cuticle presence and composition, which affects its permeability. All of these parameters vary throughout the growing season. Gallerani *et al*. (1990) found that $CaCl_2$ foliar applications did not increase the Ca concentration of apple fruit. Likewise, Bonomelli & Ruiz (2010) were not able to increase berry Ca concentration through $CaCl_2$ foliar applications nor soil applicated $CaCl_2$. According to Hanger (1979), foliar applied Ca is normally immobile, but chelation and increased Ca-concentrations apparently induced some translocation in apples. Obtaining increased translocation of applied Ca, however, does not seem to be the issue. Instead, the data from this trial seems to indicate that the reduction in Ca concentration due to berry growth, as well as a termination of Ca influx to the berries at véraison as discussed in Chapter IV, is the reason why increased berry Ca levels are not obtained from soil applied Ca. Furthermore, bunch applied Ca did not raise berry Ca concentration or Ca content, probably because its uptake reduces to negligible amounts throughout berry development as the number of stomata per surface area decrease. The epidermis and cuticle of apples was also found to thicken (Wojcik, 2001) and fruit applied Ca stays in the epidermis or cuticle as found for litchis by Huang *et al*. (2005).

Berry magnesium

In contrast to petiole Mg levels, berry Mg concentration and content were not affected by any particular treatment (data not shown). This is ascribed to the combination of mobility of Mg and low Mg content of berries, leading to berry Mg requirements being easily met even in situations of low vine Mg nutritional status.

Post-harvest quality

The only season that decay was of any significant extent, was in 2006/07, during which the control had the highest occurrence of decay. In this season, treatments containing Ca had the lowest decay, except where K was also applied (Table 10). After 2006/07 the extent of decay obtained for following seasons was too low to make any decisive conclusions regarding the effect of the treatment. Furthermore, none of the treatments seemed to increase the susceptibility of the grapes to decay, e.g. increased berry N content obtained for treatments containing N did not lead to increased decay. Since bunch rot (*Botrytis cinerea*) is

expected to be enhanced by higher N in the berries (Conradie,1986; Mundy & Beresford, 2007), the lack of increased decay for treatments N and NCa (Table 10) is somewhat unexpected, albeit that the increase in berry N was not dramatic. It might, furthermore, also be explained by the study of Christensen *et al.* (1994). They found that high vigour due to excessive N fertilisation, increasing shade and decreasing the efficiency of spraying, was the reason for increased disease incidence, rather than high N levels in berries. In this study (see previous chapters), a lack of vegetative growth responses and petiole N content increases on account of excessive N fertilisation occurred; an impact on disease occurrence was therefore unlikely.

Conradie (1986) is, however, of the opinion that the time of N application affects whether N is translocated to the crop as amino acid N (soluble N) or incorporated in proteins. According to Conradie (1986) bunch rot is assumed to be enhanced more when there is a higher soluble N fraction in the berries, especially if N is applied during the latter part of the season (from four weeks before vèraison up to harvest) instead of during the earlier part (up to four weeks before vèraison). Furthermore, lower decay obtained for the Ca treatments, including Ca-Bunch, in only one season is too little to ascribe any value to Ca in controlling decay, especially in the light of the fact that increased berry Ca concentrations were not obtained for the treatments with the lowest level of decay (Table 10).

For all the sampling times, the levels of occurrence of internal browning were far below the commercially significant level of 1% and significant differences were only obtained for the first harvest sampling in 2007/08 (Table 11). During the latter sampling time, the most internal browning occurred for KCa and K. Although not significant, internal browning of these two treatments was also highest for the third harvest in 2009/10, whereas KCa was highest for the second harvest in 2008/09 and K for the third harvest of the same season (Table 11). There therefore seems to be some indication that internal browning might be enhanced by excessive K in the berries. Similar results were obtained by Conradie (1999) who found that browning was increased by elevated levels of K fertilisation. According to Saxton (2002), permeability of cell membranes increases as the Ca:K ratio in berry flesh is reduced, potentially leading to the polyphenoloxidase enzyme coming increasingly into contact with phenols in the vacuoles.

Table 10. Effect of excessive nitrogen, potassium and calcium fertilisation on the development of decay (% of fresh mass affected) of Prime Seedless during post-harvest storage.

Treatment	2006/07	2007/08		2008/09			2009/10
	First Harvest (14.8°B)	First Harvest (14.1°B)	Second Harvest (15.6°B)	First Harvest (15.5°B)	Second Harvest (16.9°B)	Third Harvest (18.2°B)	Third Harvest (18.1°B)
Control	44.2a	0	0.15	0.05b	0	0	2.93
(Control-Bunch)	8.6b	0	0.00	0.05b	0.03	0	3.07
N	17.0b	0	0.07	0.00b	0	0.056	4.39
K	22.0ab	0.09	0.00	0.00b	0	0	5.52
Ca	11.6b	0.18	0.00	0.00b	0	0	3.21
KCa	28.6ab	0	0.07	0.12b	0	0	6.34
NCa	8.4b	0	0.63	0.45a	0	0.06	
LSD (p ≤ 0.05)	**23.3**	**NS**	**NS**	**0.28**	**NS**	**NS**	**NS**

Table 11. Effect of excessive nitrogen, potassium and calcium fertilisation on the development of internal browning (% of fresh mass affected) of Prime Seedless during post-harvest storage.

Treatment	2006/07	2007/08		2008/09			2009/10
	First Harvest (14.8°B)	First Harvest (14.1°B)	Second Harvest (15.6°B)	First Harvest (15.5°B)	Second Harvest (16.9°B)	Third Harvest (18.2°B)	Third Harvest (18.1°B)
Control	0	0.01b	0.00	0.05	0.63	0.41	0.04
(Control-Bunch)	0	0.17ab	0.10	1.13	0.29	0.06	0.16
N	0	0.06ab	0.07	0.00	0.49	0.51	0.07
K	0	0.29ab	0.04	0.85	0.37	0.83	0.26
Ca	0	0.16ab	0.05	0.08	0.22	0.86	0.17
KCa	0	0.31a	0.19	0.07	0.99	0.78	0.22
NCa	0	0.18ab	0.16	0.29	0.17	0.71	
LSD (p ≤ 0.05)	**0**	**0.28**	**NS**	**NS**	**NS**	**NS**	**NS**

Although the occurrence of external browning was higher than internal browning, it was also very low, occurring at levels that might have commercial relevance only for first harvest 2008/09 and third harvest 2009/10 (Table 12). Progressively decreasing levels of external browning when berries mature, as found for 2008/09, are not uncommon (DFPT Research, 2010). The significantly higher levels of external browning that were obtained in 2008/09 for all three sampling times for Ca-Bunch are contrary to popular believe that bunch applications of Ca reduce browning (Strydom *et al.*, 1999). In general, the data in Tables 11 and 12 seem to indicate that neither Ca fertilisation nor bunch Ca applications have the potential to reduce berry browning.

Soft tissue breakdown, often limited to one or two berries per bunch, was also found to be of very low levels during all four seasons and no consistent treatment effect to promote or reduce soft tissue breakdown was observed (Table 13). Furthermore, no significant differences between the treatments in the occurrence of berry split, loose berries or waterberry were obtained (data not shown).

Sensory evaluation

Since no differences in fruit eating quality were obtained for 2006/07, data of only the 2007/08 season are presented in Figure 1. The data indicate that treatments N and NCa were perceived to make a poorer general impression, having lower acceptable colour, being less crunchy (firm) and having tougher skins. Ruiz *et al*. (2004) furthermore found that the berries of Thompson Seedless became softer the higher the N content of both skins and pulp were. Furthermore, berry crunchiness (firmness) was enhanced by Ca-Bunch and K, compared to the control. Overall, Ca-Bunch and KCa were apparently found to be most acceptable, although it generally lacked significance compared to the control (Figure 1).

Table 12. Effect of excessive nitrogen, potassium and calcium fertilisation on the development of external browning (% of fresh mass affected) of Prime Seedless during post-harvest storage.

Treatment	2006/07	2007/08		2008/09			2009/10
	First Harvest (14.8°B)	First Harvest (14.1°B)	Second Harvest (15.6°B)	First Harvest (15.5°B)	Second Harvest (16.9°B)	Third Harvest (18.2°B)	Third Harvest (18.1°B)
Control	0	0	0.18	0.00b	0.00b	0b	0.23b
(Control-Bunch)	0	0	0.16	1.21a	0.71a	0.22a	1.27b
N	0	0	0.48	0.39ab	0.07ab	0.05b	0.88b
K	0	0	0.53	0.72ab	0.36ab	0b	1.91ab
Ca	0	0	0.69	1.10ab	0.07ab	0b	3.74a
KCa	0	0.07	1.52	1.06ab	0.17ab	0b	0.55b
NCa	0	0	0.13	0.06ab	0.00b	0b	
LSD (p ≤ 0.05)	**NS**	**NS**	**NS**	**1.19**	**0.70**	**0.16**	**2.26**

Table 13. Effect of excessive nitrogen, potassium and calcium fertilisation on the development of soft tissue breakdown (% of fresh mass affected) of Prime Seedless during post-harvest storage.

Treatment	2006/07	2007/08		2008/09			2009/10
	First Harvest (14.8°B)	First Harvest (14.1°B)	Second Harvest (15.6°B)	First Harvest (15.5°B)	Second Harvest (16.9°B)	Third Harvest (18.2°B)	Third Harvest (18.1°B)
Control	0	0.27	0.06b	0.20b	0.08	0.16b	0.18b
(Control-Bunch)	0	0.19	0.26ab	0.50b	0.09	0.12b	0.32ab
N	0	0.17	0.00b	1.68a	0.39	0.89a	0b
K	0	0.40	0.26ab	0.49b	0.28	0b	0b
Ca	0	0.23	0.18ab	0.08b	0.26	0.35ab	0.08b
KCa	0	0.29	0.19ab	0.38b	0.56	0.27ab	0.69a
NCa	0	0.13	0.55a	0.05b	0.57	0.23ab	
LSD (p ≤ 0.05)	**NS**	**NS**	**0.40**	**0.80**	**NS**	**0.71**	**0.47**

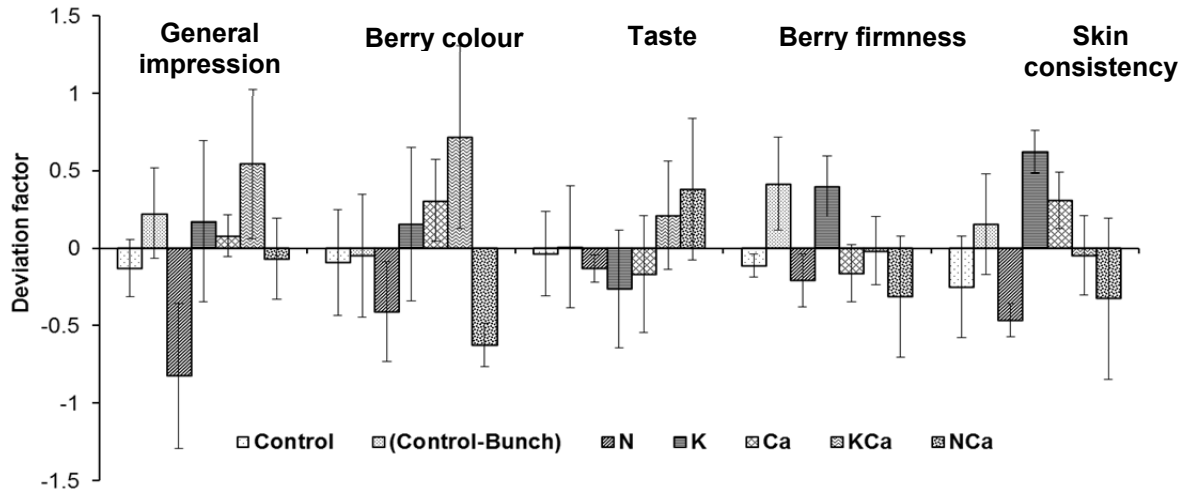

Figure 1. Evaluation results of a tasting panel of first harvest Prime Seedless grapes from a fertilisation trial conducted in Paarl during the 2007/08 season. The deviation factor indicates variance from the average scoring of the panel for a specific parameter, positive values indicate acceptability, while negative values indicate unacceptability. Bars indicate the ± standard error of the means.

CONCLUSIONS

Larger berry size obtained for N applications is ascribed to slight increases in early vegetative growth, allowing support and a better response to GA_3 treatments. The growth response was, however, not dramatic, leading to a lack of consistently significant increases in berry size. The use of GA_3 for berry enlargement is also considered the reason why K fertilisation, resulting in increased berry K levels, did not affect berry size as is often found for wine grapes.

Both treatments K and KCa had a slightly positive effect on TSS of berries, but given that fact that totally excessive levels of K were applied, there seems to be no value in applying high rates of K fertilisation on sandy soils to enhance ripening of Prime Seedless grapes. However, no K was applied after véraison, and giving the mobility of K in the vine, the effect of late K applications on sandy soil is still not clear.

Higher available NO_3^- in the soil on account of excessive N applications (treatments N & NCa) resulted in higher levels of berry N, despite sub-optimal soil pH regimes that were created by these treatments (Chapter II). High rates of K applications (treatments K & KCa) reduced berry N levels. Decay was, however, not affected by these shifts in berry N concentration. The results seem to indicate that berry N does not play a role in berry susceptibility to decay. On the other hand, a strong case for investigating the effect of late N applications (after véraison) on berry susceptibility to develop decay may be made. In

addition to this, elucidation whether there are N levels in the berries above which their susceptibility to fungal infection is increased, or specific N compounds that make berries more susceptible to decay, is required.

Berry K concentration and content were increased by K fertilisation. Rapid vine K uptake and translocation to the berries seem to negate the reduced vine nutritional status as observed in petioles for situations of over-fertilisation with N. Berry size seems to play a more important role in skin K concentration and berry skin K:flesh K content than inherent berry physiology.

Despite higher soil and soil water Ca levels, berry Ca levels were not increased by Ca fertilisation or by bunch applied Ca (Ca-Bunch). The rapid rates of berry growth, together with low rates of berry Ca uptake and Ca uptake that terminates at the onset of ripening, are assumed to be the main reasons for this result.

Low levels of decay as well as a lack of consistently increased decay were obtained for N containing treatments (N & NCa). Although the study was not focused on treatment effects on development of decay, the results pointed to a lack of enhanced post-harvest decay with high berry N levels. Validation of the role of berry N in enhancing *Botrytis cinerea* infections or other forms of decay, is required. Furthermore, potentially increased berry browning on account of high rates of K fertilisation needs to be further investigated.

LITERATURE CITED

Anonymous, 2007. Guidelines for preparing export table grapes. Capespan Exports (Pty) Ltd., Bellville.

Bell, A.A., Ough, C.S. & Kliewer, W.M., 1979. Effects on must and wine composition, rates of fermentation, and wine quality of nitrogen fertilization of *Vitis Vinifera* var. Thompson Seedless grapevines. Am. J. Enol. Vitic. 30, 124 - 129.

Bonomelli, C. & Ruiz, R., 2010. Effects of foliar and soil calcium application on yield and quality of table grape cv. “Thompson Seedless”. J. Plant Nutr. 33, 299 - 314.

Casero, T., Benavides, A.L. & Recasens, I., 2010. Interrelation between fruit mineral content and pre-harvest calcium treatments on “Golden Smoothee” apple quality. J. Plant Nutr. 33, 27 - 37.

Choné, X., Lavigne-Cruège, V., Tominaga, T., van Leeuwen, C., Castagnède, C., Saucier, C. & Dubourdieu, D., 2006. Effect of vine nitrogen status on grape aromatic potential: Flavour precursors (S-cysteine conjugates), glutathione and phenolic content in *Vitis vinifera* (L.) cv. Sauvignon Blanc grape juice. J. Int. Sci. Vigne Vin 40, 1 - 6.

Christensen L.P., Bianchi M.L., Peacock W.L. & Hirschfelt D.J., 1994. Effect of nitrogen fertiliser timing and rate on inorganic nitrogen status, fruit composition, and yield of grapevines. Am. J. Enol. Vitic. 45, 377 - 387.

Christensen, L.P. & Boggero, J.D., 1985. A study of mineral nutrition relationships of waterberry in Thompson Seedless. Am. J. Enol. Vitic. 36, 57 - 64.

Conradie, W.J., 1980. Seasonal uptake of nutrients by Chenin blanc in sand culture: I. Nitrogen. S. Afr. J. Enol. Vitic. 1, 59 - 65.

Conradie, W.J., 1981a. Seasonal uptake of nutrients by Chenin blanc in sand culture: II. Phosphorus, potassium, calcium and magnesium. S. Afr. J. Enol. Vitic. 2, 7 - 13.

Conradie, W.J., 1981b. Nutrient consumption by Chenin blanc grown in sand culture and seasonal changes in the chemical composition of leaf blades and petioles. S. Afr. J. Enol. Vitic. 2, 15 - 18

Conradie, W.J., 1986. Nitrogen nutrition of the grape-vine (*Vitis vinifera* L.), PhD Thesis, Univ. of Stellenbosch.

Conradie, W.J., 1999. Die ontwikkeling van stikstof- en kaliumbemestingsriglyne vir tafeldruifproduksie, soos beïnvloed deur grondtipe. Final research report to DFPT Research. Project WW 03/05, Infruitec-Nietvoorbij Institute for Fruit, Vine and Wine.

Conradie, W.J., 2005. Partitioning of mineral nutrients and timing of fertilizer applications for optimum efficiency. Proc. Soil Env. Vine Mineral Nutr. Symp., L.P. Christensen & D.R. Smart (eds.) American Soc. Enol. Vitic. Davis, USA, 69 - 81.

Conradie, W.J. & de Wet, T., 1985. The effect of potassium fertilisation of grapevines on yield and quality. Proc. Potassium Symp. Dept. Agric. & Water Supply, Pretoria, 1 - 3 October, 181 - 183.

Creasy, G.L., Price, S.F. & Lombard, P.B., 1993. Evidence of xylem discontinuity in Pinot noir and Merlot grapes: Dye uptake and mineral composition during berry maturation. Am. J. Enol. Vitic. 44, 187 - 192.

DFPT Research, 2010. Browning on white seedless table grapes: An update from the Browning Working Group. Fresh Notes, Oct., 1 - 4.

Drake, S.R. & Spayd, S.E., 1983. Influence of calcium treatment on “Golden Dlicious” apple quality. J. Food Sci., 48, 403 - 405.

Etchebarne, F., Ojeda, H. & Deloire, A., 2009. Influence of water status on mineral composition of berries in ‘Grenache Noir’ (*Vitis vinifera* L.). Vitis 48, 63 - 68.

Frank, D., Gould, I. & Millikan, M., 2005. Browning reactions during storage of low-moisture Australian sultanas: Effects of vine nitrogen nutrition on subsequent arginine-mediated Maillard reactions during storage of dried fruit. Austr. J. Grape Wine Res. 11, 29 - 35.

Gallerani, G., Pratella, G.C., Bertolini, P. & Marchi, A., 1990. Lack of relationship between total calcium of apple fruit and a calcium deficiency related disorder (Bitter pit): A four year report. Acta Hort. 274, 141 - 148.

Hanger, B.C., 1979. The movement of calcium in plants. Commun. Soil Sci. Plant Anal. 10, 171 - 193.

Huang, X., Wang, H., Yuana, W., Lu, J., Yin, J.H., Lou, S. & Huang, H., 2005. A study of rapid senescence of detached litchi: Roles of water loss and calcium. Postharvest Biol. Tech. 36, 177 - 189.

Iland, P.G., 1988. Grape berry ripening: the potassium story. Aust. Grapegrower & Winemaker 289, 22 - 24.

Keller, M., Arnink, K.J. & Hrazdina, G., 1998. Interaction of nitrogen availability during bloom and light intensity during véraison. I. Effects on grapevine growth, fruit development, and ripening. Am. J. Enol. Vitic. 49, 333 - 340.

Kliewer, W.M., 1977. Influence of temperature, solar radiation and nitrogen on coloration and composition of emperor grapes. Am. J. Enol. Vitic. 28, 96 - 103.

Löhnertz, O., Prior, B., Bleser, M. & Linsenmeier, A., 2000. Influence of N-supply and soil management on the nitrogen composition of grapes. Acta Hort. 512, 55 - 64.

Marcelle, R.D., 1995. Mineral nutrition and fruit quality. Acta Hort. 383, 219 - 226.

Morris, J.R., Cawthon, D.L. & Fleming, J.W., 1980. Effects of high rates of potassium fertilisation on rar product quality and changes in pH and acidity during storage of Concord grape juice. Am. J. Enol. Vitic. 31, 323 - 328.

Mpelasoka, B.S., Schachtman, D.S., Treeby, M.T. & Thomas, M.R., 2003. A review of potassium nutrition in grapevines with special emphasis on berry accumulation. Aust. J. Grape and Wine Res. 9, 154 - 168.

Mundy, D.C. & Beresford, R.M., 2007. Susceptibility of grapes to *Botrytis cinerea* in relation to berry nitrogen and sugar concentration. New Zealand Pl. Protection 60, 123 - 127.

Rogiers, S.Y., Greer, D.H., Hatfield, J.M., Orchard, B.A. & Keller, M., 2006. Mineral sinks within ripening grape berries (*Vitis vinifera* L.). Vitis 45, 115 - 123.

Ruiz, R., Moyano, S. & Navia, T., 2004. Accumulation of nitrogen compounds as related to the “soft berry” problem in table grapes. Agricultura Téchnica Chile 64, 426 - 430.

SAS, 1990. SAS/STAT user’s guide, version 8, first edition, volume 2. SAS Institute Inc., Campus drive, Cary NC 27513.

Saxton, V., 2002. Calcium in viticulture – unraveling the mystique of French terroir. Wine Ind. J. 17, 28 - 33.

Shapiro, S.S. & Wilk, M.B., 1965. An analyses of variance test for normality (complete samples). Biometrika 52, 591 - 611.

Schlegel, T.K. & Schönherr, J., 2002. Penetration of calcium chloride into apple fruits as affected by stage of fruit development. Proc. IS Foliar Nutrition, Tagliavini et al. (Eds.), Acta Hort. 594, 527 - 533.

Soil Classification Working Group, 1991. Soil classification: A taxonomic system for South Africa. Soil and Irrigation Research Institute, Dept. of Agric. Development, Pretoria, R.S.A.

Spayd S.E., Wample R.L., Stevens R.G., Evans R.G., Seymour B.J. & Nagel C.W., 1994. Nitrogen fertilisation of white riesling grapes in Washington. Must and wine composition. Am. J. Enol. Vitic. 45, 34 - 42.

Storey, R., 1987. Potassium localization in the grape berry pericarp by enery-dispersive X-ray microanalyses. Am. J. Enol. Vitic. 38, 301 - 309.

Strydom, G.J., Calitz, F.J. & de Bruyn, J.N., 1999. The effect of calcium on browning of Waltham Cross. Decid. Fruit Grower 49, S1 - S8.

Terblanche, J.H., Wooldridge, L., Hesebeck, I. & Joubert, M., 1979. The redistribution and immobilization of calcium in apple trees with special reference to bitter pit. Commun. Soil Sci. Plant Anal. 10, 195 - 215.

Wojcik, P., 2001. Effect of calcium chloride sprays at different water volumes on apple calcium concentration. J. Plant Nutr. 24, 639 - 650.

Wojcik, P., Gubbuk, H., Akgül, H., Gunes, E., Ucgun, K., Koçal. H. & Küçükyumuk, C., 2010. Effect of autumn calcium spray at a high rate on “Granny Smith” apple quality and storability. J. Plant Nutr. 33, 46 - 56.

Wermelinger, B., 1991. Nitrogen dynamics in grapevine: physiology and modeling. Proc. Int. Symp. Nitrogen in Grapes and Wine. (Ed. J.M. Rantz), Seattle, USA, pp. 23 - 31.

CHAPTER VI

CONCLUSIONS

A lack of defects is required for successful table grape marketing, which pre-suppose optimal vine performance, berry development and post-harvest quality. The supply of mineral nutrients affects vine growth, physiology and berry quality. Despite a vast amount of research conducted over decades, various unresolved issues regarding table grape vine nutrition to ensure optimal grape quality and shelve-life, remain. The result being unjustified fertilisation practices which include excessive applications of nitrogen (N), potassium (K) and calcium (Ca).

A four-year field trial was therefore conducted on a sandy soil in the Paarl district of South Africa, using cv. Prime Seedless (*Vitis vinifera* L.) grapevines, grafted onto Ramsey, and trained onto a gable trellis system. Nitrogen (N), potassium (K) and calcium (Ca) were applied, singular or in combination, at rates equal to 300% the calculated annual nutritional requirement, while the Control treatment received an annual application of 70 kg N/ha, 60 kg K/ha and 10 kg Ca/ha. The effect of these excessive applications on table grape performance under typical South African cultivation conditions was investigated, with special reference to 1) vegetative growth, 2) expression of grapevine nutrient availability through foliar analyses, 3) berry nutrient accumulation patterns of this early variety, 4) manipulation of berry nutrient content through soil and bunch directed applications and 5) the effect of berry nutrient content on its quality.

The objectives of this study were as follows: 1) To aid table grape nutrition practices as well as harvest timing and post-harvest quality prediction by investigating the nutrient accumulation patterns of Prime Seedless, a very early seedless table grape variety that are produced with minimum berry diameter of 18mm; 2) To establish if there is benefit for berry quality in applying K or Ca, in addition to the established nutritional requirements; 3) To establish whether berry Ca levels can be elevated and whether it benefits fruit quality; 4) To understand the interaction of N, K and Ca on uptake and translocation to the berries.

The N concentration of soil water extracts of treatments N and NCa reflected higher N availability. From the first season the K content of the soil was significantly increased where K was applied. While soil Ca of the treatments receiving Ca only started increasing from the second season, it never increased to levels significantly higher than the control soil. A high

rate of leaching-loss of K and Ca from the soil seems to have been stimulated by the application of excessive amounts of N, illustrated by the deficient levels of K in soil of treatments N and NCa. Significantly lower Ca was also found in the soil of treatments with N from the winter of 2008 onwards. Parallel to this, excessive N fertilisation caused a reduction of soil pH to detrimental levels. The lack of response in vegetative growth by the vines on account of the fertilisation treatments, especially N, can therefore not be explained by a lack of (increased) availability of the applied nutrients. It, however, is rather ascribed to the fact that the vineyard has a sandy soil where the lack of stimulation in vegetative growth may be due to the combined negative effect of these excessive applications on soil pH and soil nutrient composition. Foliage management also resulted in a stabilisation of primary shoot length, apical lateral shoot length as well as basal lateral shoot length. The leaf surface area results again demonstrated that, due to foliage management, the treatments had no significant effect on the vine canopy.

No clear relation between petiole N and leaf chlorophyll content could be found. The lack of an increase in chlorophyll with the N fertilisation treatments therefore seemed to have resulted from the fact that the vine N nutritional status of the control treatment was sufficient, as indicated by the petiole analyses. Although Ca reduces the rate of chlorophyll breakdown and protein degradation, no clear relationship between vine petiole Ca content and the chlorophyll content of the same leaves was found. This is ascribed to a lack of significant differences in petiole Ca content.

Average petiole N content was generally increased by N fertilisation but a lack of significant differences in the latter two seasons is difficult to explain. The low uptake of excessively applied N also explains why vegetative growth was not significantly increased by the high rate of N applications. The decreasing effect of N fertilisation on soil pH might have reduced root activity progressively, also explaining the lack of treatment effects in the latter two seasons. Excessive Ca or K and in combination did not affect petiole N concentration significantly or in a consistent manner. Petiole N concentration showed a decreasing trend throughout the season, meaning that the norms used to evaluate N nutritional status should also decrease. Comparing the norms published for fruit-set and véraison, excessive N fertilisation showed only once in four years an elevated N concentration in the petioles – a poor correlation between N concentration in petioles at harvest and the total amount of N applied to the soil was therefore obtained. This questions the value of using leaf nutrient concentrations to establish N fertilisation requirements. However, although harvest is not regarded as a reliable sampling time for foliar analyses, a significant increase in petiole N of both N fertilisation treatments that was obtained at harvest of 2006/07 and 2007/08, points to

the possibility to use this sampling time for setting of N nutritional norms. In the light of the rapid seasonal development of Prime Seedless (harvest is 120 days after budbreak), harvest petiole sampling of this variety might indeed have value. The exact time of sampling for the latter phenological stage may have to be determined by berry maturity level, otherwise it would be difficult to set comparable norms, even for a specific block from year to year.

Parallel to the significant increases in soil K content obtained for treatments K and KCa, petiole K concentration was increased by K fertilisation at all phenological stages. This also illustrates the preferential uptake of K from the soil and its rapid translocation within the plant. Excessive N and Ca did not suppress petiole K concentration significantly, although a consistent trend to lower K levels in the petioles was observed. The latter is explained by the stimulated leaching-losses of K from the soil under conditions of high rates of N application. The reduced petiole K due to Ca fertilisation is explained by competition that exists between K and Ca for uptake by the roots, but the lack of significant results are due to K uptake being more efficient than Ca uptake. It is therefore important to note that despite K fertilisation being applied in accordance to nutritional requirements, excessive N and Ca applications can progressively induce K deficiencies on a sandy soil. None of the K fertilisation treatments succeeded to raise petiole K concentrations above the maximum published norms. From these data it seems that by comparing petiole K analyses to existing norms will not necessarily reflect an excessive K supply. A true reflection of the uptake and effectiveness of K fertilisation might therefore only be obtained by comparing seasonal, parallel analyses of petioles from K fertilised vines and those that were not fertilised. A general decrease in petiole K concentration during the course of the growing season was also found.

Calcium fertilisation did not increase soil Ca content, resulting in a lack of differences in petiole Ca concentrations between treatments. Petiole Ca remained above the minimum Ca norms published for all treatments (even those with low pH and Ca in the soil). This indicates that the grapevine has low Ca nutritional requirements. From the data of this trial it seems that a comparison of petiole Ca analyses with our present norms, would not necessarily reflect conditions of low Ca supply. Low levels of Ca availability often lead to reduced growth, especially of young leaves and growing tissues. Analyses of young leaves, of which the best sampling time would probably be before tipping or topping, as indicator of Ca nutritional status, should therefore be investigated. An increase in petiole Ca concentration towards harvest was obtained. Interpretation of petiole analyses should therefore take the sampling time into account. Nutrient ratios in petioles were found to illustrate differences in nutrient supply only as far as it entails K, i.e. K:N ratios, K:Mg ratios and K:Ca ratios of treatments

containing K were increased significantly. The value of using such ratios is, however, doubtful since petiole K was anyway elevated by the same treatments.

Fresh mass increased at a rapid rate from pea berry size, averaging 0.146 g/day, and did not subside before first harvest. Berry dry mass increase showed a similar pattern, although the rapid increase was delayed until after 15 mm berry size. This is ascribed to sugar accumulation (not measured), which commenced later than the berry fresh mass increase. Initial berry growth is mainly due to cell division, while later growth is due to cell enlargement. From pea size to harvest, berry tissue dry mass increased 145%. This is ascribed to accumulation of total soluble solids (including minerals) in the flesh (pulp), since flesh mass:skin mass ratio, calculated from dry mass, increased by 61% from pea-sized berry to harvest. The lag phase often observed just prior to véraison was not observed. It is ascribed both to the absence of seed and the fact that Prime Seedless is a very early ripening variety and berry growth does not decrease during the transition phase from pre- to post-véraison because of its brevity. The GA_3 applied as enlargement treatment at 8 to 12 mm berry size would further compact and boost this growth rate, most likely further masking the well-known double sigmoid curve.

The dynamics of berry growth impacted on berry nutrient concentration. Early rapid berry growth, predominantly due to cell division and growth, was associated with the most rapid decreases in N, P and Ca concentration. Due to mobility of K and Mg in the plant that exceeds that of other nutrients, decrease in concentration of these two mineral elements was not as pronounced as that of the others. Nutrient accumulation was most rapid during the pre-véraison period, but only Ca showed definite termination during early ripening. The continued inflow of N, P, K and Mg, albeit at slower rates immediately after véraison, should be taken into consideration when fertilisation is applied. As a table grape, total accumulation of each nutrient in Prime Seedless berries also far exceeded that of other varieties studied this far. A particular difference is that the berry flesh:skin ratio is much higher than previously studied varieties, leading to higher levels of nutrient accumulation in the flesh.

For all the nutrients, the berry flesh contained the larger part of the total accumulated nutrients in the berry, although the skin concentration exceeded that of the flesh as the berry enlarged. Due to the role of the nutrient concentration, rather than total content, in berry quality, a better understanding of other dynamics that determine berry nutrient concentration is required. Furthermore, the rapid development of this early seedless variety, with berry size that far exceeds that of wine grapes, is accepted as an important factor influencing berry nutrient accumulation patterns to divert slightly from the generally established ones.

Berry flesh N concentration decreased rapidly from set to shortly before véraison, thereafter the decrease slowed down. Berry skin N concentration showed a similar trend. The initial rapid decrease in berry N concentration is ascribed to berry growth between set and véraison that exceeded N accumulation rates, probably due to cell growth demands. After véraison, the rate of decrease in N concentration slowed down. This is ascribed to the commencement of sugar accumulation, which is also associated with arginine accumulation. Up to véraison, N concentration of the flesh was higher than that of the skin, but from the start of ripening skin N concentration exceeded the flesh N concentration. This seems to indicate that N is partitioned mainly to the skin during ripening. If amino acid concentration increased mainly in the skin, then increased sensitivity to *Botrytis* is expected. Total berry N accumulation was rapid during the pre-véraison period, being associated with cell division and growth requiring N for chlorophyll, nucleotides, nucleic acids and proteins. After véraison, N accumulation slowed down, except in the 2008/09 season during which berry analyses were conducted at TSS that exceeded 16^{o}B. Rapid N accumulation commenced again at later maturity. If the latter N is indeed mainly in the form of proline, as speculated, it points to some form of ageing or other stress. The implication is that from a post-harvest storage point of view, N accumulation can be used as indicator of optimal harvest time.

Although a general decrease in K concentration of berry flesh was observed, varying changes in the patterns of K concentration were obtained over the four seasons. Potassium concentration in both tissues decreased during the early stages of berry development. Given the fact that berry size obtained for Prime in this experiment ranged between 8 g and 10 g, the initial decrease in K concentration during early stages of rapid berry growth in this study is not unexpected. Compared to small berry varieties, berry K concentration did not increase, especially in conditions where berry growth and berry K accumulation were maintained at similar rates. This implies that factors such as cultivar (berry size), crop load, and climatic conditions that determine berry growth and cultural practices that affect rate of berry growth and/or K accumulation in the berry would affect berry K concentration. It may also be stressed again that Prime Seedless is a very early ripening variety and berry growth does not decrease during the transition phase from pre- to post-véraison. Furthermore, the enlargement GA_3 treatments at 8 to 12 mm berry size boost berry growth rate, most likely further masking the well-known double sigmoid curve. Despite this, there is a very definite point of change in berry flesh K concentration dynamics before or around véraison, probably indicating a change in it ripening physiology. Total K content in the berries continued to increase throughout the season, with the most rapid rate of accumulation in the pre-véraison period, after which it slowed down during ripening. Given the fact that berry dry mass

accumulation was maintained after véraison at the same rate than pre-véraison, this work seems to indicate that dry mass accumulation was not associated with K accumulation. The difference of results to the accepted pattern might be due to the fact that K translocation and partitioning in the vine is affected by plant water status, presence of seed, and rate of berry growth with final size affecting the skin:flesh ratio extensively.

Berry Ca concentration showed a decreasing pattern throughout berry development. The rate of decrease seemed to be related to progressively reduced influx rates assosciated with berry growth, since it reduced most rapidly during the pre-véraison period for Prime Seedless, which coincided with rapid berry growth. Decrease in xylem flow after véraison reduces Ca movement into the berry. Calcium is accumulated at its most rapid rate between pea-size berry and 15 mm berry size. Furthermore, Ca uptake by the berry terminates, or reduces dramatically, after véraison. However, when sampling was done up to three weeks after first harvest, Ca accumulation was found to resume. This resumption points to a definite interruption of xylem flow, albeit temporary, and is probably related to rapid berry growth.

Slightly larger berry size was obtained for N applications and is ascribed to slight increases in early vegetative growth, allowing a better response to GA_3 treatments. The use of GA_3 for berry enlargement is also considered the reason why K fertilisation, resulting in increased berry K levels, did not affect berry size as is often found for wine grapes. The effect of the treatments on berry TSS was not constant. Both the control and treatment K, however, showed a tendency to have the highest TSS. Potassium as an osmotically active cation in the phloem sap and grape berries, contributes to phloem sap flow (sugar import) by helping to establish an osmotic potential gradient between the leaves (source) and the berries (sink). This was supported by a strong relationship found between berry K content and both sugar and dry mass accumulation. No consistent effect from either N, K or Ca applications on TA was obtained.

Higher available NO_3^- in the soil on account of excessive N applications resulted in higher levels of berry N, despite sub-optimal soil pH regimes that were created by these treatments. The increased berry N content obtained points to the mobility of N in the vine and that it is readily translocated to the berries. Treatments K and KCa furthermore reduced berry N concentration and content compared to treatment N and, in some cases, also compared to the control. This is ascribed to the reducing effect that K has on N uptake.

Berry K concentration and content were increased by K fertilisation. Rapid vine K uptake and translocation to the berries seem to negate the reduced vine nutritional status as observed in

petioles for situations of over-fertilisation with N. The generally accepted large increases in berry K after véraison were also observed. Inconsistent trends in K increase from véraison to first harvest were, however, obtained for the treatments and between seasons. The treatment with the highest K concentration or content at véraison therefore did not necessarily have the highest value at harvest. This indicates that various factors other than only K availability affects K translocation to the berries, e.g. irrigation, canopy conditions and factors that affect leaf photosynthetic activity. The many factors that affect nutrient uptake and distribution makes it difficult to predict the effect of fertilisation on berry mineral composition, even for a mobile nutrient like K. Berry Ca levels were not increased by Ca fertilisation or by bunch applied Ca. The rapid rates of berry growth, together with low rates of berry Ca uptake and Ca uptake that terminates at the onset of ripening, are assumed to be the main reasons for this result. Furthermore, uptake of bunch applied Ca is poor due to the epidermis and cuticle that thickens Ca that probably is caught up in the epidermis or cuticle.

Low levels of decay as well as a lack of consistently increased decay were obtained for N containing treatments. It must be established whether there are N levels in the berries above which their susceptibility to fungal infection is increased, or specific N compounds that make berries more susceptible to decay should be identified. Neither soil applied Ca nor bunch applied Ca improved berry quality, although Ca treatments seemed to reduce decay during the only season that it was at commercially significant levels. The latter, however, was too little evidence to ascribe to Ca any value in controlling decay, especially in the light of the fact that increased berry Ca concentrations were not obtained for the treatments with the lowest level of decay. For all the sampling times, the levels of occurrence of internal browning were far below the commercially significant level of 1% and significant differences were only obtained for the first harvest sampling in 2007/08, during which time the most internal browning occurred for KCa and K. It was also the highest level for these two treatments in various other sampling times. There therefore seems to be some indication that internal browning might be enhanced by excessive K in the berries. Treatments N and NCa made a poorer general impression with consumers, having lower acceptable colour, being less crunchy (firm) and having tougher skins. Furthermore, berry crunchiness (firmness) was enhanced by Ca(Bunch) and K, compared to the control (during one season only).

From this study it became clear that:

- Excessive N and K fertilisation has a detrimental effect on chemistry of sandy soils, which leads to inefficient fertilisation and a lack of the desired responses. A lack in vegetative growth responses and vine performance would be the result of reduced soil pH on account of excessive N fertilisation or due to imbalanced soil nutrient contents.

- Even on a sandy soil with low CEC, Ca levels were not raised significantly by annual fertilisation of up to three times the vine requirement. Soil applications to raise the Ca uptake of the vine are therefore ineffective. Likewise, in addition to foliar applications that have been proven useless in other research, bunch directed applications of Ca also do not affect berry Ca content.
- Reliable correlations between petiole nutrient concentration and berry mineral content at harvest could not be established. The only way of knowing the mineral content of berries is by measuring it directly instead of deducing it from the results of leaf or petiole analyses.
- As indicator of vine nutrient availability, petiole analysis must be evaluated in parallel with soil analyses, taking seasonal variation into consideration. The danger of being only guided by published norms for leaf nutrient concentrations when establishing fertilisation practices, has again been highlighted by this study. It was also reiterated that during interpretation of foliar analyses the phenological stage must be taken into consideration, since the concentrations of nutrients change during the course of the season.
- For a very early variety like Prime Seedless, nutrient accumulation dynamics can already start to change during the pre-véraison period in some seasons. This is due to different edaphic and climatic conditions as well as the dynamics of berry growth and eventual berry size, which leads to much higher flesh:skin ratios. Future research on table grapes would need to further develop an understanding of the various factors that determine berry nutrient concentration and accumulation of early ripeningseedless table grape cultivars with large berry size.

It is therefore recommended that:

- The use of analyses of young leaves as indicator of Ca nutritional status is investigated.
- Due to the role of the nutrient concentration on berry quality, an understanding of all the dynamics that determine berry nutrient concentration is acquired in the context of large berry cultivars.
- The forms in which N occur in the berries at different ripening stages are established.
- Whether there are different forms of N that would impact on the susceptibility of the berries to fungal infection.
- Potentially increased berry browning on account of high rates of K fertilisation is further investigated, since some indications thereof were observed.
- The role of berry growth to cause an interruption in xylem flow after onset of ripening is further investigated.