Bunch structure, rudimentary seed size and return fertility of *Vitis vinifera* L. 'Sunred Seedless' as affected by GA_3 and GA_{4+7} thinning treatments

by

Talana Claassen

Thesis presented in partial fulfilment of the requirements for the degree of

Master of Agricultural Science

at

Stellenbosch University

Department of Viticulture and Oenology, Faculty of AgriSciences

Supervisor: Mrs E Avenant

Co-supervisor: Mr JH Avenant

December 2020

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.

Date: September 2020

SUMMARY

Market requirements for export grapes are consumer-driven and based on characteristics such as bunch size, bunch colour uniformity, berry size and distribution, seedlessness, flavour profile, texture and eating quality. In order to meet these requirements, the use of plant growth regulators (PGRs) has become an essential tool in producing grapes of high export quality, while contributing to reducing labour costs required for manual thinning or girdling to increase berry size. Increased costs associated with the production of table grapes, along with high expectations to meet increasing market demands, require attention to minimise input costs with the effective use of PGRs. The response of cultivars poses a challenge, as cultivars react differently towards a PGR application. Apart from cultivar response, the application timing and concentration used for the specific PGR also contribute towards the efficacy of the treatment applied.

Limited research publications are available on the effect of GA_{4+7} used for thinning on table grapes, as well as the effect of GA_3 and GA_{4+7} applications on rudimentary seed size and return fertility of specifically Sunred Seedless, but table grapes in general as well. The study aimed to determine whether an alternative gibberellic acid structure, GA_{4+7}, could be used as a chemical thinning agent for cultivars that respond poorly to GA_3 in order to improve bunch quality without negatively affecting the return fertility.

The study was performed during the 2015/2016 and 2016/2017 growing seasons on 15-year-old *Vitis vinifera* L. cv. 'Sunred Seedless' vines, grafted onto Ramsey (*Vitis champinii*). The experimental site is situated in a commercial vineyard located on the premises of the ARC Infruitec-Nietvoorbij experimental farm at De Doorns, in the Hex River Valley, South Africa.

A standard GA_3 concentration of 5 parts per million (ppm) was evaluated against different concentrations of GA_{4+7}, ranging from 7.5 ppm to 120 ppm, adjusted over the two seasons. These treatments were applied at different phenological stages in order to determine the most effective timing for a thinning application on Sunred Seedless. Eight treatments and an untreated control were evaluated during the 2015/2016 season. The treatments consisted of four early thinning applications applied 31 October 2015 and four late thinning applications applied 4 November 2015. Both the early and late treatments were applied at 5 ppm GA_3, 7.5 ppm GA_{4+7},15 ppm GA_{4+7} and 30 ppm GA_{4+7}. The two application dates refer to a difference in the predominant phenological stage of the vineyard, which a producer would have used to determine the timing of a thinning application. The early application timing represents a predominant phenological stage of 10% berry set (10%BS) and the late application represents berry set (BS).

The treatment layout for the 2016/2017 season was adjusted to accommodate increased GA_{4+7} concentrations, as well as two sizing treatments. The nine treatments applied in this particular season consisted of an untreated control, six thinning (T) treatments (5 ppm GA_3; 7.5 ppm GA_{4+7}; 15

ppm GA_{4+7}; 30 ppm GA_{4+7}; 60 ppm GA_{4+7}; 120 ppm GA_{4+7}), a thinning and sizing (T+S) treatment (60 ppm GA_{4+7} + 60 ppm GA_{4+7}) and a sizing (S) only treatment (60 ppm GA_{4+7}).

Each treatment had four replicates and each replicate consisted of four vines, referred to as an experimental unit. Within each experimental unit the two centre vines were used as the experimental data unit. Field sampling was performed in the experimental data unit. Additionally, within each experimental data unit, bunches were categorised and marked at four phenological stages to determine the optimal phenological stage for application. The stages for the 2015/2016 season included 80-100% flowering (80-100%F), 10% berry set (10%BS), berry set (BS) and berry set plus four days (BS+4D). The stages for the 2016/2017 season included 50% flowering (50%F), 80-100%F, 10%BS and BS. Five bunches per experimental data unit were marked according to the phenological stages identified for each season. These marked bunches were used for bunch and berry evaluations at harvest and were therefore left in their natural state, with no bunch preparations applied or any berry sampling performed on them. Bunch structure assessments were performed in line with a protocol developed and applied by the Viticulture Division of ARC Infruitec-Nietvoorbij.

Applications during flowering resulted in a better thinning effect of Sunred Seedless, based on the bunch and berry mass measurements. Bunch and berry mass measurements at harvest didn't result in a specific trend concerning a specific GA concentration and application timing combination that could be recommended for effective thinning of Sunred Seedless.

Based on the subjective visual assessment of bunch compactness, applying a GA thinning treatment at 50% flowering is too early for Sunred Seedless, as it resulted in straggly bunches. However, the longer a GA thinning treatment was delayed from flowering to berry set, the less effective the thinning results were, resulting in more compact bunches if applied around berry set. These findings correspond with the results obtained for the quantitative bunch compactness measurements. The mean total and normal berries per cm of lateral length were reduced significantly by GA treatments applied during flowering. The 5 ppm GA_3 treatment applied at 80-100%F resulted in the most effective thinning, with a significantly reduced number of total berries per cm of lateral compared to the untreated control.

There was a significant increase in the mean percentage of shot berries at the 50%F and 80-100%F stages compared to the 10%BS and BS stages, for GA treatments applied during the 2016/2017 season. These results indicate that Sunred Seedless has a higher sensitivity for the formation of shot berries when GA is applied during flowering. An increase in shot berry occurrence was observed with the use of higher GA_{4+7} concentrations and double applications at the 50%F stage.

The sensitivity of Sunred Seedless towards GA applications applied during early flowering, along with poor response for GA applications applied after flowering observed in this study, confirms why GA thinning treatments for this particular cultivar do not give economically acceptable results. Reoccurring trends regarding the bunch phenological stage at the time of application were observed in this study, rather than trends regarding a specific GA treatment and treatment rates. These results

confirm that the timing of a GA applications play a fundamental role in the treatment outcome for a specific cultivar.

A trend was observed that applying GA treatments during flowering resulted in decreased average rudimentary seed mass per berry as well as an improved rudimentary seed size distribution with an increased percentage of small rudimentary seeds compared to GA applied during the early stages of berry development. No consistent trend regarding the effect of different GA_3 or GA_{4+7} application timing and rates on rudimentary seed size could be concluded over two seasons.

Commercially acceptable bud break percentages of ≥ 80% were obtained for all treatments, determined through forced budding in June 2016 and 2017 as well as through actual fertility assessments in October 2016. A reduction in the mean number of bunches per sprouted bud was reported from June 2016 to June 2017 for the potential fertility assessed through forced budding. Potential fertility assessed through bud dissections did not follow the same trend from June 2016 to June 2017 as mentioned above for forced budding. The use of GA_3 reduced the actual fertility of Sunred Seedless in this study, after one season of GA treatment application compared to the untreated control. Similar results were not observed for GA_{4+7} treatments.

There was a poor correlation between the potential fertility determined through bud dissection and forced budding were reported, compared to the actual fertility determined in the vineyard. Potential fertility assessments are therefore not advised for crop estimations, but rather to be used for verifying the pruning system used for a specific cultivar.

OPSOMMING

Die markvereistes vir uitvoerdruiwe word gedryf deur verbruikervoorkere. Dit word gebasseer op trosgrootte, eweredige troskleur, korrelgrootte en -verspreiding, pitloosheid, die geurprofiel, tekstuur en eetgehalte. Om aan hierdie vereistes te voldoen, het die gebruik van plantgroeireguleerders (PGRs) ‘n noodsaaklikke hulpmiddel geword om druiwe van ‘n hoë uitvoergehalte te produseer. Dit dra by tot verminderde arbeidskostes deur handuitdunning en korrelgrootte manipulasies grootliks te vervang. Meer aandag moet egter gegee word aan die effektiewe gebruik van PGRs, vanweë stygende produksiekostes, asook met die hoë vereistes wat deur verskillende markte gestel raak. Die PGR-konsentrasie wat toegedien word, gekombineer met die tydsberekening van daardie toediening, dra by tot die effektiwiteit van behandelings. Kultivar-spesifieke reaksies teenoor PGR-toedienings blyk egter steeds uitdagend te wees, aangesien kultivars verskillend reageer teenoor 'n PGR-behandeling.

Beperkte literatuur is beskikbaar oor die effek van GA_{4+7} op uitdunning van tafeldruiwe asook die effek van GA_3 en GA_{4+7} behandelings op pitresgrootte en opvolgvrugbaarheid van spesifiek Sunred Seedless, maar ook vir tafeldruiwe as geheel. Die doel van hierdie studie was om te bepaal of 'n alternatiewe struktuur van gibberelliensuur, GA_{4+7}, gebruik kan word as 'n chemiese uitdunmiddel vir kultivars wat swak reageer op GA_3, om sodoende trosgehalte te verbeter sonder om opvolgvrugbaarheid negatief te beïnvloed. Resultate van hierdie studie dra by tot beskikbare wetenskaplike gepubliseerde resultate wat handel oor die uitduneffek van GA_{4+7}, sowel as die effek van GA_3 en GA_{4+7} op trosstruktuur, pitresgrootte en opvolgvrugbaarheid van tafeldruiwe.

Die studie is uitgevoer gedurende 2015/2016 en 2016/2017 op 15-jarige *Vitis vinifera* L. cv. Sunred Seedless wingerd, wat op Ramsey (*Vitis champinii)* geënt is. Die proefperseel is geleë in 'n kommersiële wingerd op die perseel van die LNR Infruitec-Nietvoorbij proefplaas op De Doorns, in die Hexriviervallei, Suid-Afrika.

‘n Standaard GA_3 konsentrasie van 5 dele per miljoen (dpm) is geëvalueer teenoor verskillende GA_{4+7} konsentrasies, wat gewissel het van 7.5 dpm tot 120 dpm oor twee seisoene. Die behandelings is op verskillende fenologiese stadiums toegedien, om die mees effektiewe tydsberekening vir 'n uitdunbehandeling op Sunred Seedless te bepaal. Agt behandelings en ‘n onbehandelde kontrole is tydens die 2015/2016 seisoen geëvalueer. Die behandelings het bestaan uit vier vroeë uitduntoedienings op 31 Oktober 2015 en vier laat uitduntoedienings op 4 November 2015. Beide die vroeë en die latere toedienings is teen 5 dpm GA_3, 7.5 dpm GA_{4+7}, 15 dpm GA_{4+7} en 30 dpm GA_{4+7} toegedien. Die twee toedieningsdatums verteenwoordig verskillende fenologiese stadiums van die wingerd, wat deur ‘n produsent gebruik sou word om die tydsberekening van 'n uitdunbehandeling te bepaal. Die vroeë toedieningstyd verteenwoordig 'n oorheersende fenologiese stadium van 10% set en die latere toediening verteenwoordig set.

Die behandelings vir die 2016/2017 seisoen is aangepas om verhoogde GA_{4+7} konsentrasies, asook twee korrelvergrotingbehandlings in te sluit. Agt behandelings en ‘n onbehandelde kontrole is tydens die 2016/2017 seisoen geëvalueer. Die behandelings het bestaan uit ses uitdunbehandelings (5 dpm GA_3; 7.5 dpm GA_{4+7}; 15 dpm GA_{4+7}; 30 dpm GA_{4+7}; 60 dpm GA_{4+7}; 120 dpm GA_{4+7}), ‘n uitdun- en korrelvergrotingbehandlings (60 dpm GA_{4+7} + 60 dpm GA_{4+7}) en ‘n korrelvergrotingbehandlings (60 dpm GA_{4+7}).

Elke behandeling is vier keer herhaal en elke herhaling bestaan uit vier stokke. Hierna word gesamentlik verwys as 'n eksperimentele eenheid. Die middelste twee stokke van elke eksperimentele eenheid is as die datastokke gebruik. Die optimale fenologiese stadium vir toediening is bepaal deur die blomtrosse binne elke data eksperimentele eenheid in vier fenologiese stadiums te kategoriseer. Die 2015/2016-seisoen se stadiums het bestaan uit: 80-100% blom, 10% set, set en set plus vier dae. Daarteenoor was die 2016/2017-seisoen se stadiums: 50% blom 50% blom, 80-100% blom, 10 set en set. Vyf trosse is per data eksperimentele eenheid gemerk volgens die bogenoemde fenologiese stadiums wat vir elke seisoen geïdentifiseer is. Hierdie gemerkte trosse is gebruik vir tros- en korrelevaluasies tydens oes. Trosvoorbereidingsaksies of korrelversameling is dus nie op hierdie trosse uitgevoer nie. Trosstruktuurevaluerings is gedoen volgens 'n protokol van die Wingerdkunde-afdeling van LNR Infruitec-Nietvoorbij.

GA toedienings tydens blom het gelei tot 'n beter uitdun effek van Sunred Seedless, gebaseer op die evaluering van tros-en korrelmassa. Oes-evaluasies van tros- en korrelmassa het geen tendens gewys m.b.t. 'n spesifieke GA-konsentrasie in verhouding tot tyd van toediening van uitdunbehandelings nie.

Resultate gebaseer op die subjektiewe visuele assessering van troskompaktheid, dui aan dat 'n GA uitdunningsbehandeling op 50% blom te vroeg is vir Sunred Seedless, aangesien dit yl trosse tot gevolg het. Hoe langer 'n GA toediening vanaf blom tot set vertraag is, hoe minder effektief is Sunred Seedless uitgedun. Dit kan toegeskryf word aan die toediening wat tot meer kompakte trosse lei indien dit rondom set toegedien word. Hierdie bevindings stem ooreen met resultate wat verkry is met die kwalitatiewe evaluasies van troskompaktheid. GA behandelings tydens blom het die gemiddelde totale- en normale korrels per sentimeter laterale lengte betekenisvol verminder. Die 5 dpm GA_3 behandeling, wat toegedien is op 80-100% blom, was die mees effektiefste behandeling. Dit het gelei tot die effektiefste uitdunning en ‘n betekenisvolle vermindering in die totale korrels per sentimeter laterale lengte, teenoor die onbehandelde kontrole.

'n Betekenisvolle toename in die gemiddelde persentasie bokhaelkorrels is verkry met die 50% en 80-100% blomstadiums in vergelyking met die 10% set en set stadiums, vir GA behandelings toegedien gedurende die 2016/2017 seisoen. Sunred Seedless het dus ‘n verhoogde sensitiwiteit vir die vorming van bokhaelkorrels wanneer GA tydens blom toegedien word. 'n Toename in die voorkoms van bokhaelkorrels kan ook verwag word met die gebruik van hoër konsentrasies GA_{4+7}, asook met meer as een toediening tydens 50% blom.

Sunred Seedless se sensitiwiteit teenoor GA toedienings tydens vroeë blom in hierdie studie, tesame met die swak reaksie teenoor hierdie toedienings wat na blom toegedien word, bevestig waarom GA uitdunbehandelings nie ekonomies aanvaarbare resultate vir hierdie kultivar lewer nie. Herhalende tendense met betrekking tot die fenologiese stadium van die tros tydens toediening is waargeneem in hierdie studie, eerder as tendense met betrekking tot 'n spesifieke GA behandeling en konsentrasie toegedien. Hierdie bevindings bevestig dat tydsberekening van 'n GA toediening 'n fundamentele rol speel in die resultate verkry met GA toedienings vir 'n spesifieke kultivar.

GA behandelings wat tydens blom toegedien is, is vergelyk met toedienings tydens vroeë korrelontwikkeling. Eersgenoemde het tot ‘n afname in die gemiddelde pitresmassa per korrel gelei. Dit het ook ‘n verbeterde pitresgrootte verspreiding, met 'n verhoogde persentasie klein pitreste tot gevolg gehad. Geen konstante tendens is gevind t.o.v. van verskillende GA_3 of GA_{4+7} toedieningstye en konsentrasies op pitresgrootte oor die twee seisoene nie.

Kommersiëel aanvaarbare botpersentasies (≥ 80%) is verkry met uitbotproewe wat in Junie 2016 en 2017 gedoen is, asook met evaluerings wat in die wingerd uitgevoer is in Oktober 2016. 'n Afname in die gemiddelde aantal trosse per oogposisie is van Junie 2016 tot Junie 2017 verkry vir die potensiële vrugbaarheid bepaal deur uitbotproewe. Potensiële vrugbaarheid bepaal deur oogontledings het nie dieselfde tendens gevolg van Junie 2016 tot Junie 2017, soos gevind met die uitbotproewe nie. Die gebruik van GA_3 het Sunred Seedless se werklike vrugbaarheid laat afneem na afloop van ‘n enkele seisoen se GA behandeling, teenoor die onbehandelde kontrole. Dieselfde resultate is nie vir GA_{4+7} behandelings verkry nie.

Die potensiële vrugbaarheid wat deur uitbotproewe en oogontledings bepaal word het swak gekorreleer met die werklike vrugbaarheid wat in die wingerd bepaal is. Potensiële vrugbaarheidsassesserings word dus nie vir oesskattings aanbeveel nie, maar eerder om snoeistelsels wat gebruik word vir ‘n spesifike kultivar te verifieer.

Die potensiële vrugbaarheid wat deur uitbotproewe en oogontledings bepaal word, het swak gekorreleer met die werklike vrugbaarheid wat in die wingerd bepaal is. Potensiële vrugbaarheidsevaluerings word dus nie vir oesskattings aanbeveel nie, maar eerder om snoeistelsels wat gebruik word vir ‘n spesifike kultivar te verifieer.

This thesis is dedicated to my husband, Iván Claassen, my family with special regards to my mother, Carina Fourie, my father, Piet Fourie and my second mother, Annalise Erasmus, as well as my parents-in-law, Sunette and Anton Claassen for their support and encouragement.

BIOGRAPHICAL SKETCH

Talana Fourie was born in Oudtshoorn on the 5th of February 1993. She matriculated at Outeniqua High School in George in 2011. Talana enrolled at Stellenbosch University in 2012 where she obtained her BScAgric degree in Viticulture and Oenology in December 2015. The following year she enrolled for her MScAgric (Viticulture) degree at Stellenbosch University. In 2017 Talana started working for an agricultural chemical company, Villa Crop Protection, where she still works to date. In 2018 she married Iván Claassen and her surname changed to Claassen.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and appreciation to the following persons and institutions:

- **Our Heavenly Father**, who gave me the strength and courage to keep going.
- **My supervisor**, Mrs E Avenant, Department of Viticulture and Oenology, Stellenbosch University for her invaluable guidance, encouragement and motivation during my study.
- **My co-supervisor**, Mr JH Avenant, ARC Infruitec-Nietvoorbij, Plant Protection and Viticulture division for his guidance during my study.
- **My husband**, **family and friends,** for their love, support and encouragement throughout my studies.
- **The management and technical team** of ARC Infruitec-Nietvoorbij farm, De Doorns, for allowing me to use their vineyard for the study and the assistance they provided.
- **Pieter Kriel and his team** of the farm Nil Disperandum, De Doorns, for technical support and providing cold storage facilities for experimental grapes.
- **Technical support**, for field and laboratory measurements from students of the Department of Viticulture and Oenology, Stellenbosch University.
- **Marieta van der Rijst,** ARC Biometry, for statistical data analyses.
- **Dr Carolyn Howell,** for editing the dissertation and her valuable contributions.
- **Villa Crop Protection** for their financial support, as well as the sponsoring of products required for the study.

PREFACE

This thesis is presented as a compilation of eight chapters, including four result chapters presented in article format. Each chapter is introduced separately and is written according to the style of the South African Journal of Enology and Viticulture.

Chapter 1 **General introduction and project objectives**

Chapter 2 **Literature review**
A review of bunch structure, rudimentary seed size and return fertility of table grapes as affected by GA_3 and GA_{4+7} treatments

Chapter 3 **Materials and methods**

Chapter 4 **Research results**
The effect of GA_3 and GA_{4+7} thinning treatments on berry development and ripening of *Vitis vinifera* L. 'Sunred Seedless'

Chapter 5 **Research results**
The effect of GA_3 and GA_{4+7} thinning treatments on bunch structure, compactness and berry size distribution of *Vitis vinifera* L. 'Sunred Seedless'

Chapter 6 **Research results**
The effect of GA_3 and GA_{4+7} thinning treatments on rudimentary seed size of *Vitis vinifera* L. 'Sunred Seedless'

Chapter 7 **Research results**
The effect of GA_3 and GA_{4+7} thinning treatments on return fertility of *Vitis vinifera* L. 'Sunred Seedless'

Chapter 8 **General conclusions and recommendations**

TABLE OF CONTENTS

Chapter 1

General introduction and project objectives

CHAPTER 1:
General introduction and project objectives

1.1 INTRODUCTION

Over the last ten years, the South African table grape industry has grown by 55.9%, from 13 982 hectares in 2009 to 21 798 total hectares planted in 2019 (SATI, 2019b). A global consumer-driven increase in the demand for seedless table grapes is visible in the trend of South African table grape production, with the total production of seedless cultivars increasing from 80% in the 2014/2015 season to 91% in the 2018/2019 season (SATI, 2019b).

During the 2015/2016 season, which was to be the first season of the trial, 56.66 million 4.5 kg cartons were exported, which was a 3.2% decrease in production compared to the previous season (SATI, 2019b). The decrease in production was mainly due to the drought experienced in most of the South African table grape production regions. Record production volumes were recorded during the 2016/2017 season, with 65.45 million 4.5 kg cartons exported (SATI, 2017).

During the 2015/2016 season, Sunred Seedless was 19th on the top 20 list based on export volume and moved to 20th place during the 2016/2017 season (SATI, 2016; SATI, 2017). The cultivar Sunred Seedless was selected for the trial for the following reasons:

i. it is one of the top 20 cultivars in South Africa and therefore of economic importance,
ii. the cultivar is challenging to thin with the existing chemical thinning agent registered,
iii. the cultivar is known to develop detectable rudimentary seeds, and
iv. limited research studies have been performed on Sunred Seedless.

Sunred Seedless is a deep maroon-red, mid-season ripening cultivar with a firm and crunchy texture (SATI, 2016; SATI, 2019a). It was released in 1991 by ARC Infruitec-Nietvoorbij (Avenant, 2000; SATI, 2019a), as a cross between Datal and Ruby Seedless (SATI, 2019a). At the time of the release of the cultivar, it filled a critical window in the South African table grape production season, namely, the red seedless, mid-season window. Sunred Seedless has large, compact bunches with naturally large, oval-shaped berries, reaching an average mass of 6 g per berry (SATI, 2019a). With naturally compact bunches, a thinning action which is either manual or chemical is required to prepare bunches to an export standard. Given that Sunred Seedless responds poorly to chemical thinning with gibberellic acid (GA_3), an alternative gibberellic acid structure, GA_{4+7}, was tested in this trial. The plant growth regulator (PGR) GA_{4+7}, is registered for the reduction of calyx end russeting in apples

in South Africa, under the tradename Novagib® 10 SL (Registration holder: Universal Crop Protection (Pty) Ltd).

In the industry, a foliar nitrogen (N) application of low-biuret urea ion has shown good results for Sunred Seedless as an alternative to the conventional GA_3 treatment used for thinning (SATI, 2019a). Low-biuret urea should be applied at 1 kg per 100 ℓ water (1%), 10 days before flowering with two follow-up applications in three to four-day intervals (SATI, 2019a). During flowering, another two applications of 1% low-biuret urea can be applied with the last application applied at 10% berry set with the addition of 1.5 to 2 ppm GA_3 (SATI, 2019a). The addition of N applied to the vine through the urea application induces strong vigour at an early phenological stage, thereby creating competition between reproductive (berries) and vegetative (shoots) growth for carbohydrates (SATI, 2019a). Consequently, there is abscission of some berries during set, which leads to a less compact bunch structure.

Sunred Seedless has medium vigour and is very fertile, therefore it can be spur pruned (SATI, 2019a). In the Hex River region, however, the pruning system used consists of half-long bearers, due to the increased bud fertility observed at bud positions four to nine, combined with spurs for renewal. A total of 6619 hectares are planted in the Hex River Valley, accounting for 30.37% of the total South Africa table grape plantings (SATI, 2019b). The cultivar Sunred Seedless is harvested between week three and ten in the Hex River Valley.

Market requirements for export grapes are based on characteristics such as bunch size, bunch colour uniformity, berry size and distribution, flavour profile, texture and eating quality. The use of PGRs have become an essential tool in the production of table grapes for producing grapes of high export quality while reducing labour costs for manual thinning or girdling to increase berry size. Increased costs associated with the production of table grapes, along with high expectations to meet increasing market demands, require attention spent on effectively minimizing input costs.

PGRs are defined as synthetic compounds, with similar structures to plant hormones that occur naturally in higher plants, such as table grapes (Korkutal *et al.*, 2008; Rademacher, 2015). Plant hormones or PGRs are often described as signaling molecules, regulating plant growth and development alongside environmental factors also affecting plant growth and development (Pallardy, 2007; Korkutal *et al.*, 2008; Roubelakis-Angelakis, 2009; Rademacher, 2015). Abscisic acid, auxin, cytokinins, ethylene and gibberellins (GAs) are described as the five major plant hormones and are all registered for the use on table grapes with the exception of auxins (Roberts & Hooley, 1988; Fosket, 1994; Korkutal *et al.*, 2008; Durner, 2013).

The role of GAs in grapevines, especially GA_3, is defined as the regulation of growth and development through cell division and cell enlargement such as during the onset phases of berry development (Cahoon *et al.*, 1986; Dokoozlian, 2000; Ungsa *et al.*, 2008; Roubelakis-Angelakis, 2009; Molitor *et al.*, 2012). GA_3 is the most widely used PGR in table grape production and is mainly used for the following three objectives, namely (i) stretching to increase the length of the bunch rachis, (ii) berry thinning to improve bunch compactness through decreased berry set and (iii) berry sizing to meet the requirements of specific markets (Weaver & McCune, 1960; Cahoon *et al.*, 1986; Reynolds & de Savigny, 2004; Reynolds *et al.*, 2006; Roubelakis-Angelakis, 2009). Each desired outcome is dependent on the phenological stage of the grapevine during application and rate applied, which are both highly cultivar dependent.

In grapes, seedless berries develop through two different fruit set mechanisms, parthenocarpy or stenospermocarpy (Stout, 1936; Dokoozlian, 2000). True seedless berries are produced through parthenocarpy, and an example of such a cultivar is Black Corinth (Dokoozlian, 2000). Berries produced by stenospermocarpy are commercially considered to be seedless. This includes cultivars such as Sunred Seedless, Flame Seedless and Thompson Seedless (Dokoozlian, 2000). Stenospermocarpic fruit set is characterized by the abortion of the embryo two to four weeks after fertilization, terminating further seed development, resulting in the formation of rudimentary seeds or seed traces (Stout, 1936; Coombe, 1960; Nitsch *et al.*, 1960; Winkler *et al.*, 1962; Mullins *et al.*, 1992; Dokoozlian, 2000; Perl *et al.*, 2000; Reynolds *et al.*, 2006; Iland *et al.*, 2011). The inherent rudimentary seed size of a cultivar is linked to the timing of embryo abortion, which can be delayed in cultivars with larger rudimentary seeds (Dokoozlian, 2000). During the evaluation of cultivars and/ or selections, grapes are regarded as seedless when rudimentary seeds are soft, green and not perceptible organoleptically (Burger *et al.*, 2003).

An additional, but less common use for GA_3 is to reduce rudimentary seed occurrence. Although GA_3 is known to be less effective in the thinning of Sunred Seedless, the application of GA_3 during flowering was shown to be effective in reducing its rudimentary seed size (Avenant, 2000). The average rudimentary seed size of Sunred Seedless is 9.4 mg, but it can be as large as 22.1 mg compared to that of Sultanina which can vary between 5.3 mg and 6.7 mg per rudimentary seed (Avenant, 2000).

The international consumer market defines seedless grapes with detectable rudimentary seeds as a negative characteristic, decreasing the marketability of these grapes. Sunred Seedless is an example of a cultivar that tends to develop larger rudimentary seeds, increasing the noticeability when consumed. With increased consumer demands for seedless grapes, manipulations that reduce rudimentary seed size could have a valuable contribution from a marketing perspective.

The action of berry thinning can be achieved through either chemical berry thinning with the use of GA_3 as a full cover spray application and/or manual berry thinning, the latter being a time consuming and labour intensive practice (Christodoulou *et al.*, 1966; Gil *et al.*, 1994; Di Lorenzo *et al.*, 2011). The use of chemical thinners is essential for the longevity of cultivars that set naturally compact bunches, *i.e.* Sunred Seedless, because manual thinning is not a sustainable practice due to increasing labour costs. The use of GA_3 applications during flowering or the early stages of berry set has been widely studied and authors have reported a decrease in berry set when applied to seedless table grape cultivars (Lynn & Jensen, 1966; Weaver & Pool,1971; Dokoozlian & Peacock, 2001). However, this is not a viable option for Sunred Seedless as it responds poorly to GA_3 (SATI, 2019a).

Seeded berries develop a naturally large berry size compared to seedless grapes as seeds are a natural source of GA_3 (Dokoozlian, 2000). Authors have reported a positive correlation between berry size and seed occurrence (Coombe, 1960; Baydar & Harmankaya, 2005). Seedless cultivars are treated with an exogenous GA_3 application to increase berry size due to the lack of natural occurring GAs normally produced by seeds (Dokoozlian, 2000).

Certain seedless table grape cultivars, such as Thompson Seedless (Wolf & Loubser, 1992), require a GA_3 treatment, applied after berry set to improve berry size whereas cultivars such as Sunred Seedless have a large natural berry size which requires no berry sizing treatment. A GA_3 application for sizing may be used on cultivars with a large natural berry size to meet the requirements of specific markets (Abu-Zahra & Salameh, 2012). Increased berry size can be achieved by reducing the crop load, girdling the vine or with the use of GA_3 applications, either by a full cover spray application or by the labour-intensive practice of dipping individual bunches (Orth, 1990; Abu-Zahra & Salameh, 2012).

GA_3 applications with direct bud contact, *i.e.* full cover applications, have been associated with a decreased return fertility and increased bud necrosis the following season (Lavee *et al.*, 1981; Orth, 1990; Dokoozlian, 2000), but limited research articles are available on this aspect. Apart from decreased return fertility, additional negative responses with the use of GA_3 have been reported by authors. Examples include a decreased rate in colour accumulation and postharvest berry shatter (Retamales & Cooper, 1993; Zoffoli *et al.*, 2009).

Taking into account all available research results and practical experience referred to above, this study evolved around the following facets: to determine whether GA_{4+7} could be used as an alternative to GA_3 for berry thinning, berry sizing and reducing rudimentary seed occurrence in Sunred Seedless without negatively affecting return fertility. The field trial was conducted in the Hex River Valley during the 2015/2016 and 2016/2017 growing seasons.

1.2 PROJECT AIMS AND OBJECTIVES

1.2.1 Aims

The study aimed to determine whether GA_{4+7} could be used as an alternative chemical thinning agent for cultivars that respond poorly to a GA_3 treatment in order to improve quality without negatively affecting return fertility.

Main aim: Establish the most effective phenological stage to apply a minimum concentration of GA_{4+7} for effective thinning of table grapes.

Sub aim: Establish the effect of two gibberellin chemical structures, GA_3 and GA_{4+7}, applied on table grapes for berry thinning on bunch structure, rudimentary seed size and return fertility.

1.2.2 Objectives

Objective 1: Identify GA_{4+7} treatments for the effective thinning of table grapes (Sunred Seedless), compared to the standard GA_3 treatment, by:

- Establishing the most effective phenological stage to apply GA_{4+7}.
- Establishing the minimum GA_{4+7} concentration required for effective thinning results.

Objective 2: Compare the effect of different GA_3 and GA_{4+7} treatments applied at different phenological stages of Sunred Seedless on bunch structure, rudimentary seed size and return fertility.

The expected benefits of this study for the table grape industry:

- Reduce production costs by reducing manual thinning and manual bunch preparation.
- Contribute to meeting export requirements regarding seedlessness by promoting the development of very small, soft and undetectable rudimentary seeds.
- Obtain scientific results regarding the effect of GA_{4+7} on table grapes.
- Obtain scientific results regarding the effect of GA_3 and GA_{4+7} on the fertility of table grapes.

1.3 LITERATURE CITED

Abu-Zahra, T.R. & Salameh, N.M., 2012. Influence of Gibberellic acid and cane girdling on berry size of Black Magic grape cultivar. Middle-East J. Sci. Res. 11, 718-722.

Avenant, J.H., 2000. Effect of different cultivation practices on seed trace size of Sunred Seedless-preliminary results. Dec. Fruit Grow. 50, 16.

Baydar, N.G. & Harmankaya, N., 2005. Changes in endogenous hormone levels during the ripening of grape cultivars having different berry set mechanisms. Turk. J. Agric. For. 29, 205-210.

Burger, P., Gerber, C., Gerber, A. & Ellis, P.J.L., 2003. Breeding seedless grapes in South Africa by means of embryo rescue. Acta Hortic. 603, 565-569.

Cahoon, G. A., Kaps, M. L., & Pathak, S. P., 1986. Effects of gibberellic acid (GA_3) and daminozide (Alar) on growth and fruiting of Himrod grapes. Ohio Agric. Res. Develop Center Res. Circular. 29, 30-41.

Christodoulou, A., Pool, R. & Weaver, R., 1966. Prebloom thinning of Thompson Seedless grapes is feasible when followed by bloom spraying with gibberellin. Calif. Agric. 20, 8-10.

Coombe, B.G., 1960. Relationship of growth and development to changes in sugars, auxins, and gibberellins in fruit of seeded and seedless varieties of *Vitis vinifera*. Plant Physiol. 35, 241-250.

Di Lorenzo, R., Gambino, C. & Scafidi, P., 2011. Summer pruning in table grape. Adv. Hortic. Sci. 25, 143-150.

Dokoozlian, N.K., 2000. Grape berry growth and development. Raisin Production Manual. pp. 30-37.

Dokoozlian, N.K. & Peacock, W.L., 2001. Gibberellic acid applied at bloom reduces fruit set and improves size of “Crimson Seedless” table grapes. Hort. Sci. 36, 706-709.

Durner, E.F., 2013. Principles of Horticultural Physiology. CABI, Boston.

Fosket, D.E., 1994. Plant growth and development: a molecular approach. Academic Press Inc. San Diego, California.

Gil, G.F., Rivera, M., Veras, F. & Zoffoli, J.P., 1994. Effectiveness and mode of action of Gibberellic acid on grape berry thinning. In: Int. Symp. Table Grape Prod. Anaheim, California, pp. 43–46.

Iland, P., Dry, P., Proffitt, T. & Tyerman, S.D., 2011. The grapevine: from the science to the practice of growing vines for wine. Patrick Iland Wine Promotions Pty Ltd, Adelaide, South Australia.

Korkutal, I., Bahar, E. & Gökhan, Ö., 2008. The characteristics of substances regulating growth and development of plants and the utilization of Gibberellic Acid (GA_3) in viticulture. World J. Agric. Sci. 4, 321-325.

Lavee, S., Melamud, H., Ziv, M. & Bernstein, Z., 1981. Necrosis in grapevine buds (*Vitis vinifera* cv. Queen of Vineyard) I. Relation to vegetative vigor. Vitis 20, 8-14.

Lynn, C.D. & Jensen, F.L., 1966. Thinning effects of bloom time Gibberellin sprays on Thompson Seedless table grapes. Am. J. Enol. Vitic. 17, 283-289.

Molitor, D., Behr, M., Hoffmann, L. & Evers, D., 2012. Research note: Benefits and drawbacks of pre-bloom applications of gibberellic acid (GA_3) for stem elongation in Sauvignon blanc. S. Afr. J. Enol. Vitic. 33, 198-202.

Mullins, M.G., Bouquet, A. & Williams, L.E., 1992. Biology of Horticultural crops: Biology of the grapevine. Cambridge University Press, Cambridge.

Nitsch, J.P., Pratt, C., Nitsch, C. & Shaulis, N.J., 1960. Natural growth substances in Concord and Concord seedless grapes in relation to berry development. Am. J. Bot. 47, 566-576.

Orth, C.H.F., 1990. Effect of spraying or dipping with gibberellic acid on bud fertility of Muscat Seedless. Dec. Fruit Grow. 40(8), 289-292.

Pallardy, S.G, 2008 (3rd ed). Physiology of Woody Plants. Academic Press, London.

Perl, A., Sahar, N. & Spiegel-Roy, P., 2000. Conventional and biotechnological approaches in breeding seedless table grapes. Acta Hortic. 528, 607-612.

Rademacher, W., 2015. Plant growth regulators: Backgrounds and uses in plant production. J. Plant Growth Regul. 34, 845-872.

Retamales, J. & Cooper, T., 1993. Berry drop and fruit removal forces as related with GA_3 applications in table grapes. Acta Hortic. 329, 81-83.

Reynolds A.G. & de Savigny C., 2004. Influence of girdling and gibberellic acid on yield components, fruit composition, and vestigial seed formation of 'Sovereign Coronation' table grapes. Hort. Sci. 39, 541-544.

Reynolds A.G., Roller J.N., Forgione A. & de Savigny C., 2006. Gibberellic acid and basal leaf removal: Implications for fruit maturity, vestigial seed development, and sensory attributes of Sovereign Coronation table grapes. Am. J. Enol. Vitic. 57, 41-53.

Roberts, J.A. & Hooley, R., 1988. Plant Growth Regulators. Chapman and Hall, New York.

Roubelakis-Angelakis, K.A., 2009. Grapevine Molecular Physiology & Biotechnology. Springer, Dordrecht.

SATI., 2016. Statistics Booklet 2016. Lombardt, J. (ed). South African Table Grape Industry. South African Table Grape Industry, PO Box 2932, Paarl, 7620.

SATI., 2017. Statistics Booklet 2017. Lombardt, J. (ed). South African Table Grape Industry. South African Table Grape Industry, PO Box 2932, Paarl, 7620.

SATI., 2019a. Guidelines for the preparation of table grapes for export 2019/20. Van der Merwe, G.G. (ed). South African Table Grape Industry, PO Box 2932, Paarl, 7620.

SATI., 2019b. Statistics Booklet 2019. Lombardt, J. (ed). South African Table Grape Industry. South African Table Grape Industry, PO Box 2932, Paarl, 7620.

Stout, A., 1936. Seedlessness in grapes. NY Agr. Exp. Stn. Tech. Bull. 238.

Ungsa, M., Kato K., Takemura, K., Hori, T., Ohara, H., Ohkawa, K., Matsui, H. & Bukovac, M.J., 2008. Effects of the combination of gibberellic acid and ammonium nitrate on the growth and quality of seedless berries in 'Delaware' grape. J. Jpn. Soc. Hortic. Sci. 72, 366-371.

Weaver, R.J & McCune, B., 1960. Further studies with gibberellin on *Vitis vinifera* grapes. Bot. Gaz. 121, 155-162.

Weaver, R.J. & Pool, R.M., 1971. Chemical thinning of grape clusters (*Vitis vinifera* L.). Vitis 10, 201-209.

Winkler, A.J., Cook, J.A., Kliewer, W.M. & Lider, L.A., 1962. General Viticulture. University of California Press, California.

Wolf, E.E.H. & Loubser, J.T., 1992. Gibberellic acid levels and quality effects of Gibberellic acid in treated table grapes. S. Afr. J. Enol. Vitic. 13, 57-63.

Zoffoli, J.P., Latorre, B.A. & Naranjo, P., 2009. Preharvest applications of growth regulators and their effect on postharvest quality of table grapes during cold storage. Postharvest Biol. Technol. 51, 183-192.

Chapter 2

Literature review

A review of bunch structure, rudimentary seed size and return fertility of table grapes as affected by GA_3 and GA_{4+7} treatments

CHAPTER 2:

A review of bunch structure, rudimentary seed size and return fertility of table grapes as affected by GA_3 and GA_{4+7} treatments

2.1 INTRODUCTION

The quality of table grapes, non-climacteric fruit, is determined by various attributes, such as their visual appearance and nutritional value. The visual appearance of table grapes, such as berry shape and size, colour uniformity, rachis colour, as well as bunch shape, size and compactness (Wei *et al.*, 2002; Reisch *et al.*, 2012; Dragincic *et al.*, 2015; Zhou *et al.*, 2015; Piazzolla *et al.*, 2016), largely influence the table grape consumers' first impression and their desire to purchase the fresh product.

A substantial shift in the international consumer preference from seeded to seedless berries has been observed (Perl *et al.*, 2000), resulting in the higher market potential for seedless grapes (Varoquaux *et al.,* 2000). Due to the higher export market potential of seedless grapes, 91% of table grapes produced in South Africa are seedless (SATI, 2019b).

During the evaluation of cultivars and/ or selections, grapes are regarded seedless when rudimentary seeds are soft green and not perceptible organoleptically (Burger *et al.*, 2003). Seedless grapes with detectable rudimentary seeds are viewed by consumers as a negative characteristic, decreasing the marketability of these grapes. Manipulations that could contribute to decreasing rudimentary seed size in cultivars with detectable rudimentary seeds is therefore essential from a marketing perspective.

The use of plant growth regulators (PGRs) has become an essential tool in improving the quality parameters of table grapes in order to meet export market requirements. Gibberellic acid (GA_3) is the most widely used PGR in table grape production and is used mainly on seedless cultivars for stretching, berry thinning and berry sizing (Weaver & McCune, 1960; Cahoon *et al.*, 1986; Reynolds & de Savigny, 2004; Reynolds *et al.*, 2006; Roubelakis-Angelakis, 2009). An additional, but less common use for GA_3 is reducing rudimentary seed occurrence. Full cover applications of GA_3 have been associated with a decreased return fertility and increased bud necrosis the following season (Lavee *et al.*, 1981; Orth, 1990; Dokoozlian, 2000b), but there is limited research information available on this aspect. Manipulations, with the use of an alternative gibberellic acid structure, GA_{4+7}, on the attributes mentioned above could be a viable alternative to GA_3, is also reviewed in this Chapter. GA_{4+7} is currently used for calyx end russeting in apples, with limited information available on its use in table grapes.

In table grape production, manipulations with the use of PRGs have to be cost-effective without negatively influencing grapevine fertility. The use of GAs to improve bunch structure and seedlessness in table grapes, as well as their impact on grapevine fertility, are discussed in this Chapter.

2.2 GRAPEVINE BUD MORPHOLOGY AND PHENOLOGY

2.2.1 Grapevine bud anatomy

Grapevine bud development has been described in detail by Winkler *et al.* (1962), Khanduja and Balasubrahmanyam (1972), Pongracz (1978), Srinivasan and Mullins (1981b), Mullins (1986), May (2000), Williams (2000), Bennett (2002), Vasconcelos *et al.* (2009) and Iland *et al.* (2011).

An axillary bud complex consisting of a lateral or prompt bud (situated at the dorsal side of the shoot) and a compound bud (eye) consisting of three latent buds (situated at the ventral side of the shoot) can potentially develop at every shoot node (Morrison, 1991; Boss *et al.*, 2003; Carmona *et al.,* 2008; Vasconcelos *et al.*, 2009). The compound bud contains three latent buds, namely a primary as well as two secondary buds that will remain dormant until the required number of cold units have been met during the winter (Winkler *et al.*, 1962; Khanduja & Balasubrahmanyam, 1972; Morrison, 1991; Williams, 2000; Bennett, 2002). If all latent buds contain inflorescence primordia, a compound bud may contain up to three inflorescence primordia (Williams, 2000; Bennett, 2002; Iland *et al.*, 2011).

Under normal conditions, the more developed bud, *i.e.* the primary bud, will burst in spring but if it is damaged in any way one of the less developed secondary buds will burst (Khanduja & Balasubrahmanyam, 1972; Morrison, 1991; Bennett, 2002; Vasconcelos *et al.*, 2009; Iland *et al.*, 2011) (Fig. 2.1). These buds contain primordia (precursors) that can differentiate into one of two types of primordia, leaf primordia for infertile buds and inflorescence primordia for fertile buds (Khanduja & Balasubrahmanyam, 1972; Morrison, 1991; Bennett, 2002). Fertile bud positions differ between cultivars, serving as a guide in determining the cultivar's correct pruning method. The cultivar Sunred Seedless, for instance, has higher bud fertility towards the base of the cane and can therefore be spur pruned (SATI, 2019a).

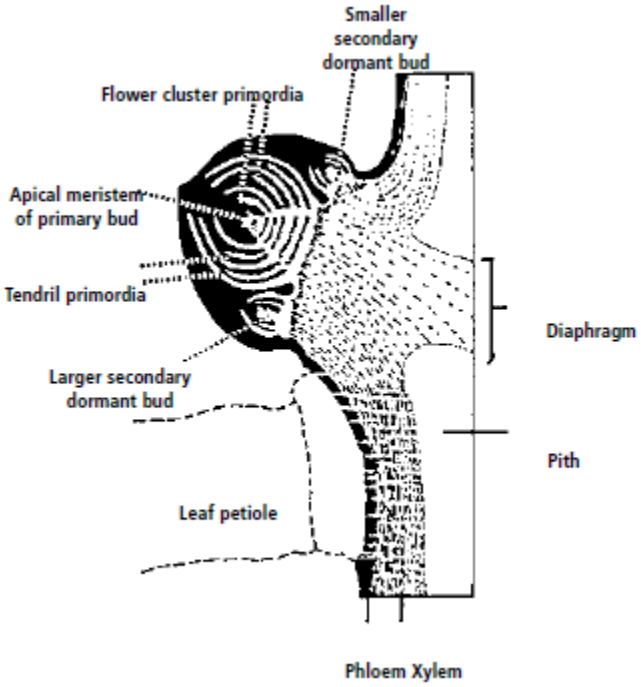

Figure 2.1: Cross-section of a compound bud, indicating a primary bud and two secondary buds. The primary bud contains leaf, tendril and cluster primordia (Williams, 2000).

2.2.2 Vegetative growth cycle

The vegetative growth cycle has been described in detail by several authors (Winkler *et al.*, 1962; Pongracz, 1978; Mullins *et al.*, 1992; Bennett, 2002; Iland *et al.*, 2011; Keller, 2015). Bud break is defined as the visibility of a green tip or leaf tissue, as described by the modified E-L (Eichhorn & Lorenz) system for grapevine phenology (Bennett, 2002). Initial growth relies on reserves, such as carbohydrates, stored in the permanent structure (roots, canes & trunk) of the vine until sufficient photosynthates can be produced to maintain a balanced sink-source relationship (Mullins *et al.*, 1992; McArtney, 1998). Leaves reach net photosynthate production once they have reached half of their final size (Bennett, 2002). During the post-harvest period, the remaining green leaves are responsible for accumulating reserves that are stored in the permanent structure of the grapevine, before entering endodormancy (Winkler *et al.*, 1962). Grapevine roots are one of the primary storage organs of nutrient reserves which promote initial growth during spring (Archer, 1981).

In grapevines, the development of new roots takes place during two periods of root growth, referred to as root flushes. New root growth is vital for water and nutrient uptake as well as the production of hormones, such as cytokinin's (Mullins *et al.*, 1992), which are linked to the differentiation of inflorescence primordia, along with auxin (Keller, 2015). The first root flush starts in spring after bud break, reaching its peak at flowering, with the second root flush occurring after harvest (Mullins *et al.*, 1992).

2.2.3 Reproductive growth cycle

The reproductive growth cycle of a grapevine occurs over a period of two consecutive seasons. Inflorescence primordia differentiation takes place during late spring and summer of the first season before the grapevine enters a period of dormancy, which is followed by bud break, flower and berry development during the second season (Dunn & Martin, 2000; Williams, 2000; Carmona *et al.*, 2008).

Inflorescence primordia formation coincides with flowering occurring in the current season (Winkler & Shemsettin, 1937; Morrison, 1991; Iland *et al.*, 2011). During the first season, the compound bud can differentiate into either a fertile bud containing inflorescence primordia with rudimentary leaves and flower clusters or an infertile bud, producing a shoot with leaves and tendrils (Khanduja & Balasubrahmanyam, 1972; Morrison, 1991; Williams, 2000; Bennett, 2002).

According to Dunn and Martin (2000) and Williams (2000), the formation of clusters through the differentiation of inflorescence primordia during the first season will determine the yield potential of the second season (Fig. 2.2). The period from initiation of inflorescence primordia until harvest is approximately 15 months, depending on factors such as cultivar and region (Bennet, 2002; Iland *et al.*, 2011).

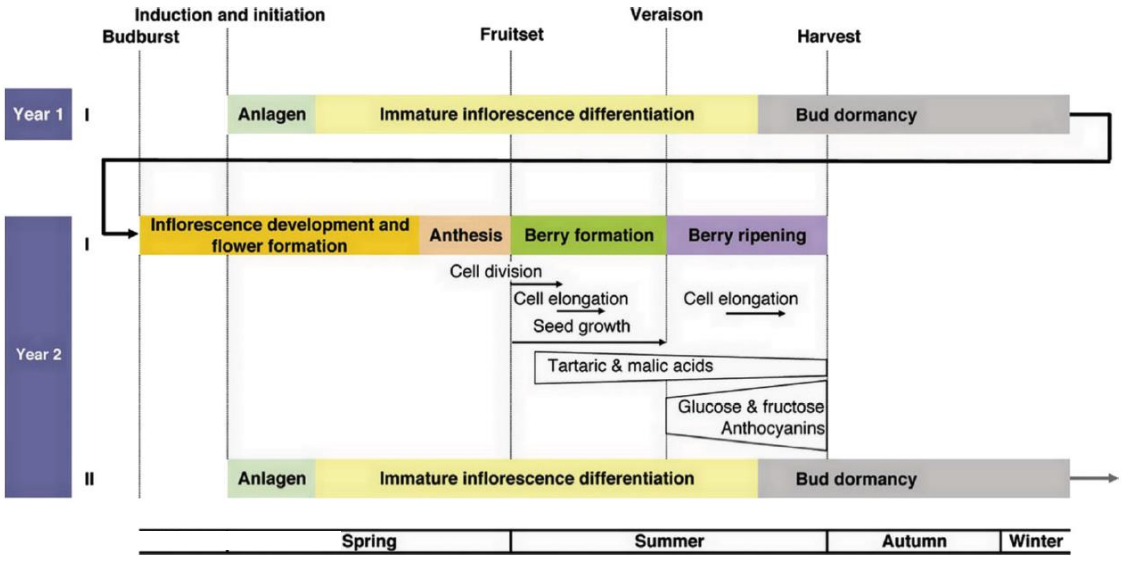

Figure 2.2: The phenological timeline of the grapevine, indicating the reproductive development growth cycle occurring over two seasons (from Li-Mallet *et al.* (2016) which was reproduced from Coombe and Iland (2004) and thereafter Carmona *et al.* (2008)).

2.2.3.1 Inflorescence formation

Inflorescence formation can be divided into three main processes:

i. Anlagen formation

Leaf primordia are formed during a short period of vegetative growth, followed by reproductive growth, resulting in the formation of the first lateral meristem (Srinivasan & Mullins, 1981b; Swanepoel & Archer, 1988; Mullins *et al.*, 1992; Williams, 2000). The lateral meristem, also known as anlagen or uncommitted primordia, is formed by the shoot apical meristem of latent buds (Tucker & Hoefert, 1968; Gerrath & Posluszny, 1988; Vasconcelos *et al.*, 2009). Structural differences between anlagen and leaf primordia are visible with anlagen forming shorter, club-shaped structures (Vasconcelos *et al.*, 2009). The process of leaf primordia and anlagen formation are repeated to form between one to three anlagen, depending on the cultivar and environmental factors (Srinivasan & Mullins, 1976; Srinivasan & Mullins, 1981b; Vasconcelos *et al.*, 2009).

Rapid shoot growth favours tendril formation but depending on conditions, anlagen can mature into either inflorescence, a tendril or an intermediate structure (Boss & Thomas, 2002; Boss *et al.*, 2003, Vasconcelos *et al.*, 2009). The timing and rate of anlagen formation are influenced by the cultivar as well as the position of the winter bud on the cane (Mullins, 1986; Watt *et al.*, 2008; Vasconcelos *et al.*, 2009).

ii. Inflorescence primordia formation

During further development, the anlagen branches into two arms, a larger inner and smaller outer arm. Inflorescence primordia are formed by the differentiation of the branched anlagen (Srinivasan & Mullins, 1981b; Swanepoel & Archer, 1988; Mullins *et al.*, 1992; Williams, 2000; Iland *et al.*, 2011). Differentiation of the inner arm contributes to the formation of the inflorescences' main body, whereas the outer arm contributes to a winged branch at the top of the inflorescence (Mullins, 1986; Mullins *et al.*, 1992; Vasconcelos *et al.*, 2009; Iland *et al.*, 2011).

The first inflorescence primordia are formed two to three weeks after the first anlagen formation. A steady decrease in the acropetal branching of the inner arm contributes to the conical shape of the inflorescence primordia, resembling a small bunch of grapes. The completion of this phase is marked four days after the appearance of the fully developed inflorescence (Srinivasan & Mullins, 1981b; Swanepoel & Archer, 1988; Mullins *et al.*, 1992; Williams, 2000; Bennett, 2002; Iland *et al.*, 2011). The initiation of the second anlagen commences during the last few days of the differentiation of the first anlagen (Swanepoel & Archer, 1988).

After the formation of inflorescence primordia, the latent bud enters dormancy (Winkler & Shemsettin, 1937; Srinivasan & Mullins, 1981b; Mullins *et al.*, 1992; Sommer *et al.*, 2000; Vasconcelos *et al.*, 2009; Iland *et al.*, 2011). Shoots turning from green to a yellow-brown colour can be used as an indication of the development of dormancy, developing over two to three weeks (Vasconcelos *et al.*, 2009).

Environmental factors during differentiation contribute to grapevine fertility, as differentiation during the first season determines the second season's bud fertility potential (Khanduja & Balasubrahmanyam, 1972*).* Cultivation practices during the first season, such as the application of PGRs and canopy management, have also been reported to affect fertility in the second season (Khanduja & Balasubrahmanyam, 1972; Mullins *et al.*, 1992; Dry, 2000; Williams, 2000; Iland *et al.*, 2011). Factors affecting grapevine fertility are discussed in detail in Section 2.5.1.

iii. *Final differentiation of the inflorescences*

Final differentiation of the inflorescence/ individual flowers takes place from shortly before bud break until flowering in the second season (Srinivasan & Mullins, 1981b; Swanepoel & Archer, 1988; Morrison, 1991; Mullins *et al.*, 1992; Dunn & Martin, 2000; Sommer *et al.*, 2000; Williams, 2000; Iland *et al.*, 2011).

2.2.3.2 Flower development

Flowering, pollination and fertilization

The process of flowering indicates the commencement of inflorescence primordia initiation for the following season and the end of inflorescence development for the current season (Coombe & Dry, 1988; Bennett, 2002). Flowering, also referred to as bloom or anthesis, occurs during spring (6-8 weeks after bud break) when the calyptra separates to reveal the stamens (male organs) and pistil (female organ) (Dokoozlian, 2000b; Bennett, 2002; Iland *et al.*, 2011). The function of the calyptra is to protect these organs before flowering. The rate at which flowering occurs increases under favourable conditions of 29-35°C, but decreases when temperatures are below 18.5°C (Dokoozlian, 2000b). Full flowering or 100% flowering is reached once all the calyptras of the flowers on the cluster have separated from the base of flowers. Berry shattering, a natural thinning process where flowers drop to the ground, occurs 8-12 days after full flowering (Dokoozlian, 2000b).

Pollination occurs when pollen released by the anthers lands on the stigma. Germinating pollen develops a pollen tube that connects to the ovary, enabling the sperm to travel down the tube and fertilize the eggs (Dokoozlian, 2000b). Favourable environmental conditions range from 26.7-32.2°C and vice versa for conditions below 15.6°C or above 37.8°C (Dokoozlian, 2000b).

2.2.3.3 Fruit set and seed development

Fruit set indicates the start of fruit development and is characterized by two occurrences, namely the completion of berry shattering and grape berries achieving diameters of 1.6 to 3.2 mm (Winkler *et al.*, 1962; Pongracz, 1978; Dokoozlian, 2000b; Iland *et al.*, 2011). During fruit set, active cell division promotes the development of ovaries into berries (Mullins *et al.*, 1992; Dokoozlian, 2000a; Bennett, 2002; Bangerth, 2004; Iland *et al.*, 2011). Fruit set is influenced by various factors, including carbohydrates (Weaver & McCune, 1960), temperature (Pongracz, 1978; Bennett, 2002) and PGRs such as auxins, cytokinins and gibberellins (Weaver *et al.*, 1962; Srinivasan & Mullins, 1981b; Mullins *et al.*, 1992; Bennett, 2002).

In grapes, seedless berries can develop through two different fruit set mechanisms, parthenocarpy or stenospermocarpy (Stout, 1936; Dokoozlian, 2000b). In parthenocarpy, fruit set is followed by pollination, resulting in the production of seedless berries in the absence of ovule fertilization (Stout, 1936). Berries of parthenocarpic fruit set are smaller in size and therefore more suitable for raisin production. True seedless berries are produced through parthenocarpy and an example of such a cultivar is Black Corinth (Dokoozlian, 2000b).

In stenospermocarpy, normal fruit set occurs up to fertilization, followed by abortion of the zygotic embryo two to four weeks after fertilization, thereby terminating further seed development (Stout, 1936; Coombe, 1960; Nitsch *et al.*, 1960; Winkler *et al.*, 1962; Mullins *et al.*, 1992; Dokoozlian, 2000b; Perl *et al.*, 2000; Reynolds *et al.*, 2006; Iland *et al.*, 2011). The result is berries with rarely detectable, slender and soft seeds referred to as seed traces or rudimentary seeds. Berries produced by stenospermocarpy are commercially considered as seedless and this includes cultivars such as Sunred Seedless, Prime Seedless, Flame Seedless and Thompson Seedless (Dokoozlian, 2000b). Berry size manipulation practices often applied on stenospermocarpic cultivars include girdling, exogenous GA applications and/ or manual thinning.

Seedless grapes have a higher market potential due to consumer preferences (Varoquaux *et al.,* 2000), therefore decreasing rudimentary seed size in cultivars with noticeable rudimentary seeds is an essential manipulation from a marketing perspective.

2.2.3.4 Rudimentary seeds

Changes in the physiological conditions during the early stages of seed development have been found to result in embryo abortion, due to increased phytohormone levels around flowering (Coombe, 1960; Nitsch *et al.*, 1960; Iwahori *et al.*, 1968).

Temperatures below average during flowering and early fruit development of stenospermocarpic cultivars could delay embryo abortion and result in an increased noticeability of rudimentary seeds (Dokoozlian, 2000b). Temperature contributes to the size of rudimentary seeds as found by Avenant (2000) who reported larger rudimentary seeds for Sunred Seedless produced in the earlier, warmer production region of Upington compared to Sunred Seedless produced in the late, colder production region of Stellenbosch. The average rudimentary seed size of Sunred Seedless is 9.4 mg, but it can be as large as 22.1 mg compared to that of Sultanina which can vary between 5.3 mg and 6.7 mg per rudimentary seed (Avenant, 2000).

Seasonal differences in the number, as well as mass of rudimentary seeds, have been reported by Reynolds and de Savigny (2004). During two consecutive seasons with lower rainfall, there was a higher average rudimentary seed number per berry (1.88 & 1.10) and mass (24.4 mg & 15.5 mg) of control treatments compared to the rudimentary seed number (0.12) and mass (0.9 mg) during a higher rainfall season (Reynolds & de Savigny, 2004). Similar results were reported by Reynolds *et al.* (2006).

Limited published research results regarding the number of rudimentary seeds per berry and rudimentary seed mass are available. Results of a study performed by Christensen *et al.* (1983) on rudimentary seed development in Fiesta is given in Table 2.1. Besides seasonal conditions, the choice in rootstock also influences rudimentary seed size as different rootstocks contribute to different levels in hormone production (Christensen *et al.*, 1983). Another factor includes the age of the vineyard and older vines may produce less rudimentary seeds (Christensen *et al.*, 1983; Dokoozlian, 2000b).

Table 2.1: The effect of rootstock and seasonal conditions on rudimentary seed development in 'Fiesta' raisins (from Christensen *et al*. (1983)).

Trial Location	Rootstock	Number of Traces per Berry				Total Weight of Traces per Berry Mg Dry Wt.			Average Weight per Trace Mg Dry Wt.		
		1977	1978	1979	1981	1978	1979	1981	1978	1979	1981
Fowler	Own	1.48 a*	1.67 ab	1.55 a	2.32 a	3.27 b	7.15 b	4.08 b	1.96 b	4.61 b	1.83 b
	Thompson	1.69 a	1.95 b	2.20 a	2.43 a	2.28 a	2.97 a	3.58 a	1.16 a	1.35 a	1.43 a
	Harmony	1.85 a	1.32 a	2.00 a	2.07 a	3.83 b	6.15 b	4.94 bc	2.90 b	3.08 b	2.34 b
	Freedom	1.88 a	1.51 a	1.77 a	2.46 a	3.72 b	6.33 b	6.31 c	2.46 b	3.58 b	2.59 b
Biola	Own	1.89 a	2.28 a	2.08 a	2.51 a	6.92 b	5.25 b	4.66 b	3.04 b	2.52 b	1.91 b
	Thompson	2.09 a	1.97 a	1.68 a	2.37 a	4.16 a	1.81 a	2.19 a	2.11 a	1.08 a	0.95 a
	Harmony	1.81 a	2.29 a	1.92 a	2.31 a	8.27 b	5.87 b	5.32 b	3.61 b	3.06 b	2.31 b
	Freedom	2.01 a	2.04 a	1.91 a	2.64 a	7.08 b	5.18 b	6.05 b	3.47 b	2.71 b	2.32 b
Rolinda	Own	1.93 a	1.86 bc	1.45 a	1.74 a	4.11 a	3.62 a	2.56 b	2.21 a	2.50 a	1.46 b
	Thompson	2.10 a	1.51 a	1.47 a	1.95 a	3.60 a	2.74 a	1.92 a	2.38 a	1.86 a	0.89 a
	Harmony	1.83 a	1.57 ab	1.72 a	1.95 a	4.86 ab	5.97 b	4.17 c	3.10 b	3.47 b	2.15 c
	Freedom	1.81 a	1.98 c	1.79 a	1.86 a	6.02 b	6.52 b	5.37 c	3.04 b	3.64 b	2.90 d

Role of plant hormones in seeded vs seedless cultivars

The size of stenospermocarpic berries are smaller than that of seeded grape berries but can be increased through genetic selection or exogenous GA applications (Weaver, 1958; Mavrikios, 1977 as cited by Bouquet & Danglot, 1996). GAs contribute to the growth and development of seedless grapes, setting naturally as well as parthenocarpically with GA applied around flowering (Crane, 1964; Matsiu *et al.*, 1986).

A positive correlation between berry size and seed occurrence has linked seed size and the number of seeds to the production of hormones such as gibberellins, thereby improving berry size (Coombe, 1960; Lavee & Nir, 1986; Baydar & Harmankaya, 2005). High endogenous GA levels in seeded cultivars contribute to the development of a naturally large berry (Lavee, 1960; Kato *et al.*, 1998; Agüero *et al.*, 2000; Perez *et al.*, 2000). Low endogenous GA levels, present in the rudimentary seeds of stenospermocarpic cultivars, contribute to the development of smaller berries (Iwahori *et al.*, 1968; Cheng *et al.*, 2013). Two peaks in GA concentration have been observed during seeded berry development, the first of these being during fruit set followed by a second peak five weeks later (Scienza *et al.*, 1978).

Authors have reported an increased occurrence of seedless berries with an GA_3 application 12-17 days before full bloom (DBFB) in Delaware (Sugiura & Inaba, 1966), 14 DBFB in Muscat Bailey (Kimura *et al.*, 1996), 5 days after bloom in Emperador (Agüero *et al.*, 2000), 16 DBFB in Kyoho and 18 DBFB in Red Globe (Cheng *et al.*, 2013). An exogenous GA_3 application restricted seed growth in seeded cultivars such as Red Globe, Kyoho and Emperatriz, whereas increased seed growth was observed for seedless cultivars, such as Thompson Seedless (Kimura *et al.*, 1996; Cheng *et al.*, 2013). A GA_3 application after flowering resulted in decreased rudimentary seed mass and increased berry size of Orlando Seedless (Halbrooks & Mortensen, 1987).

Cheng *et al.*, (2013) reported the timing of embryo abortion in Kyoho, Red Globe and Thompson Seedless to be between 9 and 15 days after full bloom (DAFB), whereas Tang *et al.* (2009) previously reported embryo abortion for Thompson Seedless between 40 and 55 DAFB.

To date, little is known about the mechanism increasing the occurrence of seedless berries with a GA_3 application during flowering. Authors reported a reduction in pollen germination and pollen tube growth observed after an exogenous GA_3 application at or before full flowering, which could be ascribed to the occurrence of pollen tube inhibitors resulting in unfertilized ovules (Motomura & Ito, 1972; Fukunaga & Kurooka, 1987; Kimura *et al.*, 1996; Okamoto & Miura, 2005).

2.2.3.5 Berry development

Berry growth is defined by changes in berry mass, volume or diameter that follow a double-sigmoidal curve resulting from three growth stages (Harris *et al.*, 1968; Coombe & McCarthy, 2000; Dokoozlian, 2000b; Bennett, 2002; Sonnekus, 2015) which are highly dependent on factors such as cultivar (seeded or seedless), cultivation practices and environmental conditions (Coombe, 1973).

i. *Stage I*: starts after flowering and is known as the first period of rapid berry growth (Dokoozlian, 2000b). Berry growth during this stage is mostly due to the contribution of cell enlargement and partially cell division (Winkler *et al.*, 1962; Harris *et al.*, 1968; Mullins *et al.*, 1992; Coombe & McCarthy, 2000; Dokoozlian, 2000b; Bennett, 2002; Conde *et al.*, 2007). The largest contribution of cell division is 5-10 days prior and post-flowering but will continue for up to three weeks after flowering, during which the number of cells in the berry is determined (Winkler *et al.*, 1962; Harris *et al.*, 1968; Mullins *et al.*, 1992; Dokoozlian, 2000b; Iland *et al.*, 2011).

ii. *Stage II*: the period where berry growth starts to slow down and is also known as the lag phase. The lag phase of seedless cultivars is less prominent or even absent compared to seeded cultivars, resulting in a growth curve with less definition between the growth stages (Pratt, 1971; Coombe & Hale, 1973; Raath, 2012; Sonnekus, 2015; Van der Vyver, 2016).

iii. The onset of *Stage III* is characterized by véraison, where berry firmness decreases and berry colour starts to develop (Dokoozlian, 2000b). *Stage III* marks the second period of berry growth and the initiation of fruit ripening. During this stage, berry growth can solely be ascribed to cell enlargement, lasting 6-8 weeks, during which berry shape will be expressed inherent to the cultivar, either as oval, round or long (Winkler *et al.*, 1962; Harris *et al.*, 1968; Robinson & Davies, 2000; Dokoozlian, 2000b). Berry ripening is observed through the simultaneous increase in sugar content and decrease in organic acid content (Harris *et al.*, 1968; Dokoozlian, 2000b).

2.3 BUNCH COMPACTNESS

The density or morphological volume of a bunch, also referred to as bunch compactness, is mainly determined by the length of the rachis, the number of berries and their size (Tello & Ibáñez, 2018). The number of berries per bunch is determined by the flower number per inflorescence as well as the success of fruit set (Carmona *et al.*, 2008) whereas berry size is determined mainly by the growth of the berry in stage I and stage III (Coombe & McCarthy, 2000; Robinson & Davies, 2000), described above in Section 2.2.3.4.

Table grape consumers' first impression is based on the visual appearance of the berries and bunch, with their preferences based on berry shape and size, colour uniformity, rachis colour as well as the

bunch shape, size and compactness (Wei *et al.*, 2002; Reisch *et al.*, 2012; Dragincic *et al.*, 2015; Zhou *et al.*, 2015; Piazzolla *et al.*, 2016). Bunch compactness is, therefore, an important factor determining the marketability of table grapes.

The quality of grapes produced, especially seedless table grapes, can be compromised for cultivars that tend to set too compact bunches, as a reduction in berry size and deformed berries can occur due to less spatial area for berries to develop optimally (Dokoozlian & Peacock, 2001; Molitor *et al.*, 2012b; Domingos *et al.*, 2016; Tello & Ibáñez, 2018). Such bunches have a greater risk of developing bunch rot in favourable conditions (Dokoozlian & Peacock, 2001; Molitor *et al.*, 2012b; Tello & Ibáñez, 2018).

2.3.1 Methods for determining bunch compactness

Bunch compactness can be evaluated through both subjective and objective methods.

2.3.1.1 Subjective methods

Subjective evaluations are based on visual inspections of the bunch appearance, assigning observations to defined categories (Tello & Ibáñez, 2018). Various bunch density descriptors exist based on the visibility of the rachis and berry mobility, which mostly consists of five categories, namely, very loose, loose, medium, compact and very compact (Tello & Ibáñez, 2018). Examples of these classification systems include the OIV (Organisation Internationale de la Vigne et du Vin) descriptor code 204, UPOV (International Union for the Protection of New Varieties of Plants) descriptor 33 and IPGRI (International Plant Genetic Resources Institute, now Biodiversity International) descriptor 6.2.3. Various other visual classification systems have been developed and are given in Table 2.2 in a summary created by Tello and Ibáñez (2018).

In addition to visual bunch evaluations, an indirect measurement of bunch compactness characteristics has been developed based on the flexibility of a bunch (Tello & Ibáñez, 2018). The density index developed by Ipach *et al.* (2005) (as cited by Tello & Ibáñez (2018)) takes the degree to which the bunch stem can be bent and the distance between berries into consideration to form five categories:

i. Very loose – No contact between berries and 90° stem bending is possible
ii. Loose – Contact between berries and 45-90° stem bending is possible
iii. Dense – Flexible berries and 10-45° stem bending is possible
iv. Compact – Berries not flexible and 10° stem bending is possible
v. Very compact – Berries not flexible and no stem bending is possible

Methods determining the space between randomly selected berries on a bunch have also been explored as a method of determining bunch compactness (Zabadal & Dittmer, 1998).

Table 2.2: Visual classification systems used for determining bunch compactness based on predefined categories (from: Tello & Ibáñez (2018)).

No. categories	Categories	Reference
3	1, Very loose; 2, moderately compact; 3, very compact	Kasimatis et al. (1971)
3	1, Very tight; 2, loose; 3, very loose	Miele et al. (1978)
3	1, Very loose; 2, medium loose; 3, very compact	Roberto et al. (2015)
3	1, Compact; 2, moderate; 3, loose	Ristic et al. (2016)
4	1, Very loose; 2, moderately loose; 3, well filled; 4, compact	El-Banna and Weaver (1978)
5	1, Bunches excessively loose; 2, bunches very loose; 3, most desirable degree of looseness; 4, bunches somewhat compact; 5, bunches excessively compact	Christodoulou et al. (1967)
5	1, Excessively loose; 2, loose; 3, moderately loose; 4, slightly loose; 5, tightly compacted	Hopping (1975)
5	1, Ragged; 2, loose; 3: well-filled; 4, compact; 5, very compact	Firoozabady and Olmo (1987)
5	1, Very loose; 3, loose; 5, medium; 7, dense; 9: very dense	Organisation Internationale de la Vigne et du Vin (2007)
6	1, Rigid, unable to move berries on bunch; 2, some movement of berries; 3, able to manually separate berries; 4, loose, occasional berries not touching others; 5, uniformly loose with many berries not touching each other, able to see some gaps through the bunch; 6, large holes or gaps visible in the bunch	Zabadal and Dittmer (1998)

2.3.1.2 Objective methods

Objective measurements are based on measurable variables of the bunch. The most common objective method used to determine bunch compactness is to divide the number of berries per bunch by the length of the rachis (Vail & Marois, 1991; Pommer *et al.*, 1996; Hed *et al.*, 2009; Hed *et al.*, 2011; Bavaresco *et al.*, 2010; Sabbatini & Howell, 2010; Abd El-Razek *et al.*, 2011; Palliotti *et al.*, 2011; Palliotti *et al.*, 2012; Kotseridis *et al.*, 2012). To ensure a more time-efficient calculation of bunch compactness, authors have adapted this method by measuring only a few laterals of the bunch and determining the number of berries per centimetre of the rachis (Christodoulou *et al.*,1967; Dokoozlian & Peacock, 2001).

The use of automated systems, such as image analysis techniques for calculating bunch compactness have been explored by authors over the past few years, allowing for fast and accurate calculations (Kicherer *et al.*, 2014; Cubero *et al.*, 2015; Ivorra *et al.*, 2015; Schöler & Steinhage, 2015; Tello *et al.*, 2016).

2.3.2 Practices for manipulating bunch compactness

2.3.2.1 Viticultural practices

Studies and cultivar production guidelines have shown the use of cultural practices to improve bunch compactness, such as tipping or topping, shortening of bunches (before or during flowering) or leaf removal before flowering or at full-flowering (Molitor *et al.,* 2011a; Tello & Ibáñez, 2018; SATI, 2019a). Leaf removal during these periods has an impact on fruit set as decreased leaf photosynthesis results in a reduced amount of photoassimilates available for developing inflorescences. This promotes flower drop and therefore the number of berries per bunch is reduced (Lebon *et al.*, 2008; Vaillant-Gaveau *et al.*, 2011; Tello & Ibáñez, 2018). Leaf removal at full-flowering and two weeks after full-flowering is

recommended for improving bunch compactness (Candolfi-Vasconcelos & Koblet, 1990). Additional viticultural practices that have been studied to improve bunch compactness include vine shading (Domingos *et al.*, 2015), brushing off flowers (Abdel-Fattah *et al.*, 2010), bunch thinning as well as sectional bunch or berry thinning (Tardáguila *et al.*, 2008; Molitor *et al.*, 2012b; Gatti *et al.*, 2015; Roberto *et al.*, 2015), crop load adjustments, rootstock selection and the use of different pruning systems (Ferreira & Marais, 1987; Zabadal & Dittmer, 1998; Weyand & Schultz, 2006; Archer & van Schalkwyk, 2007).

2.3.2.2 Chemical control

Various PRGs such as forchlorfenuron (CPPU), GA and prohexadione-calcium have been studied to evaluate their effect on bunch structure (Table 2.3), with GA most commonly used and commercially applied in table grape production. Experimental applications of PGRs not yet registered in South Africa, such as cytokinin and auxin containing products, are also being evaluated to improve set.

Early studies indicated that the improvement of bunch compactness with the use of GA was due to the elongation of the rachis (Weaver & McCune, 1962, Miele *et al.*, 1978). Improved bunch compactness can be observed with GA applied at full flowering due to a reduction in the fruit set rate, resulting in a reduced number of berries per bunch (Dokoozlian & Peacock, 2001). Applying GAs after flowering promotes bunch compactness by improving berry size, a common practice used in commercial table grape production for improving the marketability of the grapes (Zabadal & Dittmer, 2000; Casanova *et al.*, 2009).

The efficacy of a GA treatment depends on various factors, such as phenological stage at time of application, cultivar, application rate and number, as well as environmental conditions (Hopping, 1976; Casanova *et al.*, 2009; Hed *et al.*, 2011). The use of GAs for bunch elongation and berry thinning is discussed further in more detail in Sections 2.4.5.1 and 2.4.5.2.

The use of prohexadione-calcium at full-flowering inhibits the active GA biosynthesis, disturbing the balance between active (GA_1) and inactive (GA_{20}) GAs, resulting in increased flower and berry abscission (Molitor *et al.*, 2011b). In contrast to GAs and prohexadione-calcium, the use of CPPU increases bunch compactness by promoting fruit set and berry size (Table 2.3) (Zabadal & Bukovac, 2006).

Table 2.3: The effect of forchlorfenuron, ethephon, gibberellins and prohexadione-calcium applications on bunch compactness and architecture (from: Tello & Ibáñez (2018)).

Treatment	Cultivar	Application timing	Effect on bunch compactness†	Effect on bunch architecture	Reference
CPPU (5–15 mg/L)	Concord	B4	None	No effect	Zabadal and Bukovac (2006)
CPPU (5–15 mg/L)	Himrod	B4	↑	↑ Berry size	Zabadal and Bukovac (2006)
CPPU (5–15 mg/L)	Lakemont Seedless	B4	None	No effect	Zabadal and Bukovac (2006)
CPPU (5–15 mg/L)	Niagara	B4	None	No effect	Zabadal and Bukovac (2006)
CPPU (5–15 mg/L)	Vanessa	B4	↑	↑ Berry size, ↑ Berry number	Zabadal and Bukovac (2006)
Ethephon (100–500 mg/L)	Thompson Seedless	V	None	No effect	El-Banna and Weaver (1978)
Gibberellins (1–5 mg/L)	Carignane	PrF	↓	Not reported	Weaver et al. (1962)
Gibberellins (Gibb3, 10 mg/L)	Sauvignon Blanc	PrF	↓	↑ Bunch length	Molitor et al. (2012a)
Gibberellins	Thompson Seedless	PrF	↓	↑ Bunch length	Weaver and McCune (1962)
Gibberellins (1–5 mg/L)	Tinta Madeira	PrF	↓	Not reported	Weaver et al. (1962)
Gibberellins (1–5 mg/L)	Zinfandel	PrF	↓	Not reported	Weaver et al. (1962)
Gibberellins (5–50 mg/L)	Zinfandel	PrF	↓	↓ Fruitset, ↓ Berry size, ↑ Bunch length, ↑ Pedicel length	Miele et al. (1978)
Gibberellins (Pro-Gibb, 5–25 mg/L)	Chardonnay	F	↓	↓ Fruitset	Hed et al. (2011)
Gibberellins (Pro-Gibb, 10–25 mg/L)	Chardonnay	F	↓	↓ Berry number	Hed et al. (2015)
Gibberellins (Pro-Gibb, 2.5–25 g/ha)	Crimson Seedless	F	↓	↓ Fruitset	Dokoozlian and Peacock (2001)
Gibberellins (Gibb3, 800 L/ha)	Pinot Noir	F	↓	↓ Fruitset	Evers et al. (2010)
Gibberellins (5–40 mg/L)	Seibel 5455	F	↓	↓ Fruitset	Hopping (1975)
Gibberellins	Sultanina	F	↓	↑ Pedicel length	Sarooshi (1977)
Gibberellins (10–20 mg/L)	Thompson Seedless	F	↓	↓ Berry number	Lynn and Jensen (1966)
Gibberellins (20 mg/L)	Thompson Seedless	F	↓	↓ Berry number	Christodoulou et al. (1968)
Gibberellins (5–25 mg/L)	Thompson Seedless	F	↓	↓ Berry number	Mosesian and Nelson (1968)
Gibberellins (KGA_3, 15–25 mg/L)	Thompson Seedless	F	↓	↓ Fruitset	Miele et al. (1978)
Gibberellins (Pro-Gibb, 5–40 mg/L)	Vignoles	F	↓	↓ Fruitset	Hed et al. (2011)
Gibberellins (KGA_3, 5–50 mg/L)	Thompson Seedless	PoF	↑	↑ Berry size	El-Banna and Weaver (1978)
Prohexadione-Ca (Regalis, 1.5 kg/ha)	Grüner Veltliner	F	↓	↓ Berry size	Schildberger et al. (2011)
Prohexadione-Ca (Regalis, 1.5 kg/ha)	Pinot Blanc	F	↓	Not reported	Molitor et al. (2011b)
Prohexadione-Ca (Regalis, 1.5 kg/ha)	Pinot Gris	F	↓	Not reported	Molitor et al. (2011b)

†↓ Indicates significant reduction of bunch compactness; ↑ indicates significant increase of bunch compactness. B4, berries 4 mm; F, flowering; PoF, post-flowering; PrF, pre-flowering; V, veraison.

2.4 PLANT GROWTH REGULATORS IN TABLE GRAPE PRODUCTION

PGRs are defined as synthetic compounds, with similar structures to plant hormones that occur naturally in higher plants such as table grapes (Korkutal *et al.*, 2008; Rademacher, 2015). Plant hormones or PGRs are often described as signalling molecules, regulating plant growth and development, alongside environmental factors that also affect plant growth and development (Pallardy, 2008; Korkutal *et al.*, 2008; Roubelakis-Angelakis, 2009; Rademacher, 2015). The primary source of endogenous plant hormones includes growth tips, leaves and root tips (Pallardy, 2008).

In table grapes, some PGRs contribute to processes involving the division, enlargement and differentiation of cells that indirectly coordinate berry development processes such as flowering and berry set (Rademacher, 2015). Exogenous applications of PGRs are used to alter the development of the grapevine in order to promote certain characteristics regarding the quality of the produce. The desired outcome of a PGR application is dependent on the cultivar as well as the phenological stage of the crop during the time of application (Dokoozlian, 2000b; Christensen, 2000; Roubelakis-Angelakis, 2009; Hed *et al.*, 2011; Molitor *et al.*, 2012a; Domingos *et al.*, 2016; SATI, 2019a).

Abscisic acid (ABA), auxin, cytokinins, ethylene and gibberellins are described as the five major plant hormones and are all registered for the use on table grapes except for auxins (Roberts & Hooley, 1988; Fosket, 1994; Kende & Zeevaart, 1997; Vivanco & Flores, 2000; Korkutal *et al.*, 2008; Durner, 2013). A list of the five major PGRs is given in Table 2.4, along with their primary functions and an indication of their registered use with the corresponding timing of application for table grapes.

Table 2.4: A list of the five major plant hormones with their site of biosynthesis, mode of translocation, primary functions as well as their use and timing of application for table grapes (adapted from Durner (2013) and Van der Vyver (2016)).

Hormone	Primary site of biosynthesis	Primary mode of translocation	Primary function(s)	Registered use in table grapes	Recommended phenological stage at application
Auxin	Young meristematic tissue	Mass flow in phloem; polar transport	Cell elongation; vascular differentiation; root initiation; apical dominance; stimulates ethylene production	Not registered for use on table grapes in South Africa	
Cytokinin	Root tips: developing seeds	Xylem	Stimulates cell division; overcomes apical dominance; stimulates leaf blade growth; stimulates cell expansion	Berry enlargement and decreased berry shatter	After set, at about 4-6 mm berry diameter
Gibberellin	Root and shoot apical meristems; young leaves; young fruit; developing seeds	Often synthesized at site of action; phloem; xylem; cell to cell	Stimulates stem elongation; replaces vernalization requirement of some long-day plants; affects floral sex expression; stimulates hydrolases in some germinating seeds; inhibits leaf senescence; inhibits root growth	Bunch stretching	Before flowering when the inflorescence is about 6-9 cm in length
				Berry thinning	Flowering to 10% berry set
				Berry enlargement	After set, at about 4-10 mm berry diameter
Ethylene	All living plant tissue	Diffusion	Promotes fruit ripening, senescence and abscission; promotes leaf abscission; promotes (*Ananas*) or delays (*Prunus*) flowering; promotes the production of female flowers; induces epinasty	Colour improvement	Véraison
Abscisic acid	Mature leaves and roots; developing seeds	Mostly phloem	Induces stomatal closure; induces cessation of embryo growth in developing seeds; induces storage of seed proteins and development of desiccation tolerance in seeds	Colour improvement	Véraison

2.4.1 Gibberellins and their role in the grapevine

The first interaction with gibberellins was recorded in Japan in a quest to find the source responsible for a disease of rice crops which increased stem length and decreased production, (Roberts & Hooley, 1988; Fosket, 1994; Srivastava, 2002; Pallardy, 2008). The disease was called 'foolish seedling' disease. The word gibberellin was derived from naming the active ingredient found during the extraction of the fungus *Gibberella fujikuroi* responsible for this particular disease (Srivastava, 2002). After developing their method for extracting the active substance from the fungus *G. fujikuroi*, the Western world called it gibberellic acid (GA), instead of gibberellin (Srivastava, 2002). GA_3 and a mixture of GA_4 and GA_7 is produced by fermentation of *G. fujikuroi* (Rademacher, 2015).

The discovery of GA in higher plants initiated the movement of further research to uncover more gibberellins and the possible outcome they have on plants (Srivastava, 2002). MacMillan and Takahashi developed a system for allocating names by awarding an A-number for the discovery of new GAs (Mander & Liu, 2010). To date, over 136 A-numbers have been allocated, with only a few biologically active GAs among the list (Bömke & Tudzynski, 2009; Gao *et al.*, 2017). Active GAs includes GA_1 and GA_4, as well as GA_3, GA_5 and GA_7 in certain higher plants (Yamaguchi, 2008; Giacomelli *et al.*, 2013).

Gibberellins are diterpenoids, containing the same basic ring structure called an *ent*-gibberellane (Mander & Liu, 2010; Pallardy, 2008) as depicted in Figure 2.3. Gibberellins are divided into two groups, according to the number of carbon atoms the structure contains, with either 20 or 19 carbon atoms (Srivastava, 2002; Pallardy, 2008). The C_{19} group of GAs contains a lactone and are defined as the physiologically active form in higher plants, contributing to further differentiate the C_{19} GA group from the C_{20} GA group (Srivastava, 2002).

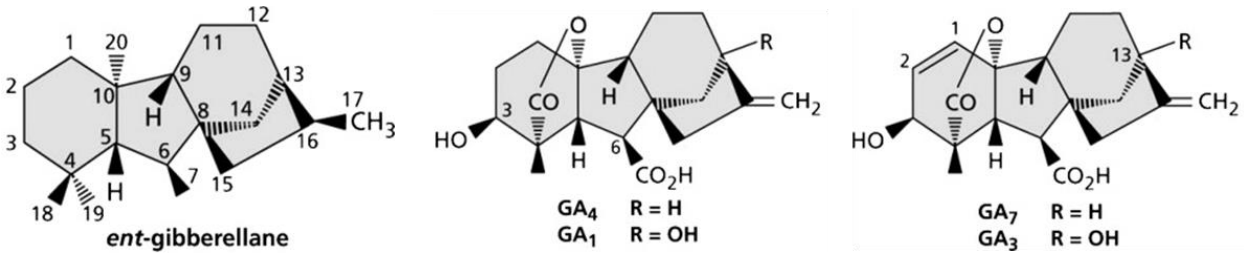

Figure 2.3: The basic ring structure, ent-gibberellane and the structures of GA_4, GA_1, GA_7 and GA_3 (from Bömke & Tudzynski (2009) which were reprinted from Sponsel (2006)).

The most abundant forms of GAs found in grapevines are GA_1, GA_3 and GA_{20} (Roberts & Hooley, 1988; Wolf & Loubser, 1992; Durner, 2013). Wolf and Loubser (1992) reported a similar trend in the breakdown of GA during berry development in grapes treated with GA and untreated grapes. Gibberellin is synthesized on different sites throughout the vine, including young root and shoot apical meristems,

anthers, pollen and developing seeds (Coombe, 1959, as cited by Weaver & McCune, 1960; Jackson, 2008; Durner, 2013).

An important role of GAs in grapevines, especially GA_3, is to regulate early berry growth and development through means of cell division and cell enlargement (Cahoon *et al.*, 1986; Ungsa *et al.*, 2008; Roubelakis-Angelakis, 2009; Molitor *et al.*, 2012a). From the start of flowering, an increase in GA is observed within berries of seeded cultivars, decreasing again after early berry development (Zhang *et al.*, 2003; Baydar & Harmankaya, 2005; Symons *et al.*, 2006).

Depending on the time of application, concerning the stage of development, exogenous applications of GAs are used in the table grape industry for promoting an increase in bunch length, decreasing bunch compactness, increasing fruit size and reducing rudimentary seed occurrence (Weaver & McCune, 1960; Cahoon *et al.*, 1986; Reynolds & de Savigny, 2004; Reynolds *et al.*, 2006; Roubelakis-Angelakis, 2009). Although firmer berries can be obtained with a GA treatment three weeks before harvest, this increases the possibility of exceeding the maximum residue level (MRL) allowed for GA on table grapes (Wolf & Loubser, 1992). In South Africa, a local MRL of 0.2 mg/kg has to be considered for gibberellic acid use (Source: Agri-Intel: www.agri-intel.com).

Although GA is widely known for its use on seedless table grapes, in some instances GA is also used to improve the quality of seeded table grapes. For Waltham Cross, a single GA application is used after set to decrease the occurrence of uneven berries throughout a bunch caused by the set of seedless berries (Wolf & Loubser, 1992). Guidelines for the preparation of Red Globe for export recommend a sizing spray of 20 ppm GA_3 at 12-14mm berry size (SATI, 2019a).

Along with all the positive responses related to the use of GA, a few negative responses have been reported. These include a lower rate of colour accumulation and post-harvest berry shatter, with a positive correlation reported between berry shatter and GA application rates (Retamales & Cooper, 1993; Zoffoli *et al.*, 2009).

As mentioned previously, bunch appearance and characteristics such as bunch structure, bunch compactness, berry size and even bunch colour play an important role in contributing to the perceived quality and therefore value of the grapes produced (Xia *et al.*, 2010; Tello & Ibáñez, 2014). All the characteristics mentioned above, except for bunch colour, can be improved with the use of GA (Dokoozlian & Peacock, 2001).

2.4.2 The mechanism and role of GA_{4+7}

The activity of gibberellins differs between crops. GA_4 and GA_7 have been found to be very active in apples, less active in grapes and inactive in stone fruit such as plums and peaches (Nakagawa, *et al.*,

1968). In South Africa, GA_{4+7} is mainly used in the apple industry for reduction of calyx end russeting. It is also used in combination with the cytokinin, 6-benzyladenine, for fruit thinning and to improve apple fruit quality, through affecting the shape and size (Rademacher, 2015). Apple seeds are also a source of gibberellin: Dennis and Nitsch (1966) identified at least eleven GAs in apple seeds with predominantly GA_4 and GA_7 present.

The similar chemical structures of GA_4 and GA_7 render it difficult to separate the two chemical structures from each other in fermentation extracts (Rademacher, 2015). Therefore, the GA_7 content in products can differ by as much as 40% GA_7 to insignificant amounts. The GA_7 content in products from Fine Agrochemicals Ltd. has been found to be exceptionally low (Rademacher, 2015). Fungi other than *G. fujikuroi* have been tested for their GA-producing capability, especially for the production of GA_4 unaccompanied by GA_7 but none have resulted in an alternative that could be produced on a commercial scale (Rademacher, 2015). The production of GAs is possible through chemical synthesis, but this process is not economically viable for the use on a commercial scale (Rademacher, 2015).

Russeting in apples is controlled primarily by the GA_4 component, whereas the GA_7 component has lasting effects which could decrease the return fertility by reducing flower bud induction (Rademacher, 2015). As mentioned above, these components are difficult to separate and produce for commercial use, therefore low GA_7 content preparations are beneficial for the fertility of the crop.

2.4.3 GA_{4+7} response in table grapes

A study performed by Chundawat *et al.* (1971) on Kyoho (*Vitis vinifera* L. x *Vitis labrusca* L.), a seeded cultivar, evaluated GA_{4+7} applied four days before flowering at concentrations of 50, 75 and 100 ppm to determine the effect on fruit set, seedlessness and fruit quality. There was a significant increase in the number of berries per bunch as well as an increased bunch weight with the 75 and 100 ppm GA_{4+7} treatments compared to the 50 ppm GA_{4+7} treatment (Chundawat *et al.*, 1972). GA_{4+7} treatments applied at 75 and 100 ppm induced 100% seedlessness through parthenocarpy compared to the 50 ppm GA_{4+7} treatment resulting in 95% seedlessness (Chundawat *et al.*, 1972). A similar occurrence of seedless berries in Kyoho grapes was reported by Nakamura *et al.* (1974) with the use of 100 ppm GA_{4+7} treatments under field conditions, as well as in Campbell Early and Delaware grapes tested under laboratory conditions (Kato *et al.,* 1998). An increase in phenylalanine ammonia-lyase activity was recorded in the rachis of Kyoho grapes when treated with both GA_{4+7} and GA_3 treatments, resulting in a thicker and harder rachis due to the increased lignin content (Nakamura *et al.*, 1974).

2.4.4 The mechanism and role of GA_3

The occurrence of flowers and berries that drop naturally has been linked to the competition between developing berries (Gil *et al.*, 1994). The mechanism of GA_3 as a chemical berry thinning agent has

been reported to induce competition between sinks, such as berries and growth points, accompanied by the occurrence of decreased nutrient availability (Gil *et al.*, 1994). Limiting the availability of nutrients during flowering can be obtained by removing leaves in the bunch zone, creating a shortage in nutrients available for the flowering process (Intrieri *et al.*, 2008; Tardaguila *et al.*, 2008; Molitor *et al.*, 2011a). The practice of removing leaves in the bunch zone during flowering, along with a GA_3 thinning application can be considered in cultivars that are less reactive to a GA_3 thinning application on its own. The movement of photosynthate within the vine is affected by exogenous GA applications by favouring photosynthate translocation to the sites of GA application (Quinlan & Weaver, 1970). GA stimulates cell division and enlargement, creating an increased requirement for nutrients/ assimilates, which contribute to making the flower/berry a stronger sink and promoting an influx of photosynthate to the berries.

The response of seeded table grape cultivars to an external GA thinning application have been reported by Boll *et al.* (2009) to be linked to the internal production of GA, especially GA_8. A decreased thinning effect on bunches was recorded for cultivars producing more GA, indicating a lowered cultivar sensitivity towards an external GA thinning application, and *vice versa* (Boll *et al.*, 2009). In this particular study, the quantity of cultivar specific GA produced could also be related to the number of pollen tubes occurring during flowering. A transcriptomic analysis performed by Chai *et al.* (2014) found that applying GAs to Centennial Seedless grapes after flowering led to expression changes of the terminal cell-wall enzymes that stimulate cell enlargement due to changes generated in the hormone signaling network by temporal and multi-level cross talk.

2.4.5 GA_3 response in table grapes

2.4.5.1 Bunch elongation

Less compact bunches can be achieved through an increase in rachis length (Gil *et al.*, 1994). The use of a GA_3 application prior to flowering has been reported to increase bunch length caused by an elongation of the rachis (Weaver & McCune, 1962; Miele *et al.*, 1978; Molitor *et al.*, 2012a). In cultivars such as Sultana and Exotic, Shavrukov *et al.* (2004) reported that the contributing mechanism causing rachis elongation was cell expansion rather than cell division. Molitor *et al.* (2012a) recorded the most significant results when GA_3 was applied with the unfolding of seven leaves.

2.4.5.2 Berry thinning

The objective of a berry thinning action is to increase the spatial area available for larger berries to develop into, resulting in less compact bunches (Weaver & Pool, 1971; Reynolds *et al.*, 2006; Domingos *et al.*, 2016). The action of berry thinning can be achieved through either chemical berry thinning and/or manual berry thinning, with the aim of reducing the number of berries per bunch (Di Lorenzo *et al.*,

2011). Chemical berry thinning involves the application of GA and is predominantly applied by means of a spray application in the table grape industry, with a direct spray that covers the whole canopy (Gil *et al.*, 1994). Manual berry thinning is a time-consuming process requiring intensive labour inputs. The combination of time and labour makes this an expensive practice (Winkler *et al.*, 1962; Christodoulou *et al.*, 1966).

The use of GA for berry thinning is a common practice used in seedless table grape production, but the effectivity depends on internal factors, namely cultivar and phenological stage at the time of application and external factors during flowering, namely water and nutrient status, temperature and humidity (Weaver & Pool, 1971; Dokoozlian & Peacock, 2001). A higher berry thinning success rate is achieved with cultivars that produce less GA_3. These cultivars have been found to be more sensitive towards an external GA application, resulting in a more significant response (Boll *et al.*, 2009).

The use of GA_3 for berry thinning increases the number of shot berries for specific cultivars (Lynn & Jensen, 1966; Hed *et al.*, 2015). More recently, other PGRs such as ABA (Padmalatha *et al.*, 2017; Mohamed *et al.*, 2019) and ethylene have also been evaluated as thinning agents for table grapes, but are not registered for this purpose on table grapes.

Application timing and rates for berry thinning

The use of GA_3 applications during flowering has been widely studied and has been shown to reduce berry set when applied to seedless table grapes cultivars (Lynn & Jensen, 1966; Weaver & Pool, 1971; Dokoozlian & Peacock, 2001). GA_3 application at full-flowering is effective in reducing the fruit set rate, resulting in fewer berries per bunch (Christodoulou *et al.*, 1966; Lynn & Jensen, 1966; Miele *et al.*, 1978; Dokoozlian & Peacock, 2001).

Determining the optimal timing and application for the use of GA for berry thinning has been extensively researched on various cultivars (Jensen, 1994; Dokoozlian, 1998 as cited by Dokoozlian & Peacock, 2001). Apart from work done by Avenant (2000), no other studies to determine the optimal timing and application rate for the cultivar Sunred Seedless have yet been performed.

Dokoozlian and Peacock (2001) evaluated various timings for berry thinning applications on Crimson Seedless. This included six different stages of flowering (1%; 5%; 20-30%; 50-60%; 80-90% & 100%), all applied at a rate of 2 $g.ha^{-1}$. No significant differences were found in fruit set when applied at the different stages of flowering. In contrast, an increase in berry longitudinal length and berry mass was observed as the flowering stages increased. Based on their results of an increase in berry longitudinal length and berry mass, along with a 24% reduction in the number of berries per cm lateral length, compared to the untreated control, Dokoozlian and Peacock (2001) recommended applying GA_3 between 80-100% flowering at a rate of 2.5 $g.ha^{-1}$

A second experiment performed by Dokoozlian and Peacock (2001) included different GA_3 rates (2.50, 6.25, 12.50, 18.75 & 25 $g.ha^{-1}$) applied at 80% flowering. An increase in the appearance of shot berries was observed as the rate of GA_3 applied during flowering increased (Dokoozlian & Peacock, 2001). Furthermore, sensitivity to the formation of shot berries after a GA_3 treatment was noted to be cultivar dependent. Similarly, Christodoulou *et al.* (1966) also reported an increase in shot berries with increased GA rates, as well as when more than one GA_3 application was applied during flowering.

2.4.5.3 Berry sizing

Specific seedless table grape cultivars, such as Thompson Seedless (Wolf & Loubser, 1992), require a GA treatment, applied after berry set to improve berry size. In contrast, a cultivar such as Sunred Seedless has a large natural berry size, which requires no berry sizing treatment. A GA application for sizing can be used to produce grapes for specific markets with large berry size requirements in order to increase the economic value of the grapes.

Naturally produced gibberellins are limited in stenospermocarpic cultivars due to gibberellins only being produced before embryo abortion. This results in a smaller natural berry size in these seedless cultivars compared to seeded cultivars (Pérez *et al.*, 2000). In order to meet export market requirements, an exogenous GA application is required and therefore the use of GA_3 during early berry development has become a common practice in table grapes to increase berry size (Singh *et al.*, 1978; Zabadal & Dittmer, 2000; Casanova *et al*., 2009).

The practice of improving berry size with the use of GA_3 is often misused, with as many as up to five applications per season due to the potentially large economic gain (Casanova *et al.*, 2009; Zoffoli *et al.*, 2009). Depending on the cultivar, the overuse of GA_3 could lead to increased berry shatter (Retamales & Cooper, 1993), delayed ripening (Guelfat-Reich & Safran, 1973) as well as the possibility of decreased return fertility (Dokoozlian, 2000a; Raban *et al*., 2013).

Various factors contribute to the success of the berry sizing application, including the cultivar, the rate and timing of the application as well as endogenous factors such as the hormonal and nutritional status of the vine and exogenous factors which include prevailing environmental conditions (Orth, 1990; Coombe & Dry, 1992; Mullins *et al*., 1992; Dokoozlian, 2000a; Ojeda *et al*., 2001; Casanova *et al*., 2009). Increased GA application rates deliver larger berries along with an elevated sensitivity to defects developing on the surface of the berries (Wolf & Loubser, 1992).

The action of berry sizing can be achieved through a full cover spray application of GA_3 or by manually dipping bunches into a GA_3 solution (Orth, 1990; Coombe & Dry, 1992; SATI, 2019a).

2.5 GRAPEVINE FERTILITY

Grapevine fertility or fruitfulness is defined by either the number of inflorescence primordia per bud (Swanepoel & Baard, 1988; Ferrer *et al.*, 2004) or the number of bunches per shoot (Iland *et al.*, 2011). Inflorescence primordia initiate within the bud during the previous growing season, during which various factors such as physiological and environmental factors, influence bud fertility (May, 2000; Ferrer *et al.*, 2004). Seasonal variations in fertility are linked to the disparity in yield observed in each season – usually exceeding 15%, and sometimes as much as 35% (May, 1961; Chloupek *et al.*, 2004; Keller & Mills, 2004; Clingeleffer, 2010), with the number of berries per bunch and berry size being less sensitive variations linked to yield fluctuations (Vasconcelos *et al.*, 2009).

Quantifying the potential fertility of the grapevine during dormancy of the current season is possible, due to inflorescence primordia that have already initiated within the bud during the previous growing season (Barnard, 1932; Swanepoel & Archer, 1988; Swanepoel & Baard, 1988; Iland *et al.*, 2011; Molitor *et al.*, 2012a).

2.5.1 Factors affecting grapevine fertility

2.5.1.1 Genetic factors

Diversity within the *Vitis* genus, due to genotype variability (This *et al.*, 2006), allows for the breeding of new cultivars better adapted to specific cultivation regions whilst ensuring optimal productivity (Dai *et al.*, 2011). Grapevine genotypes (cultivars or clones) differ in bud fertility according to the position of these buds on the cane, as some genotypes have higher bud fertility at the base of the cane, whilst for others, it might be more towards the middle of the cane (Khanduja & Balasubrahmanyam, 1972; Bennett, 2002; Ferrer *et al.*, 2004; Sánchez & Dokoozlian, 2005).

The bud fertility characteristic of a cultivar plays a vital role in determining the cultural practices required, such as the pruning system with regards to the type and intensity of pruning (Ferrer *et al.*, 2004). Pruning systems for table grapes in South Africa are based on the cultivar and cultivation region. Fertile cultivars are spur pruned, while less fertile cultivars are pruned with half long bearers or canes (Lombard *et al.*, 2006). Recommended pruning system have been assigned to cultivars based on their fertile bud positions and are summarised in Table 2.5. The cultivar Sunred Seedless experiences a higher bud fertility towards the base of the cane and can therefore be spur pruned (SATI, 2019a). The percentage of buds which differentiate into flower primordia is also cultivar dependent (Khanduja & Balasubrahmanyam, 1972), with a 30-40% differentiation rate in Sultana and 100% in Alphonse Lavallee (Lavee *et al.*, 1967).

Table 2.5: Recommended table and raisin grape pruning system, based on their fertile bud positions (adapted from Van der Vyver (2016); SATI (2019a) and Avenant & Avenant (2020)).

Spur (2 buds)	Half long bearer (4-8 buds)	Cane (12-16 buds)
Table grapes		
Alphonse Lavallée	Autumn Crisp (Sugra 35)	Autumn Royal (10)
Allison	Allison	Adora (Sugra 34)
Arra 13	Barlinka (6-8)	Crimson Seedless (10)
Arra 14	Bien Donné (4-6)	Midnight Beauty
Autumn Crisp (Sugra 35)	Bonheur (4-6)	Ralli Seedless (10)
Bien Donné	Crimson Seedless (8-10)	Sugraone
Bonheur	Dan-ben-Hannah (4-6)	Sultanina
Ebony Star	Dauphine (6-8)	
Flame Seedless	Flame Seedless (6-8)	
Krissy	Krissy	
La Rochelle	Majestic (4-6)	
Midnight Beauty	New Cross (4-6)	
Muska, Pirobella	Prime (4-6)	
Queen of the Vineyard	Red Globe (6-8)	
Prime	Waltham Cross (8)	
Regal Seedless	White Gem (4-6)	
Ronelle		
Sable Seedless		
Starlight		
Sundance		
Sunred Seedless		
Victoria		
Raisin grapes		
Datal		Black Monucca
Muscat d'Alexandrie (Hanepoot)		Merbein Seedless
Currants		Sultanina

2.5.1.2 Morphological factors

The relationship between bud fertility and the morphology of the grapevine cane includes factors such as length, diameter and the length of internodes (Khanduja & Balasubrahmanyam, 1972). Studies performed by various authors on wine grape cultivars (Pinot gris, Riesling & Sauvignon blanc) showed that there was a reduction in the number of inflorescences per shoot as the cane diameter decreased (Vasconcelos *et al.,* 2009). Furthermore, pencil thickness is a general measure used in viticulture as a reference for the minimum cane diameter required to ensure optimal fertility (Vasconcelos *et al.*, 2009).

2.5.1.3 Physiological factors

Plant hormones

The process of flowering is highly dependent on two hormonal regulators, namely gibberellins and cytokinins (Vasconcelos *et al.*, 2009). External applications of these hormonal regulators can influence grapevine fertility. Inflorescences can convert to tendrils when treated with gibberellins and the opposite occurs when uncommitted anlagen or young tendrils treated with cytokinins convert to inflorescence (Srinivasan & Mullins, 1981b; Mullins *et al.*, 1992).

In the grapevine, GAs contribute to anlagen formation and determine the development of the anlagen (Srinivasan & Mullins, 1980). The role of GAs are determined by the stage of bud development. During the early stages, GA will promote fertility since anlagen formation requires GA but during flower formation GA act as an inhibitor by directing differentiating anlagen to form tendrils (Khanduja & Balasubrahmanyam, 1972; Srinivasan & Mullins, 1981b; Mullins *et al.*, 1992; Williams, 2000; Bennett, 2002; Vasconcelos *et al.*, 2009; Iland *et al.*, 2011).

Endogenous gibberellins can be detected in the xylem sap of grapevines (Srinivasan & Mullins, 1981b; Bennett, 2002). Grapevine sensitivity towards exogenous gibberellins applied at concentrations as low as 3µmol/L can inhibit the formation of inflorescences from the anlagen due to an induced bursting effect on the latent buds (Srinivasan & Mullins, 1981b; Mullins, 1986). Exogenous GA applied before the initiation process directs the anlagen formation to tendrils and inhibits flower bud formation. However, applying GA after flower bud formation had little impact (Khanduja & Balasubrahmanyam, 1972; Mullins *et al.*, 1992; Williams, 2000).

Cytokinins are synthesized in the roots and translocated to the developing inflorescence *via* xylem sap where it contributes to the regulation of flower differentiation (Mullins & Rajasekaran, 1981; Srinivasan & Mullins, 1981a; Srinivasan & Mullins, 1981b; Lavee, 1987; Mullins *et al.*, 1992; Bennett, 2002). Cytokinins influence grapevine fertility by regulating the development of inflorescence primordia during bud break and flower formation and an increased cytokinin concentration is found in the xylem sap at these particular times (Khanduja & Balasubrahmanyam, 1972; Srinivasan & Mullins, 1979; Mullins, 1986; Mullins *et al.*, 1992; Williams, 2000; Bennett, 2002; Iland *et al.*, 2011). A constant supply of endogenous cytokinins into the anlagen is required for the formation of flowers in the grapevine (Srinivasan & Mullins, 1980; Srinivasan & Mullins 1981b).

Carbohydrates

Carbohydrates are essential for the reproductive cycle of the grapevine (Caspari *et al.*, 1998), where they contribute from the development of inflorescence to flower initiation (Lebon *et al.*, 2008). Latent

buds receive carbohydrates from shoot reserves and leaves on the same side of the shoot. Compared to developing flowers, bunches and growth points are weak sinks for carbohydrates (Hale & Weaver, 1962). During their early development, young leaves are sink organs (Lebon *et al.*, 2008). Consequently, carbohydrate reserves remain the primary source of energy for the development of buds and inflorescences until the leaves become a source of assimilates. Leaf removal during the early part of the growing season or a high percentage of leaf removal can contribute to reduced accumulation of carbohydrate reserves, thereby reducing the potential bud fertility for the following season (Candolfi-Vasconcelos & Koblet, 1990; Duchêne *et al.*, 2003a; Duchêne *et al.*, 2003b; Bennett *et al.*, 2005).

A negative impact on bud fertility is observed with a reduction in photosynthesis during or shortly after flowering (Candolfi-Vasconcelos & Koblet, 1990; Bennett, 2002). Studies have reported a positive correlation between the accumulation of carbohydrate reserves in the annual wood and bud fertility the following season, regarding the number of inflorescences and flower number per inflorescence (Candolfi-Vasconcelos & Koblet, 1990; Howell *et al.*, 1994; Keller & Koblet, 1995, Duchêne *et al.*, 2003a, Duchêne *et al.*, 2003b; Bennett *et al.*, 2005; Lebon *et al.*, 2008). Early bunch development is influenced by the carbohydrate reserve content in latent buds and contributes to a positive mean primordia size during inflorescence initiation (Antcliff & Webster, 1955).

Nutrients

An adequate nutrient status, especially for nitrogen (N), is required for inflorescence primordia formation and flower differentiation (Khanduja & Balasubrahmanyam, 1972; Mullins *et al.*, 1992; Williams, 2000; Vasconcelos *et al.*, 2009; Iland *et al.*, 2011). The oversupply of N during flower primordia initiation has been reported to reduce the number of differentiated inflorescences (Winkler *et al.*, 1962; Khanduja & Balasubrahmanyam, 1972; Srinivasan & Mullins, 1981b; Mullins *et al.*, 1992; Vasconcelos *et al.*, 2009). With an adequate phosphate (P) nutrient status, bud fertility is promoted as phosphate is required for the support of initiated inflorescence primordia (Skinner & Matthews, 1989; Vasconcelos *et al.*, 2009). In the grapevine, potassium (K) plays an essential role in the activation of enzymes and the movement of carbohydrates (Bouard, 1968). The correction of a K deficiency with an exogenous application of K to the soil has been found to increase bud fertility (Christensen, 1975, as cited by Li-Mallet *et al.*, 2016). High levels of cytokinin produced by the roots are associated with an adequate N, P and K nutrient status within the grapevine (Jako, 1976; Srinivasan & Mullins, 1981a; Srinivasan & Mullins, 1981b).

Micronutrients, such as zinc play a role in pollen formation and a deficit could inhibit pollination (Keller, 2010). Boron is essential for ovule fertilization as it plays a role in pollen germination and tube growth (Alva *et al.*, 2015). These micronutrient deficits may lead to excessive flower drop, resulting in reduced fruit set and promoting the development of shot berries (Keller, 2010).

2.5.1.4 Environmental conditions

Temperature

Favourable conditions for the formation of inflorescence primordia is reported at high temperatures (Khanduja & Balasubrahmanyam, 1972; Srinivasan & Mullins, 1981b; Mullins *et al.*, 1992; Dunn & Martin, 2000; Iland *et al.*, 2011). These temperatures range from above 20°C to 35°C, depending on the cultivar and its origin (Winkler *et al.*, 1962; Khanduja & Balasubrahmanyam, 1972; Srinivasan & Mullins, 1981b; Moncur *et al.,* 1989; Dunn & Martin, 2000; Sommer *et al.*, 2000; Sánchez & Dokoozlian, 2005). The optimum temperature for inflorescence primordia formation in *Vitis vinifera* cultivars range from 25°C to 28°C (Buttrose, 1970; Srinivasan & Mullins, 1981b; Mullins *et al.,* 1992). Temperatures over 35°C between flowering and berry set resulted in reduced berry set in Pinot noir and Carignane (Kliewer, 1977). Early spring temperatures of below 20°C were associated with an increased formation of tendril primordia, resulting in reduced fertility (Buttrose, 1970). During the critical time of inflorescence primordia induction and differentiation, a daily period of four to five hours of high temperature is required to promote the formation of inflorescence primordia (Buttrose, 1970; Srinivasan & Mullins, 1981b; Dunn & Martin, 2000).

Temperature plays a vital role in grapevine fertility. Relationships have been reported between the maximum temperature in season one and flower number per shoot in season two (Palma & Jackson, 1989), as well as the temperature at initiation in season one and bunches per shoot in season two (Vasconcelos *et al.,* 2009).

Light

Sufficient light in the renewal zone during fruit bud initiation is essential for optimal bud fertility as shading can reduce bud fertility of the following season by decreasing both the number and size of inflorescence primordia formed in the primary bud (Khanduja & Balasubrahmanyam, 1972; Srinivasan & Mullins, 1981b; Lavee, 1987; Smart & Smith, 1988; Williams, 2000; Sánchez & Dokoozlian, 2005; Vasconcelos *et al.*, 2009; Iland *et al.*, 2011). May *et al.* (1976), as well as Perez and Kliewer (1990), reported that buds located inside the canopy were less fertile than buds located on the outside of the canopy. Light can influence fertility either directly by affecting the bud itself, with shading increasing the occurrence of bud necrosis (Perez & Kliewer, 1990) or indirectly by affecting photosynthesis and carbohydrate availability (Vasconcelos *et al.*, 2009; Li-Mallet *et al.*, 2016). The availability of carbohydrate reserves plays a role in the flower number per inflorescence and number of inflorescences per vine of the next season (Bennett *et al.*, 2005; Eltom *et al.*, 2017).

Optimal bud fertility for wine grapes is reached at a light intensity of 800 $\mu E.m^{-2}.s^{-1}$ (Archer, 2011). Light exposure requirements for optimal fertility is different for each cultivar (Sánchez & Dokoozlian, 2005).

Increased light exposure to shoots of Flame Seedless and Thompson Seedless increased their fertility (Sánchez & Dokoozlian, 2005). A positive correlation between light exposure and the diameter of inflorescence primordia in primary buds has been recorded (Sánchez & Dokoozlian, 2005).

Water

Inflorescence development is highly influenced by water stress, especially during the first four weeks of flowering (Winkler *et al.*, 1962; Srinivasan & Mullins, 1981b; Mullins *et al.*, 1992; Williams, 2000; Vasconcelos *et al.*, 2009). The influence of water stress on bud fertility is higher early during the growing season (Myburgh, 2003). The impact of water stress on fertility can be indirect by influencing photosynthesis, plant hormonal balance and carbohydrate availability or direct through water availability during cell division and enlargement (Vasconcelos *et al.*, 2009). Potential berry growth is affected through a reduction in the number of cells per berry due to the impeding effect of water stress on the cell division process (Matthews *et al.*, 1987; McCarthy, 1997).

Extended periods of water stress can reduce bud fertility the following season by decreasing the number and size of inflorescence primordia. This is a likely explanation for the higher yields associated with irrigated vines compared to dryland vines (Winkler *et al.*, 1962; Srinivasan & Mullins, 1981b; Mullins *et al.*, 1992; Iland *et al.*, 2011). Water stress during bud break has been associated with decreased and inconsistent bud break (Vasconcelos *et al.*, 2009).

2.5.1.5 Viticultural practices

Trellis system, row direction, canopy management and pruning

Canopy microclimate is influenced by the amount and angle of light interception within the canopy, in turn affecting bud fertility (Dry, 2000; Poni *et al.*, 2003; Iland *et al.*, 2011). Canopy management practices, namely the type of trellis system used, row orientation, spacing and shoot orientation, number of shoots, tipping and topping of shoots and leaf removal can be used to manipulate light interception within the canopy (Dry, 2000; Iland *et al.*, 2011).

The correlation between trellis system and yield can be ascribed to the exposure of leaves to solar radiation (Sanchez-Rodriguez & Spósito, 2020). Trellis systems like the Gable trellis or Geneva double curtain system improve light exposure due to the split canopy architecture, improving bud fertility and grapevine yield (Winkler *et al.*, 1962; Srinivasan & Mullins, 1981b; Mullins *et al.*, 1992; Iland *et al.*, 2011; Shoeib, 2012).

Hunter *et al.* (2018) defined row orientation as a driver of grapevine yield and berry composition as it affects the fertility of buds, development of berries, lignification of shoots as well as the overall plant health. Shoot thinning and leaf removal around the bunch zone can be used to decrease canopy density

in order to reduce excessive shading, allowing light exposure and air penetration through the canopy (Bravetti *et al.*, 2010; Silvestroni *et al.*, 2016).

In grapevines, winter pruning is performed between leaf fall and bud break (Winkler *et al.*, 1962; Dunn & Martin, 2000). It provides the opportunity to optimize yield by selecting the correct pruning system based on the cultivar's fertile bud positions (Table 2.5) and improving bud formation by awarding the optimal number of canes per vine, in order to prevent overcropping (Winkler *et al.*, 1962). The intensity of winter pruning influences the size of inflorescences and the number of flowers, Dunn and Martin (2007) attributed this phenomenon to the decreased number of carbon sinks.

Rootstock selection

The choice of rootstock influences the vegetative growth and yield of the scion cultivar (Keller *et al.*, 2001; Sommer *et al.*, 2001). Selecting the wrong rootstock could negatively affect grapevine fertility (Winkler *et al.*, 1962; Khanduja & Balasubrahmanyam, 1972; Mullins *et al.*, 1992; Iland *et al.*, 2011). Increased canopy density due to the use of a vigorous rootstock could decrease fertility as a result of excessive shading of buds, inhibiting inflorescence induction and initiation (Sommer *et al.*, 2000). The rootstock Ramsey is known to increase vegetative growth of the scion cultivar, resulting in dense canopies if poor canopy management is practiced (Sommer *et al.*, 1993).

Plant growth regulators

The effect of PGRs on grapevine fertility is discussed in Section 2.5.3.

2.5.2 Methods for determining potential grapevine fertility

The assessment of grapevine bud fertility during the winter serves as a measure of the number of inflorescence primordia that could potentially develop into bunches (Dry, 2000). The potential fertility can be determined during grapevine dormancy through the use of two methods, namely bud dissection and forced budding (Sommer *et al.*, 2000). The potential fertility only represents an estimate of the actual grapevine fertility. The actual fertility should be determined in spring by recording the number of bunches present after bud break as soon as they are clearly visible (Khanduja & Abbas, 1973; Swanepoel & Baard, 1988; Candolfi-Vasconcelos & Koblet, 1990; Williams, 2000) and before commercial crop control (removal of some of the bunches) is applied.

2.5.2.1 Bud dissection

The assessment of bud fertility, as well as crop forecasting in table grapes, are possible with the use of stereomicroscope bud dissections (Barnard, 1932; Khanduja & Abbas, 1973; Swanepoel & Baard, 1988; Sommer *et al.*, 2000). Potential fertility through bud dissection is determined by opening and examining

buds under a microscope and counting the number of inflorescence primordia present (Antcliff & Webster, 1955; Swanepoel & Baard, 1988; Iland *et al.*, 2011).

2.5.2.2 Forced budding

Single-node cuttings are placed in a glasshouse under controlled climatic conditions (˚C) promoting bud break in order to determine the potential fertility by counting the number of inflorescences present (Buttrose, 1969; Khanduja & Abbas, 1973; May & Antcliff, 1973; Shulman *et al.*, 1983; Swanepoel & Baard, 1988; Palma & Jackson, 1989; Dunn & Martin, 2000; Iland *et al.*, 2011).

Potential fertility can be used to determine the correct pruning system for each cultivar and to make adjustments for specific seasons, depending on the level of fertility. Pruning methods promoting optimal yields can be selected during low potential fertility seasons and pruning methods that eliminate overcropping can be used in high potential fertility seasons (Antcliff & Webster, 1955; Khanduja & Abbas, 1973).

2.5.3 The effect of gibberellic acid applications on grapevine return fertility

Research on the impact of plant growth regulators on the fruitfulness or return fertility of the grapevine is limited and extremely limited for GA_{4+7}. The effect of GA_{4+7} on the return fertility of the grapevine have not yet been reported on, but Davis (2002) found that GA_{4+7} treatments decreased apple flower bud formation. Studies have reported a reduced number of bunches the year after application (Weaver, 1960), an inhibition and reduction in the formation of inflorescences (Palma & Jackson, 1989) as well as an increase in the occurrence of bud necrosis (Lavee *et al.*,1981). All of these were associated with a reduction in return fertility due to the application of GA_3. Variability in the degree of decreased return fertility caused by a GA_3 application can be ascribed to various factors such as cultivar, phenological stage at the time of application, application rate, number of applications and seasonality (Lavee *et al.*, 1981; Dokoozlian, 2000a).

Most of the causes, as reported by authors, of reduced fertility associated with an GA_3 application can be ascribed to four possible contributing factors: application timing, rate of GA_3 applied, along with the number of applications and lastly the method of GA_3 application. Most studies found the reduction in fertility to be related with one or a combination of those factors.

2.5.3.1 Application timing

Authors have associated the reduction in the return fertility of the vine with the timing of GA_3 applications, both for stretching and thinning. The use of a stretching application with GA_3 for increased bunch length is usually applied before flowering when bunches are between 6 and 9 cm long, according to registration guidelines. According to Christensen (2000), such an application falls within the time when the potential

fertility of the following season is being determined and therefore has the most significant impact on the vine's fertility in the following season. Molitor *et al.* (2012a) concluded that the possibility that a GA_3 application 5-7 weeks after bud break, coinciding with the initiation and differentiation of primordia, could promote the differentiation of anlagen to tendrils rather than inflorescences. A pre-flowering GA application could, therefore reduce the number of inflorescences per shoot. Thinning applications of GA_3, applied during flowering (Mullins *et al.*, 1992) or shortly afterwards (Weaver & McCune, 1959, cited by Lavee *et al.*, 1993), have been found by researchers to affect the vine's fertility in the season thereafter negatively.

2.5.3.2 Application rate and number of applications

Studies have found that the use of a full cover GA_3 application (Coombe & Dry, 1992) at increased rates can decrease the fertility of the vine in the following season for cultivars such as Thompson Seedless (Jawanda *et al.*, 1974) and Ruby Seedless (Peacock, 1998). A decrease in vine fertility in the season of application, as well as the following season, have been recorded by Korkutal *et al.* (2008) and this was due to the use of high GA_3 concentrations.

No decrease in vine fertility was observed in a thinning trial with GA_3 concentrations up to 25 ppm, applied before flowering and during the late stages of flowering on Chardonnay and Vignoles wine grapes (Hed *et al.*, 2011). Furthermore, the authors ascribed decreased fertility reported not only to be associated with the use of much higher concentrations than the 25 ppm used in their experiment but also depending on the cultivar and phenological stage of the vine during the application.

2.5.3.3 A combination of application timing and application rate

A GA_3 application applied at high rates can lead to a reduction in the return fertility of the vine if applied during the time when the potential fertility of the following season is being determined through the initiation of flower primordia (Elgendy *et al.*, 2012).

A decrease in return fertility was observed in Riesling for two seasons following a GA_3 application applied at 50 ppm during 50-80% flowering (Weyand & Schultz, 2005). Compared to the recommended dosage rates for table grape cultivars in South Africa (Table 2.6), ranging from 1 ppm to 7.5 ppm, except for 30 ppm for Thompson Seedless, a rate of 50 ppm applied during flowering is exceptionally high. Dokoozlian and Peacock (2001) also reported a decrease in return fertility for two consecutive seasons following GA_3 applications applied at rates ranging from 6.25 to 25 $g.ha^{-1}$ during 80% flowering in Crimson Seedless. Lavee (1987) suggested that increased levels of GA_3 found in vigorous vines, especially after flowering, promote the development of primary bud necrosis. Berry sizing applications have also been associated with a reduction in fertility, but mostly due to the increased application rates associated with a GA_3 sizing application, along with the continuous yearly use of high GA_3 rates (Dokoozlian, 2000a).

Table 2.6: Cultivar specific recommendations regarding timing of application and dosage rate for GA_3 thinning and berry sizing applications under South African conditions (SATI, 2019a).

Cultivar	Thinning		Berry sizing	
	Application timing	Dosage Rate (ppm)	Application timing (berry size in mm)	Dosage Rate (ppm)
Autumn Crisp (Sugra 35)			7-8 mm	3 ppm
Crimson Seedless	10% berry set	1 ppm		
Prime	10% berry set	1-2 ppm	9-10 mm	15-20 ppm (dip)
Thompson Seedless	50% flowering	10 ppm	50% of berries at 4-5mm	20-30 ppm
	80% flowering	10 ppm	75% of berries at 4-5mm	20-30 ppm
	10% berry set	10 ppm	100% of berries at 4-5 mm	20-30 ppm
Sable Seedless	10% berry set	1.5 ppm	8-10 mm	6 ppm
	3 days later	1.5 ppm		
Starlight	10% berry set	1-2 ppm	9-12 mm	15-20 ppm (dip)
Sheegene 20 (Allison)	10% berry set	1-2 ppm	6-8 mm	2-3 ppm
Tawny Seedless	10% berry set	1 ppm	6-7 mm	5-7.5 ppm
Red Globe			12-14 mm	20 ppm
Arra 13	10% berry set	1.5 ppm	6-7 mm	20 ppm
Flame Seedless	80% flowering	5-7.5 ppm	7-8 mm	20-30 ppm
			8-9 mm	20-30 ppm

2.5.3.4 Application method

According to a study performed on the method of application, a decrease in return fertility was observed in Muscat Seedless where a GA_3 treatment was applied as a full cover spray compared to the dipping of bunches in a GA_3 solution (Orth, 1990). Lavee *et al.* (1981) also found increased bud necrosis for GA_3 applications with direct bud contact.

2.6 CONCLUSION

In grapevines, seeded berries develop through normal set, whereas seedless berries develop through either parthenocarpy or stenospermocarpy. Table grape cultivars that set through stenospermocarpy include Crimson Seedless, Sunred Seedless, Prime, Thompson Seedless, Scarlotta Seedless, Midnight Beauty, Flame Seedless and Early Sweet. Specific seedless cultivars that set through stenospermocarpy tend to set detectable rudimentary seeds which decrease the eating quality, as well as marketability of these grapes. An increased occurrence of seedless berries has been reported by authors with the use of GA_3 during flowering, on stenospermocarpic cultivars.

Depending on the cultivar, the use of PGRs in table grape production has become a standard practice, assisting in meeting market requirements. Consumer preferences with regards to seedlessness, berry shape and size, colour uniformity, rachis colour as well as bunch shape, size and compactness, determine these market requirements. GA_3 is the most widely used PGR in table grapes and is mainly used on seedless cultivars for berry sizing as well as bunch stretching and berry thinning on cultivars that tend to set too compact bunches. The intended outcome for the use of PGRs is influenced by factors such as the cultivar, phenological stage at application, applications rate and the number of applications.

The following season's fertility is affected by cultural or chemical practices applied in the current season. The formation of clusters through the initiation and differentiation of inflorescence primordia, during spring and summer of the current season, will determine the yield potential for the following season. Observations in the table grape industry have indicated that the use of GA_3 is associated with decreased bud fertility. The availability of published research articles on the use of GA_3 leading to decreased return fertility are minimal and non-existing for GA_{4+7}.

2.7 LITERATURE CITED

Abd El-Razek, E., Treutter, D., Saleh, M.M.S., El-Shammaa, M., Fouad, A.A. & Abdel-Hamid, N., 2011. Effect of nitrogen and potassium fertilization on productivity and fruit quality of 'crimson seedless' grape. Agric. Biol. J. N. Am. 2, 330-340.

Abdel-Fattah, M.E., Amen, K.A., Alaa, A.B., Eman, A.A. & Zeed, A., 2010. Effect of berry thinning, CPPU spraying and pinching on cluster and berry quality of two grapevine cultivars. Assiut. J. Agric. Sci. 40, 92-107.

Agüero, C., Vigliocco, A., Abdala, G. & Tizio, R., 2000. Effect of gibberellic acid and uniconazole on embryo abortion in the stenospermocarpic grape cultivars Emperatriz and Perlon. Plant Growth Regul. 30, 9-16.

Alva, O., Roa-Roco, R.N., Pérez-Díaz, R., Yáñez, M., Tapia, J., Moreno, Y., Ruiz-Lara, S. & González, E., 2015. Pollen morphology and boron concentration in floral tissues as factors triggering natural and GA-induced parthenocarpic fruit development in grapevine. PloS One, 10.

Antcliff, A.J. & Webster. W.J. 1955. Studies on the Sultana vine. I. Fruit bud distribution and budburst with reference to forecasting potential crop. Aust. J. Agric. Res. 6, 565-588.

Archer, E., 1981. Classification, Anatomy and Morphology (in Afrikaans). In: Burger, J. & Deist, J. (eds). Wingerdbou in Suid-Afrika. ARC Infruitec-Nietvoorbij, Private Bag X5026, 7599 Stellenbosch, South Africa.

Archer, E., 2011. Increasing yield from wine grapes. Wynboer Technical Yearbook, pp. 60-84.

Archer, E. & Van Schalkwyk, D., 2007. The effect of alternative pruning methods on the viticultural and oenological performance of some wine grape varieties. S. Afr. J. Enol. Vitic. 28, 107-139.

Avenant, J.H., 2000. Effect of different cultivation practices on seed trace size of Sunred Seedless-preliminary results. Dec. Fruit Grower 50, 16.

Avenant, J.H. & Avenant, E., 2020. Pruning of table and raisin grapes. SATI/ ARC Infruitec-Nietvoorbij Modular Course in Table and Raisin Grape Production 2020. SATI, PO Box 2932, Paarl, 7620.

Bangerth, F.K., 2004. Internal regulation of fruit growth and abscission. Acta Hortic. 636, 235-248.

Barnard, C., 1932. Fruit bud studies: I, The Sultana. An analysis of the distribution and behaviour of the buds of the Sultana vine, together with an account of the differentiation. J. Counc. Sci. Ind. Res. Aust. 47-52.

Bavaresco, L., de Macedo, M.I.V.Z., Gonçalves, B., Civardi, S., Gatti, M. & Ferrari, F., 2010. Effects of traditional and new methods on overcoming lime-induced chlorosis of grapevine. Am. J. Enol. Vitic. 61, 186-190.

Baydar, N.G. & Harmankaya, N., 2005. Changes in endogenous hormone levels during the ripening of grape cultivars having different berry set mechanisms. Turk. J. Agric. For. 29, 205-210.

Bennett, J.S., 2002. Relationships between carbohydrate supply and reserves and the reproductive growth of grapevines (*Vitis vinifera* L.). Thesis, Lincoln University, PO Box 85084, 7647 Lincoln (Canterbury), New Zealand.

Boll, S., Lang, T., Hofmann, H. & Schwappac, P., 2009. Correspondence between gibberellin-sensitivity and pollen tube abundance in different seeded vine varieties. Mitt. Klosterneuburg 59, 129-133.

Bömke, C. & Tudzynski, B. 2009. Diversity, regulation, and evolution of the gibberellin biosynthetic pathway in fungi compared to plants and bacteria. Phytochemistry 70, 1876-1893.

Boss, P.K., Buckeridge, E.J., Poole, A. & Thomas, M.R., 2003. New insights into grapevine flowering. Funct. Plant Biol. 30, 593-606.

Boss, P.K. & Thomas, M.R., 2002. Association of dwarfism and floral induction with a grape 'green revolution' mutation. Nature 416, 847-850.

Bouard, J., 1968. The influence of the carbohydrate and nutrient element content of the canes of the vine on the production of grapes. Potash. Rev. 29, 1-7.

Bouquet, A. & Danglot, Y., 1996. Inheritance of seedlessness in grapevine (*Vitis vinifera* L.). Vitis 35, 35-42.

Bravetti, B., Lanari, V., Manni, E. & Silvestroni, O., 2010, August. Canopy density modification and crop control strategies on 'Montepulciano' (*Vitis vinifera* L.). Acta Hortic. 931, 331-337.

Burger, P., Gerber, C., Gerber, A. & Ellis, P.J.L., 2003. Breeding seedless grapes in South Africa by means of embryo rescue. Acta Hortic. 603, 565-569.

Buttrose, M.S., 1969. Fruitfulness in grapevines: Effects of light intensity and temperature. Bot. Gaz. 130, 166-173.

Buttrose, M.S., 1970. Fruitfulness in grapevines: The response of different cultivars to light, temperature and daylength. Vitis 9, 121–125.

Cahoon, G. A., Kaps, M. L. & Pathak, S. P., 1986. Effects of gibberellic acid (GA_3) and daminozide (Alar) on growth and fruiting of Himrod grapes. Ohio Agric. Res. Develop. Center Res. Circular. 29, 30-41.

Candolfi-Vasconcelos, M.C. & Koblet, W., 1990. Yield, fruit quality, bud fertility and starch reserves of the wood as a function of leaf removal in *Vitis vinifera*. Evidence of compensation and stress recovering. Vitis 29, 199-221.

Carmona, M.J., Chaïb, J., Martínez-Zapater, J.M. & Thomas, M.R., 2008. A molecular genetic perspective of reproductive development in grapevine. J. Exp. Bot. 59, 2579-2596.

Casanova, L., Casanova, R., Moret, A. & Agustí, M., 2009. The application of gibberellic acid increases berry size of 'Emperatriz' seedless grape. Span. J. Agric. Res. 7, 919-927.

Caspari, H.W., Lang, A. & Alspach, P. 1998. Effects of girdling and leaf removal on fruit set and vegetative growth in grape. Am. J. Enol. Vitic. 49, 359-366.

Chai, L., Li, Y., Chen, S., Perl, A., Zhao, F. & Ma, H., 2014. RNA sequencing reveals high resolution expression change of major plant hormone pathway genes after young seedless grape berries treated with gibberellin. Plant Sci. 229, 215-224.

Cheng, C., Xu, X., Singer, S.D., Li, J., Zhang, H., Gao, M., Wang, L., Song, J. & Wang, X., 2013. Effect of GA_3 treatment on seed development and seed-related gene expression in grape. PLoS One 8, 1-2.

Chloupek, O., Hrstkova, P. & Schweigert, P., 2004. Yield and its stability, crop diversity, adaptability and response to climate change, weather and fertilisation over 75 years in the Czech Republic in comparison to some European countries. Field Crops Res. 85, 167-190.

Christensen, P., 1975. Long-term responses of "Thompson Seedless" vines to potassium fertilizer treatment. Am. J. Enol. Vitic. 26, 179–183.

Christensen, L.P., 2000. Growth Regulators. Raisin Production Manual. UCANR Publications, California, pp. 139-141.

Christensen, P., Ramming, D. & Andris, H., 1983. Seed trace content of Fiesta grapes. Am. J. Enol. Vitic. 34, 257-259.

Christodoulou, A., Pool, R. & Weaver, R., 1966. Prebloom thinning of Thompson Seedless grapes is feasible when followed by bloom spraying with gibberellin. Calif. Agric. 20, 8-10.

Christodoulou, A., Weaver, R.J. & Pool, R.M., 1967. Response of Thompson Seedless grapes to prebloom thinning. Vitis 6, 303-308.

Chundawat, B. S., Takahashi, E. & Nagasawa, K., 1971. Effect of gibberellic acid, B-nine and kinetin on fruit set, parthenocarpy and quality of Kyoho grapes. J. Japan. Soc. Hort. Sci. 40, 105-109.

Clingeleffer, P.R., 2010. Plant management research: status and what it can offer to address challenges and limitations. Aust. J. Grape Wine Res. 16, 25-32.

Conde, C., Silva, P., Fontes, N., Dias, A.C.P., Tavares, R.M., Sousa, M.J., Agasse, A., Delrot, S. & Gerós, H., 2007. Biochemical changes throughout grape berry development and fruit and wine quality. Food 1, 1–22.

Coombe, B.G., 1959. Physiological aspects of fruit set and development in *Vitis vinifera*. Thesis, University of California, Davis, 1 Shields Ave, Davis, CA 95616, United States.

Coombe, B.G., 1960. Relationship of growth and development to changes in sugars, auxins, and gibberellins in fruit of seeded and seedless varieties of *Vitis vinifera*. Plant Physiol. 35, 241-250.

Coombe, B.G., 1973. The regulation of set and development of the grape berry. Acta Hortic. 34, 261-273.

Coombe, B.G. & Dry, P.R., 1988. Viticulture. Volume 1, Resources in Australia. Australian Industrial Publishers, Adelaide.

Coombe, B.G. & Dry, P.R., 1992. Viticulture. Volume 2, Practices. Winetitles, Adelaide.

Coombe, B.G. & Hale, C.R., 1973. The hormone content of ripening grape berries and the effects of growth substance treatments. Plant Physiol. 51, 629-634.

Coombe, B.G. & Iland, P., 2004. Grape berry development and wine grape quality. In: Dry, P.R. & Coombe, B.G. (eds). Viticulture. Vol. 1. Resources. Winetitles, Adelaide, South Australia, pp. 210-248.

Coombe, B.G. & McCarthy, M.G., 2000. Dynamics of grape berry growth and physiology of ripening. Aust. J. Grape Wine Res. 6, 131-135.

Crane, J.C., 1964. Growth substances in fruit setting and development. Annu. Rev. Plant Physiol. 15, 303-326.

Cubero, S., Diago, M.P., Blasco, J., Tardáguila, J., Prats-Montalbán, J.M., Ibáñez, J., Tello, J. & Aleixos, N., 2015. A new method for assessment of bunch compactness using automated image analysis. Aust. J. Grape Wine Res. 21, 101-109.

Dai, Z.W., Ollat, N., Gomès, E., Decroocq, S., Tandonnet, J.P., Bordenave, L., Pieri, P., Hilbert, G., Kappel, C., van Leeuwen, C. & Vivin, P., 2011. Eco, physiological, genetic, and molecular causes of variation in grape berry weight and composition: A review. Am. J. Enol. Vitic. 62, 413-425.

Davis, D.E., 2002. Inhibition of flower bud initiation and development in apple by defoliation, gibberellic acid and crop load manipulation. Thesis, Virginia Polytechnic Institute and State University, 800 Washington Street, SW, Suite 150, Blacksburg, VA 24061, United States.

Dennis, F.G. & Nitsch, J.P., 1966. Identification of gibberellins A_4 and A_7 in immature apple seeds. Nature 211, 781-782.

Di Lorenzo, R., Gambino, C. & Scafidi, P., 2011. Summer pruning in table grape. Adv. Hortic. Sci. 25, 143-150.

Dokoozlian, N.K., 1998. Use of plant growth regulators in table grape production in California. In: Dokoozlian, N.K. (ed). Proc. Univ. of California Table Grape Production Short Course. Visalia, California, pp. 200–210.

Dokoozlian, N.K., 2000a. Plant Growth Regulator use for table grape production in California. In: Proc. 4th Int. Symp. Table Grape. La Serena, Chile, pp. 129–143.

Dokoozlian, N.K., 2000b. Grape berry growth and development. In: Christensen, L.P. (ed). Raisin production manual, Vol. 3393. UCANR Publications, California. pp. 30-37.

Dokoozlian, N.K. & Peacock, W.L., 2001. Gibberellic acid applied at bloom reduces fruit set and improves size of “Crimson Seedless” table grapes. Hort. Sci. 36, 706-709.

Domingos, S., Nobrega, H., Raposo, A., Cardoso, V., Soares, I., Ramalho, J.C., Leitão, A.E., Oliveira, C.M. & Goulao, L.F., 2016. Light management and gibberellic acid spraying as thinning methods in seedless table grapes (*Vitis vinifera* L.): Cultivar responses and effects on the fruit quality. Sci. Hortic. 201, 68-77.

Domingos, S., Scafidi, P., Cardoso, V., Leitao, A.E., Di Lorenzo, R., Oliveira, C.M. & Goulao, L.F., 2015. Flower abscission in *Vitis vinifera* L. triggered by gibberellic acid and shade discloses difference in the underlying metabolic profiles. Front. Plant Sci. 6, 457.

Dragincic, J., Korac, N. & Blagojevic, B., 2015. Group multi-criteria decision making (GMCDM) approach for selecting the most suitable table grape variety intended for organic viticulture. Comput. Electron. Agric. 111,194-202.

Dry, P., 2000. Canopy management for fertility. Aust. J. Grape Wine Res. 6, 109-115.

Duchêne, E., Jaegli, N. & Saliber, R. 2003a. Effects of the leaf / fruit ratio during a year N on the bud fertility in the year N+2: results for Pinot noir and Gewurztraminer grapevines (*Vitis vinifera* L.) in the greenhouse. J. Int. Sci. Vigne. Vin. 37, 257-260.

Duchêne, E., Monamy, C., Langellier, F., Jaegli, N., Salber, R., Meluc, D. & Panigai, L. 2003b. Effects of the ripening conditions in the vineyard on growth and yield components in the following season. J. Int. Sci. Vigne. Vin. 37, 103-106.

Dunn, G.M. & Martin, S.R., 2000. Do temperature conditions at budburst affect flower number in *Vitis vinifera* L. cv. Cabernet Sauvignon? Aust. J. Grape Wine Res. 6, 116-124.

Durner, E.F., 2013. Principles of Horticultural Physiology. CABI, Boston.

Elgendy, R.S.S., Shaker, G.S.H. & Ahmed, O.A., 2012. Effect of foliar spraying with GA_3 and/or CPPU on bud behavior, vegetative growth, yield and cluster quality of Thompson seedless grapevines. J. Am. Sci. 8, 21-34.

Eltom, M., Trought, M.C., Agnew, R., Parker, A. & Winefield, C.S., 2017. Pre-budburst temperature influences the inner and outer arm morphology, phenology, flower number, fruitset, TSS accumulation and variability of *Vitis vinifera* L. Sauvignon Blanc bunches. Aust. J. Grape Wine Res. 23, 280-286.

Ferreira, J.H.S. & Marais, P.G., 1987. Effect of rootstock cultivar, pruning method and crop load on *Botrytis cinerea* rot of *Vitis vinifera* cv. Chenin blanc grapes. S. Afr. J. Enol. Vitic. 8, 41-44.

Ferrer, M., Abella, J.M., Sibille, I., Camussi, G. & González-Neves, G. 2004. Determination of bud fertility as a simple method for the determination of harvesting volume in *Vitis vinifera* L. cv. Tannat, using two pruning systems. OENO One 38, 49-53.

Fosket, D.E., 1994. Plant growth and development: a molecular approach. Academic Press Inc. San Diego, California.

Fukunaga, S. & Kurooka, H., 1987. Studies of seedlessness of 'Kyoho' grapes induced by gibberellin in combination with Streptomycin. Bul. Univ. Osaka Prefec. 40, 1-10.

Gao, X., Zhang, Y., He, Z. & Fu, X., 2017. Gibberellins. In: Li, J., Li, C., & Smith, S.M. (eds)., 2017. Hormone metabolism and signaling in plants. Academic Press. pp.107-160

Gatti, M., Garavani, A., Cantatore, A., Parisi, M.G., Bobeica, N., Merli, M.C., Vercesi, A. & Poni, S., 2015. Interactions of summer pruning techniques and vine performance in the white *Vitis vinifera* cv. Ortrugo. Aust. J. Grape Wine Res. 21, 80-89.

Gerrath, J.M. & Posluszny, U., 1988. Morphological and anatomical development in the Vitaceae. I. Vegetative development in *Vitis riparia*. Can. J. Bot. 66, 209-224.

Giacomelli, L., Rota-Stabelli, O., Masuero, D., Acheampong, A.K., Moretto, M., Caputi, L., Vrhovsek, U. & Moser, C., 2013. Gibberellin metabolism in *Vitis vinifera* L. during bloom and fruit-set: functional characterization and evolution of grapevine gibberellin oxidases. J. Exp. Bot. 64, 4403-4419.

Gil, G.F., Rivera, M., Veras, F. & Zoffoli, J.P., 1994. Effectiveness and mode of action of Gibberelllic acid on grape berry thinning. In: Int. Symp. Table Grape Prod. Anaheim, California, pp. 43–46.

Guelfat-Reich, S. & Safran, B., 1973. Maturity responses of Sultanina grapes to Gibberellic acid treatments. Vitis 12, 33-37.

Halbrooks, M.C. & Mortensen, J.A., 1987. Influence of gibberellic acid and various management practices on berry, seed and cluster development in 'Orlando Seedless' grape. Proc. Flo. State Hort. Soc. 100, 312-315.

Hale, C.R. & R.J. Weaver., 1962. The effect of developmental stage on direction of translocation of photosynthate in *Vitis vinifera*. Hilgardia 33, 89-141.

Harris, J.M., Kriedemann, P.E. & Possingham, J.V., 1968. Anatomical aspects of grape berry development. Vitis, 106-109.

Hed, B., Ngugi, H.K. & Travis, J.W., 2009. Relationship between cluster compactness and bunch rot in Vignoles grapes. Plant Dis. 93, 1195-1201.

Hed, B., Ngugi, H.K. & Travis, J.W., 2011. Use of gibberellic acid for management of bunch rot on Chardonnay and Vignoles grape. Plant Dis. 95, 269-278.

Hed, B., Ngugi, H.K. & Travis, J.W., 2015. Short-and long-term effects of leaf removal and gibberellin on Chardonnay grapes in the Lake Erie region of Pennsylvania. Am. J. Enol. Vitic. 66, 22-29.

Hopping, M.E., 1976. Effect of bloom applications of gibberellic acid on yield and bunch rot of the wine grape 'Seibel 5455'. New Zeal. J. Exp. Agric. 4, 103-107.

Howell, G.S., Candolfi-Vasconcelos, M.C. & Koblet, W., 1994. Response of Pinot noir grapevine growth, yield, and fruit composition to defoliation the previous growing season. Am. J. Enol. Vitic. 45, 188-191.

Hunter, K., Volschenk, C. & Zorer, R., 2018. Canopy management: Grapevine row orientation: A factor in microclimate, physiology, growth and yield. Wine Vitic. J. 33, 40-46.

Iland, P., Dry, P., Proffitt, T. & Tyerman, S.D., 2011. The grapevine: from the science to the practice of growing vines for wine. Patrick Iland Wine Promotions Pty Ltd, Adelaide, South Australia.

Intrieri, C., Filippetti, I., Allegro, G., Centinari, M. & Poni, S., 2008. Early defoliation (hand vs mechanical) for improved crop control and grape composition in Sangiovese (*Vitis vinfera* L.). Aust. J. Grape Wine Res. 14, 25-32.

Ivorra, E., Sánchez, A.J., Camarasa, J.G., Diago, M.P. & Tardáguila, J., 2015. Assessment of grape cluster yield components based on 3D descriptors using stereo vision. Food Control 50, 273-282.

Iwahori, S., Weaver, R.J. & Pool, R.M., 1968. Gibberellin-like activity in berries of seeded and seedless Tokay grapes. Plant Physiol. 43, 333-337.

Jackson, R.S., 2008. Wine science: principles and applications. Academic press.

Jako, N., 1976. The relationship between nitrogen phosphorus, potassium and magnesium nutrition and growth of grapevines and cytokinin production by the roots. Szoleszet Borazet 1, 35-47.

Jawanda, J.S., Singh, R. & Pal, R.N., 1974. Effect of growth regulators on floral bud drop, fruit characters and quality of Thompson Seedless grape (*Vitis vinifera* L.). Vitis 13, 215-221.

Jensen, F.L., 1994. Table grape production in California. In: Rantz, J.M. (ed). Proc. First Intl. Symp. on Table Grape Production, Anaheim, California, pp. 26-30.

Kato, K., Ohara, H., Takahashi, E., Matsui, H. & Nakayama, M., 1998. Endogenous gibberellin-induced parthenocarpy in grape berries. Acta Hortic. 514, 69-74.

Keller, M., 2010 (1st ed). The science of grapevines: Anatomy and physiology. Academic Press, London.

Keller, M., 2015 (2nd ed). The science of grapevines: Anatomy and physiology. Academic Press, London.

Keller, M. & Koblet, W., 1995. Dry matter and leaf area partitioning, bud fertility and second season growth of *Vitis vinifera* L.: Responses to nitrogen supply and limiting irradiance. Vitis 34, 77-83.

Keller, M., Kummer, M. & Candolfi-Vasconcelos, M.C., 2001. Reproductive growth of grapevines in response to nitrogen supply and rootstock. Aust. J. Grape. Wine Res. 7, 12-18.

Keller, M. & Mills, L., 2004. Crop load management in Concord grapes using different pruning techniques. Am. J. Enol. Vitic. 55, 35.50.

Kende, H. & Zeevaart, J., 1997. The five" Classical" plant hormones. In: The plant cell, 9(7), pp.1197-1210.

Khanduja, S.D. & Abbas, S., 1973. Estimating fertility of grape-vine buds by forced bursting in summer in the tropics of India. Vitis 12, 23-25.

Khanduja, S. D. & Balasubrahmanyam, V. R., 1972. Fruitfulness of grape vine buds. Economic Bot. 26, 280-294.

Kicherer, A., Roscher, R., Herzog, K., Förstner, W. & Töpfer, R., 2014. Image based evaluation for the detection of cluster parameters in grapevine. Acta Hortic. 1082, 335-340.

Kimura, P.H., Okamoto, G. & Hirano, K., 1996. Effects of gibberellic acid and streptomycin on pollen germination and ovule and seed development in Muscat Bailey A. Am. J. Enol. Vitic. 47, 152-156.

Kliewer, W.M., 1977. Effect of high temperatures during the bloom-set period on fruit-set, ovule fertility, and berry growth of several grape cultivars. Am. J. Enol. Vitic. 28, 215-222.

Korkutal, I., Bahar, E. & Gökhan, Ö., 2008. The characteristics of substances regulating growth and development of plants and the utilization of Gibberellic Acid (GA_3) in viticulture. World J. Agric. Sci. 4, 321-325.

Kotseridis, Y., Georgiadou, A., Tikos, P., Kallithraka, S. & Koundouras, S., 2012. Effects of severity of post-flowering leaf removal on berry growth and composition of three red *Vitis vinifera* L. cultivars grown under semiarid conditions. J. Agric. Food Chem. 60, 6000-6010.

Lavee, S., 1960. Effect of gibberellic acid on seeded grapes. Nature 185, 395.

Lavee, S., 1987. Necrosis in grapevine buds (*Vitis vinifera* cv. Queen of Vineyard) III. Endogenous gibberellin levels in leaves and buds. Vitis 26, 225-30.

Lavee, S., Melamud, H., Ziv, M. & Bernstein, Z., 1981. Necrosis in grapevine buds (*Vitis vinifera* cv. Queen of Vineyard) I. Relation to vegetative vigor. Vitis 20, 8-14.

Lavee, S. & Nir, G., 1986. Grape. In: Monselise S.P. (ed). Handbook of fruit set and development. CRC Press, Florida. pp. 167-191.

Lavee, S., Ziv, M., Melamud, H. & Berstein, Z., 1993. The involvement of Gibberellins in controlling bud development of grape vines (*Vitis vinifera* L.). Acta Hortic. 329, 177-182.

Lebon, G., Wojnarowiez, G., Holzapfel, B., Fontaine, F., Vaillant-Gaveau, N. & Clémen, C., 2008. Sugars and flowering in the grapevine (*Vitis vinifera* L.). J. Exp. Bot. 59, 2565-2578

Li-Mallet, A., Rabot, A. & Geny, L., 2016. Factors controlling inflorescence primordia formation of grapevine: Their role in latent bud fruitfulness? A review. Bot. 94, 147-163.

Lombard, P.J., Cook, N.C. & Bellstedt, D.U., 2006. Endogenous cytokinin levels of table grape vines during spring budburst as influenced by hydrogen cyanamide application and pruning. Sci. Hortic. 109, 92-96.

Lynn, C.D. & Jensen, F.L., 1966. Thinning effects of bloom time Gibberellin sprays on Thompson Seedless table grapes. Am. J. Enol. Vitic. 17, 283-289.

Mander, L. & Liu, H.W., 2010. Comprehensive natural products II: Chemistry and Biology (Vol. 1). Elsevier Science.

Matthews, M.A., Anderson, M.M. & Schultz, H., 1987. Phenologic and growth responses to early and late season. Vitis 26, 147-160.

Matsui, S., Ryugo, K. & Kliewer, W.M., 1986. Growth inhibition of Thompson Seedless and Napa Gamay berries by heat stress and its partial reversibility by applications of growth regulators. Am. J. Enol. Vitic. 37, 67-71.

May, P., 1961. The value of an estimate of fruiting potential in the Sultana. Vitis 3,15-26.

May, P., 2000. From bud to berry, with special reference to inflorescence and bunch morphology in *Vitis vinifera* L. Aust. J. Grape Wine Res. 6, 82-98.

May, P. & Antcliff, A.J., 1973. The fruitfulness of grape buds. I. Measuring bud fertility on forced single-node cuttings. Ann. l'Amélioration des Plantes 23, 1-12.

May, P., Clingeleffer, P.R. & Brien, C.J., 1976. Sultana (*Vitis vinifera* L.) canes and their exposure to light. Vitis 14, 278-288.

McCarthy, M.G., 1997. The effect of transient water deficit on berry development of cv. Shiraz (*Vitis vinifera* L.). Aust. J. Grape Wine Res. 3, 2-8.

McArtney, S.J., 1998. Remobilization of stored carbohydrates and nitrogen and their importance for initial development of grapevine shoots. Thesis, The Ohio State University, 281 W Lane Ave, Columbus, OH 43210, United States.

Miele, A., Weaver, R.J. & Johnson, J., 1978. Effect of potassium gibberellate on fruit-set and development of Thompson Seedless and Zinfandel grapes. Am. J. Enol. Vitic. 29, 79-82.

Mohamed, A.K.A., El-Salhy, A.M., Mostafa, R.A.A., El-Mahdy, M.T. & Hussein, A.S., 2019. Effect of Exogenous Abscisic Acid (ABA), Gibberellic Acid (GA_3) and Cluster Thinning on Yield of some Grape Cultivars. J Plant Prod. 10, 101-105.

Molitor, D., Behr, M., Fischer, S., Hoffmann, L. & Evers, D., 2011a. Timing of cluster-zone leaf removal and its impact on canopy morphology, cluster structure and bunch rot susceptibility of grapes. J. Tnt. Sci. Vigne. Vin. 45, 149-159.

Molitor, D., Behr, M., Hoffmann, L. & Evers, D., 2012a. Research note: Benefits and drawbacks of pre-bloom applications of gibberellic acid (GA_3) for stem elongation in Sauvignon blanc. S. Afr. J. Enol Vitic. 33, 198-202.

Molitor, D., Behr, M., Hoffmann, L. & Evers, D., 2012b. Impact of grape cluster division on cluster morphology and bunch rot epidemic. Am. J. Enol. Vitic. 63, 508-514.

Molitor, D., Rothmeier, M., Behr, M., Fischer, S., Hoffmann, L. & Evers, D., 2011b. Crop cultural and chemical methods to control grey mould on grapes. Vitis 50, 81-87.

Moncur, M.W., Rattigan, K., Mackenzie, D.H. & Mc Intyre, G.N., 1989. Base temperatures for budbreak and leaf appearance of grapevines. Am. J. Enol. Vitic. 40, 21-26.

Morrison, J.C., 1991. Bud development in *Vitis vinifera* L. Bot. Gaz. 152, 304-315.

Motomura, Y. & Ito, H., 1972. Exogenous gibberellin as responsible for the seedless development of grapes. Tohoku J. Agr. Res. 23, 15-31.

Mullins, M.G., 1986. Hormonal regulation of flowering and fruit set in the grapevine. Acta Hortic. 179, 309-315.

Mullins, M.G., Bouquet, A. & Williams, L.E., 1992. Biology of Horticultural crops: Biology of the grapevine. Cambridge University Press, Cambridge.

Mullins, M.G. & Rajasekaran, K., 1981. Fruiting cuttings: Revised method for producing test plants of grapevine cultivars. Am. J. Enol. Vitic. 32, 35-40.

Myburgh, P.A., 2003. Responses of *Vitis vinifera* L. cv. Sultanina to water deficits during various pre- and post-harvest phases under semi-arid conditions. S. Afr. J. Enol. Vitic. 24, 25-33.

Nakagawa, S., Bukovac, M.J., Hirata, N. & Kurokawa, H., 1968. Morphological studies of gibberellin-induced parthenocarpic and asymmetric growth in apple and Japanese pear fruits. J. Japan Soc. Hort. Sci. 37, 9-19.

Nakamura, M., Takahashi, E. & Noda, T., 1974. Rachis lignification and seedless berry production in seeded grape cultivar “Kyoho” as influenced by gibberellin and quercetin. Technical Bulletin of the Faculty of Horticulture, Chiba University 22, 7-12

Nitsch, J.P., Pratt, C., Nitsch, C. & Shaulis, N.J., 1960. Natural growth substances in Concord and Concord seedless grapes in relation to berry development. Am. J. Bot. 47, 566-576.

Ojeda, H., Deloire, A. & Carbonneau, A., 2001. Influence of water deficits on grape berry growth. Vitis 40, 141-145.

Okamoto, G. & Miura, K., 2005. Effect of pre-bloom GA application on pollen tube growth in cv. Delaware grape pistils. Vitis 44, 157-159.

Orth, C.H.F., 1990. Effect of spraying or dipping with gibberellic acid on bud fertility of Muscat Seedless. Dec. Fruit Grower 40, 289-292.

Padmalatha, K., Weksler, H., Mugzach, A., Acheampong, A.K., Zheng, C., Halaly-Basha, T. & Or, E., 2017. ABA application during flowering and fruit set reduces berry number and improves cluster uniformity. Am. J. Enol. Vitic. 68, 275-282.

Pallardy, S.G, 2008 (3rd ed). Physiology of Woody Plants. Academic Press, London.

Palliotti, A., Gatti, M. & Poni, S., 2011. Early leaf removal to improve vineyard efficiency: gas exchange, source-to-sink balance, and reserve storage responses. Am. J. Enol. Vitic. 62, 219-228.

Palliotti, A., Gardi, T., Berrios, J.G., Civardi, S. & Poni, S., 2012. Early source limitation as a tool for yield control and wine quality improvement in a high-yielding red *Vitis vinifera* L. cultivar. Sci. Hortic. 145, 10-16.

Palma, B.A. & Jackson, D.I., 1989. Inflorescence initiation in grapes - responses to plant growth regulators. Vitis 28, 1-12.

Peacock, W., 1998. Influence of GA_3 sizing sprays on Ruby Seedless. Univ. Calif. Coop. Extension, Tulare Cty. pp. 897

Perez, J. & Kliewer, W.M., 1990. Effect of shading on bud necrosis and bud fruitfulness of Thompson Seedless grapevines. Am. J. Enol. Vitic. 41, 168-175.

Perez, F. J., Viani, C. & Retamales, J., 2000. Bioactive gibberellins in seeded and seedless grapes: identification and changes in content during berry development. Am. J. Enol. Vitic. 51, 315-318.

Perl, A., Sahar, N. & Spiegel-Roy, P., 2000. Conventional and biotechnological approaches in breeding seedless table grapes. Acta Hortic. 528, 607-612.

Piazzolla, F., Pati, S., Amodio, M.L. & Colelli, G., 2016. Effect of harvest time on table grape quality during on-vine storage. J. Sci. Food Agric. 96, 131-139.

Pommer, C.V., Pires, E.J.P., Terra, M.M. & Passos, I.R.S., 1996. Streptomycin-induced seedlessness in the grape cultivar Rubi (Italia Red). Am. J. Enol. Vitic. 47, 340-342.

Pongracz, D.P., 1978. Practical viticulture. David Philip Publisher (Pty.) Ltd., Cape Town.

Poni, S., Magnanini, E. & Bernizzoni, F., 2003. Degree of correlation between total light interception and whole-canopy net CO_2 exchange rate in two grapevine growth systems. Aust. J. Grape Wine Res. 9, 2-11.

Pratt, C., 1971. Reproductive anatomy in cultivated grapes - A Review. Am. J. Enol. Vitic. 22, 92-109.

Quinlan, J.D. & Weaver, R.J., 1970. Modification of the photosynthate movement within and between shoots of *Vitis vinifera* L. Plant Physiol. 46, 527-530.

Raath, P.J., 2012. Effect of varying levels of nitrogen, potassium and calcium nutrition on table grape vine physiology and berry quality. Thesis, Stellenbosch University, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Raban, E., Kaplunov, T., Zutahy, Y., Daus, A., Alchanatis, V., Ostrovsky, V., Lurie, S. & Lichter, A., 2013. Rachis browning in four table grape cultivars as affected by growth regulators or packaging. Postharvest Biol. Technol. 84, 88-95.

Rademacher, W., 2015. Plant growth regulators: Backgrounds and uses in plant production. J. Plant Growth Regul. 34, 845-872.

Reisch, B.I., Owens, C.L. & Cousins, P.S., 2012. Grape. In: Badenes, M.L. & Byrne, D.H. (eds). Fruit breeding. Springer, Boston, pp. 225-262.

Retamales, J. & Cooper, T., 1993. Berry drop and fruit removal forces as related with GA_3 applications in table grapes. Acta Hortic. 329, 81-83.

Reynolds A.G. & de Savigny C., 2004. Influence of girdling and gibberellic acid on yield components, fruit composition, and vestigial seed formation of 'Sovereign Coronation' table grapes. Hort. Sci. 39, 541-544.

Reynolds A.G., Roller J.N., Forgione A. & de Savigny C., 2006. Gibberellic acid and basal leaf removal: Implications for fruit maturity, vestigial seed development, and sensory attributes of Sovereign Coronation table grapes. Am. J. Enol. Vitic. 57, 41-53.

Roberto, S.R., Borges, W.F.S., Colombo, R.C., Koyama, R., Hussain, I. & de Souza, R.T., 2015. Berry-cluster thinning to prevent bunch compactness of 'BRS Vitoria', a new black seedless grape. Sci. Hortic. 197, 297-303.

Roberts, J.A. & Hooley, R., 1988. Plant Growth Regulators. Chapman and Hall, New York.

Robinson, S.P. & Davies, C., 2000. Molecular biology of grape berry ripening. Aust. J. Grape Wine Res. 6, 175-188.

Roubelakis-Angelakis, K.A., 2009. Grapevine Molecular Physiology & Biotechnology. Springer, Dordrecht.

Sabbatini, P. & Howell, G.S., 2010. Effects of early defoliation on yield, fruit composition, and harvest season cluster rot complex of grapevines. Hort. Sci. 45, 1804-1808.

Sánchez, L.A. & Dokoozlian, N.K., 2005. Bud microclimate and fertility in *Vitis vinifera* L. Am. J. Enol. Vitic. 56, 319-329.

Sanchez-Rodriguez, L.A. & Spósito, M.B., 2020. Influence of the trellis/training system on the physiology and production of *Vitis labrusca* cv. Niagara Rosada in Brazil. Sci. Hortic. 261.

SATI., 2019a. Guidelines for the preparation of table grapes for export 2019/20. Van der Merwe, G.G. (ed). South African Table Grape Industry, PO Box 2932, Paarl, 7620.

SATI., 2019b. Statistics Booklet 2019. Lombardt, J. (ed). South African Table Grape Industry. SATI, PO Box 2932, Paarl, 7620.

Schöler, F. & Steinhage, V., 2015. Automated 3D reconstruction of grape cluster architecture from sensor data for efficient phenotyping. Comput. Electron. Agric. 114, 163-177.

Scienza, A., Miravalle, R., Visai, C. & Fregdni, M., 1978. Relationships between seed number, gibberellin and abscisic acid levels and ripening in 'Cabernet Sauvignon' grape berries. Vitis 17, 361-368.

Shavrukov, Y.N., Dry, I.B. & Thomas, M.R., 2004. Inflorescence and bunch architecture development in *Vitis vinifera* L. Aust. J. Grape Wine Res. 10, 116-124.

Shoeib, M.M., 2012. Effect of supporting systems on vines growth and root distribution of Flame Seedless grapevines. J. Plant Prod. 3, 349-365.

Shulman, Y., Nir, G., Fanberstein, L. & Lavee, S., 1983. The effect of cyanamide on the release from dormancy of grapevine buds. Sci. Hortic 19, 97-104.

Silvestroni, O., Lanari, V., Lattanzi, T., Palliotti, A. & Sabbatini, P., 2016. Impact of crop control strategies on performance of high-yielding Sangiovese grapevines. Am. J. Enol. Vitic. 67, 407-418.

Singh, K., Weaver, R.J. & Johnson, J.O., 1978. Effect of applications of gibberellic acid on berry size, shatter, and texture of Thompson Seedless grapes. Am. J. Enol. Vitic. 29, 258-262.

Skinner, P.W., & Matthews, M.A., 1989. Reproductive development in grape (*Vitis vinifera* L.) under phosphorus-limited conditions. Sci. Hortic. 38, 49-60.

Smart, R.E. & Smith, S.M., 1988. Canopy management: identifying the problems and practical solutions. In: Anon. (ed). Proc. 2nd International Cool Climate Viticulture and Oenology Symposium, Auckland, pp. 109-115.

Sommer, K.J., Clingeleffer, P.R. & Ollat, N.,1993. Effects of minimal pruning on grapevine canopy development, physiology and cropping level in both cool and warm climates. Vitic. Enol. Sci. 48, 135-139.

Sommer, K.J., Islam, M.T. & Clingeleffer, P.R., 2000. Light and temperature effects on shoot fertility in *Vitis vinifera* L. cv. Sultana: Influence of trellis type and grafting. Aust. J. Grape Wine Res. 6, 99-108.

Sommer, K.J., Islam, M.T. & Clingeleffer, P.R., 2001. Sultana fruitfulness and yield as influenced by season, rootstock and trellis type. Aust. J. Grape Wine. Res. 7,19-26.

Sonnekus, N., 2015. Development and change that occurs in table grape berry composition during growth. Thesis, Stellenbosch University, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Sponsel, V.M. & Hedden, P., 2004. Gibberellin biosynthesis and inactivation. In: Davies, P.J. (ed). In: Plant Hormones. Biosynthesis, Signal transduction, Action! Springer, Dordrecht, pp. 63–94.

Stout, A., 1936. Seedlessness in grapes. NY Agr. Exp. Stn. Tech. Bull. 238.

Srinivasan, C. & Mullins, M.G., 1976. Reproductive anatomy of the grape-vine (*Vitis vinifera* L.): Origin and development of the anlage and its derivatives. Ann. Bot. 40, 1079-1084.

Srinivasan, C. & Mullins, M.G., 1979. Flowering in *Vitis*: Conversion of tendrils into inflorescences and bunches of grapes. Planta 145(2), 187–192.

Srinivasan, C. & Mullins, M.G., 1980. Effects of temperature and growth regulators on formation of anlagen, tendrils and inflorescences in *Vitis vinifera* L. Ann. Bot. 45(4), 439–446.

Srinivasan, C. & Mullins, M.G., 1981a. Induction of precocious flowering in grapevine seedlings by growth regulators. Agron. EDP Sci. pp. 1–5.

Srinivasan, C. & Mullins, M.G., 1981b. Physiology of flowering in the grapevine-A review. Am. J. Enol. Vitic. 32, 47-63.

Srivastava, L.M., 2002. Plant growth and development: hormones and environment. Academic press.

Sugiura, A. & Inaba, A., 1966. Studies on the mechanism of gibberellin-induced seedlessness of Delaware grapes. I. J. Jpn. Soc. Hortic. Sci. 35, 233-241.

Symons, G.M., Davies, C., Shavrukov, Y., Dry, I.B., Reid, J.B. & Thomas, M.R., 2006. Grapes on steroids. Brassinosteroids are involved in grape berry ripening. Plant Physiol. 140, 150-158.

Swanepoel, J.J. & Archer, E., 1988. The ontogeny and development of *Vitis vinifera* L. cv. Chenin blanc inflorescence in relation to phenological stages. Vitis 27, 133-141.

Swanepoel, J.J. & Baard, F., 1988. The correlation between potential and actual fertility in the grapevine. Dec. Fruit Grower 38, 231-235.

Tang, D., Wang, Y., Cai, J. & Zhao, R., 2009. Effects of exogenous application of plant growth regulators on the development of ovule and subsequent embryo rescue of stenospermic grape (*Vitis vinifera* L.). Sci. Hortic. 120, 51-57.

Tardáguila, J., Blanco, J.A., Poni, S. & Diago, M.P., 2012. Mechanical yield regulation in winegrapes: Comparison of early defoliation and crop thinning. Aust. J. Grape Wine Res. 18, 344-352.

Tardáguila, J., Diago, M.P., de Toda, F.M., Poni, S. & Vilanova, M., 2008. Effects of timing of leaf removal on yield, berry maturity, wine composition and sensory properties of cv. Grenache grown under non irrigated conditions. OENO One 42, 221-229.

Tello, J., Cubero, S., Blasco, J., Tardaguila, J., Aleixos, N. & Ibáñez, J., 2016. Application of 2D and 3D image technologies to characterise morphological attributes of grapevine clusters. J. Sci. Food Agric. 96, 4575-4583.

Tello, J. & Ibáñez, J., 2014. Evaluation of indexes for the quantitative and objective estimation of grapevine bunch compactness. Vitis 53, 9-16.

Tello, J. & Ibáñez, J., 2018. What do we know about grapevine bunch compactness? A state-of-the-art review. Aust. J. Grape Wine Res. 24, 6-23.

This, P., Lacombe, T. & Thomas, M., 2006. Historical origins and genetic diversity of wine grapes. Trends Genet. 22, 511-519.

Tucker, S.C. & Hoefert, L.L., 1968. Ontogeny of the tendril in *Vitis vinifera*. Am. J. Bot. 55, 1110-1119.

Ungsa, M., Kato K., Takemura, K., Hori, T., Ohara, H., Ohkawa, K., Matsui, H. & Bukovac, M.J., 2008. Effects of the combination of gibberellic acid and ammonium nitrate on the growth and quality of seedless berries in 'Delaware' grape. J. Jpn. Soc. Hortic. Sci. 72, 366-371.

Vail, M.E. & Marois, J.J., 1991. Grape cluster architecture and the susceptibility of berries to Botrytis cinerea. Phytopathology 81, 188-191.

Vaillant-Gaveau, N., Maillard, P., Wojnarowiez, G., Gross, P., Clément, C. & Fontaine, F., 2011. Inflorescence of grapevine (*Vitis vinifera* L.): a high ability to distribute its own assimilates. J. Exp. Bot. 62, 4183-4190.

Van der Vyver, L., 2016. The influence of gibberellic acid (GA_3) for berry thinning and berry sizing on table grape production, quality and fertility of Prime. Thesis, Stellenbosch University, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Varoquaux, F., Blanvillain, R., Delseny, M. & Gallois, P., 2000. Less is better: new approaches for seedless fruit production. Trends Biotechnol. 18, 233-242.

Vasconcelos, M.C., Greven, M., Winefield, C.S., M.C.T., T. & Raw, V., 2009. The flowering process of *Vitis vinifera*: A Review. Am. J. Enol. Vitic. 60, 411-434.

Vivanco, J.M. & Flores, H.F., 2000. Control of root formation by plant growth regulators. In: Basra, A.S. (ed). Plant growth regulators in agriculture and horticulture. Their role and commercial use. Food Products Press, New York, pp. 1-16.

Watt, A.M., Dunn, G.M., May, P.B., Crawford, S.A. & Barlow., E.W.R., 2008. Development of inflorescence primordia in *Vitis vinifera* L. cv. Chardonnay from hot and cool climates. Aust. J. Grape Wine Res. 14, 46-53.

Weaver, R.J., 1960. Gibberellins on grape. Calif. Agric. 14, 3.

Weaver, R.J & McCune, S.B., 1960. Further studies with gibberellin on *Vitis vinifera* grapes. Bot. Gaz. 121, 155-162.

Weaver, R.J., McCune, S.B. & Hale, C.R., 1962. Effect of plant regulators on set and berry development in certain seedless and seeded varieties of *Vitis vinifera* L. Vitis 3, 84–96.

Weaver, R.J. & McCune, S.B., 1962. Studies on prebloom sprays of gibberellin to elongate and loosen clusters of Thompson Seedless grapes. Am. J. Enol. Vitic. 13, 15-19.

Weaver, R.J. & Pool, R.M., 1971. Chemical thinning of grape clusters (*Vitis vinifera* L.). Vitis 10, 201-209.

Wei, X., Sykes, S.R. & Clingeleffer, P.R., 2002. An investigation to estimate genetic parameters in CSIRO's table grape breeding program. 2. Quality characteristics. Euphytica 128, 343-351.

Weyand, K.M. & Schultz, H.R., 2005. Physiological responses of minimal pruning systems to Gibberellic acid. Acta Hortic. 689, 117-124.

Weyand, K.M. & Schultz, H.R., 2006. Regulating yield and wine quality of minimal pruning systems through the application of gibberellic acid. OENO One 40, 151-163.

Williams, L.E., 2000. Bud development and fruitfulness of grapevines. In: Christensen, L.P. (ed). Raisin production manual, Vol. 3393. UCANR Publications, California. pp. 24-29.

Winkler, A.J., Cook, J.A., Kliewer, W.M. & Lider, L.A., 1962. General Viticulture. University of California Press, California.

Winkler, A.J. & Shemsettin, E.M., 1937. Fruit-bud and flower formation in the Sultanina grape. Hilgardia 10, 589-611.

Wolf, E.E.H. & Loubser, J.T., 1992. Gibberellic acid levels and quality effects of Gibberellic acid in treated table grapes. S. Afr. J. Enol. Vitic. 13, 57-63.

Xia, E.Q., Deng, G.F., Guo, Y.J. & Li, H.B., 2010. Biological activities of polyphenols from grapes. Int. J. Mol. Sci. 11, 622–646.

Yamaguchi, S., 2008. Gibberellin metabolism and its regulation. Annu. Rev. Plant Biol. 59, 225-251.

Zabadal, T.J. & Bukovac, M.J., 2006. Effect of CPPU on fruit development of selected seedless and seeded grape cultivars. Hort. Sci.41, 154-157.

Zabadal, T.J. & Dittmer, T.W., 1998. Vine management systems affect yield, fruit quality, cluster compactness, and fruit rot of Chardonnay' Grape. Hort. Sci. 33, 806-809.

Zabadal, T.J. & Dittmer, T.W., 2000. Influence of gibberellic acid sprays on berry size and shot berry on 'Vanessa' grapevines. Acta Hortic. 527, 153-157.

Zhang, X., Luo, G., Wang, R., Wang, J. & Himelrick, D.G., 2003. Growth and developmental responses of seeded and seedless grape berries to shoot girdling. J. Am. Soc. Hortic. Sci. 128, 316-323.

Zhou, J., Cao, L., Chen, S., Perl, A. & Ma, H., 2015. Consumer-assisted selection: the preference for new table grape cultivars in China. Aust. J. Grape Wine Res. 21, 351-360.

Zoffoli, J.P., Latorre, B.A. & Naranjo, P., 2009. Preharvest applications of growth regulators and their effect on postharvest quality of table grapes during cold storage. Postharvest Biol. Technol. 51, 183-192

Chapter 3

Materials and methods

CHAPTER 3:
Materials and methods

3.1 SITE DESCRIPTION

The experiment was conducted over the 2015/2016 and 2016/2017 growing seasons in a commercial vineyard located on the premises of the ARC Infruitec-Nietvoorbij experimental farm in De Doorns (33°27'56"S, 19°39'44"E). The experimental vineyard consisted of 15-year-old *Vitis vinifera* L. cv. Sunred Seedless vines, grafted onto Ramsey (*Vitis champinii*). The vigour of the block is considered moderate. The vines were planted with a 3.0 m × 1.8 m spacing in an East-West row direction and trained onto a Trentina trellis system. Grapevines were irrigated through micro-sprinklers during the growing season on a fixed schedule. The Fernwood soil form found at the experimental site is representative of the Hex River region with grey, sandy soils (Myburgh & Howell, 2007). The sandy soil at the site contained predominantly medium (42.44%), fine (30.8%) and coarse sand (23.7%), with low clay (2.0%) and silt (0.6%) content (Myburgh & Howell, 2007).

The vines were pruned with an average of six half long bearers (nine to ten buds per cane) and twelve spurs (two to three buds) per vine. Standard canopy management practices were applied, which included suckering, leaf removal and crop control. Leaf removal around the bunch zone was applied before the application of plant growth regulators. The crop load was reduced to 28 bunches per vine before flowering as part of crop control practices.

3.1.1 Long term weather data

The weather station, Normandi, was used as the source of weather data for the 2015/2016 and 2016/2017 seasons (Source: Ileaf: www.ileaf.co.za). Normandi is situated approximately 3.27 km from the experimental site. The Hex River Valley has a Mediterranean climate with warm, dry summer periods and cold, wet winter periods.

The average monthly minimum and maximum temperatures from January 2015 to March 2017 are given in Figure 3.1. For the 2015/2016 season, the average minimum and maximum temperatures for November to January was 12.6°C and 29.5°C, respectively, compared to 12.2°C and 30.6°C, for the same period of the 2016/2017 season. January 2017 had lower minimum temperatures compared to January 2016. The warmest month of the year in the region, January is also the month in which Sunred Seedless ripens. Grapes were harvested on the same date in each season. During August to October 2016, notably lower minimum temperatures occurred compared to the same period in 2015. November 2016 had noticeably higher maximum temperatures compared to

November 2015. Apart from the exceptions mentioned above, the minimum and maximum temperatures over the 2015/2016 and 2016/2017 season followed similar trends (Fig. 3.1).

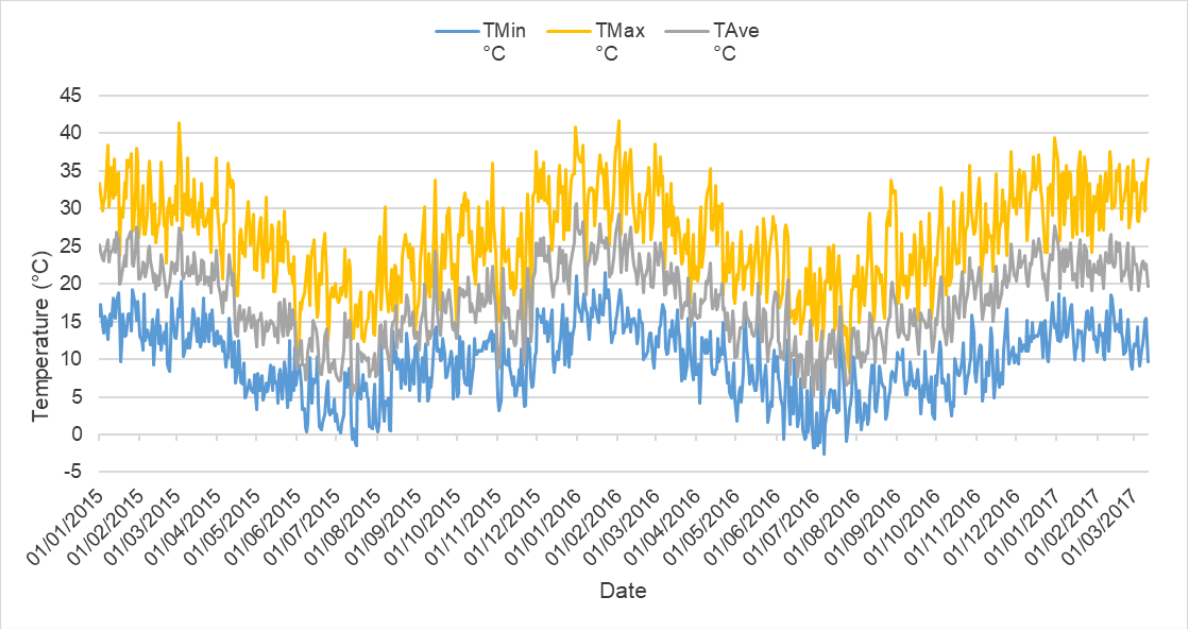

Figure 3.1: Average monthly minimum and maximum temperature for De Doorns, Hex River Valley, for the 2015/2016 and 2016/2017 seasons (Source: Ileaf: www.ileaf.co.za).

The Hex River Valley is a winter rainfall region. The total monthly rainfall for De Doorns recorded at the Normandi weather station from June 2015 to August 2017 is given in Figure 3.2. Rainfall differed substantially between the two seasons. The months of June to August 2015 had a total rainfall of 700.6 mm compared to 103.6 mm in 2016 and 39.1 in 2017. The rainfall of 2015 was typical of a winter rainfall region, with the highest rainfall months recorded from June to August. The rainfall for 2016 ranged from January to August with very low rainfall recorded over the winter months (Fig. 3.2). As expected for the region, there was very low rainfall in the month of harvest (January) with an average of 0.25 mm and 0.04 mm recorded for 2015/2016 and the 2016/2017 seasons, respectively.

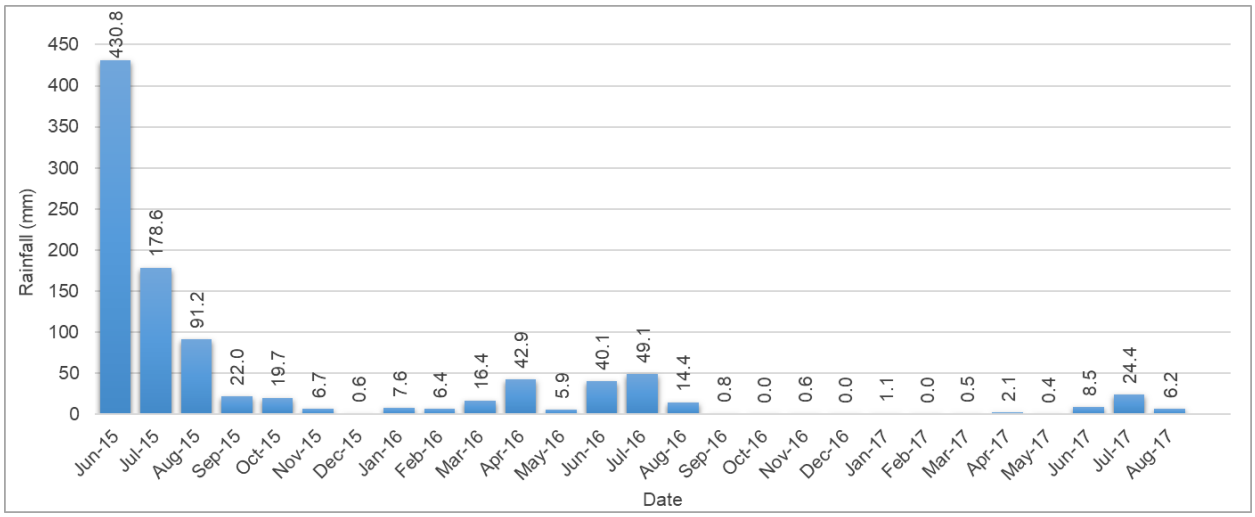

Figure 3.2: Total monthly rainfall for De Doorns, Hex River Valley, from June 2015 to August 2017 (Source: Ileaf: www.ileaf.co.za).

The chill unit accumulation for De Doorns, from May to August for 2015, 2016 and 2017, is given in Figure 3.3. The chill unit accumulation is expressed as Richardson or Utah units (Richardson *et al.*, 1974), as well as Infruitec or Daily Positive Chilling Units (DPCU) (Linsley-Noakes & Allan, 1994) as cited by (Luedeling, 2012).

A study performed by Dokoozlian (1999) indicated that a minimum of 200 hours is required at temperatures ranging between 0°C and 10°C to obtain high (≥ 80%) bud break, while 400 hours in this temperature range is required to obtain both high and even bud break. Long term temperature data, as researched by Avenant and Avenant (2014), indicates that De Doorns accumulates more than 400 chill units from May to August. From May to August for 2015, 2016 and 2017, De Doorns received more than the required cold units in order to ensure high and even bud break (Fig. 3.3). Therefore, the use of rest breaking agents in the De Doorns area to acquire high percentages of bud break is not required, but it could still be used to promote even bud break.

The Winkler index for viticulture serves as a classification for growth potential, based on the climatic potential of the production area (Winkler *et al.*, 1974). De Doorns or the Hex River Valley falls within region III of the Winkler index, which entails moderately warm temperatures ideal for producing grapes of colour (Winkler *et al.*, 1974).

The daily, as well as total heat units expressed in degree hours, with a base temperature of 10°C, is given in Figure 3.4. The heat unit accumulation for the 2015/2016 season reached 67085-degree hours compared to 67448 for the 2016/2017 season. Although the heat accumulation for the two seasons is very similar, significant heat unit fluctuations for the 2015/2016 season were observed during the end of October (around full bloom), until early December (before the start of véraison) (Fig. 3.4). The 2016/2017 season had more fluctuations starting towards the end of March, after the harvest period.

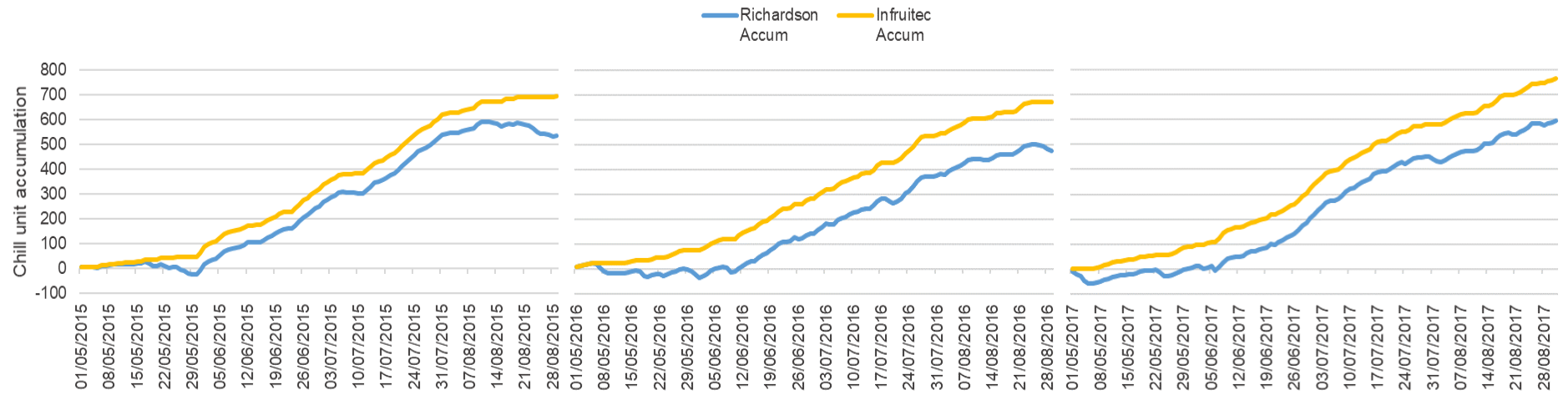

Figure 3.3: Chill unit accumulation for De Doorns, Hex River Valley, from May to August for 2015, 2016 and 2017 (Source: Ileaf: www.ileaf.co.za).

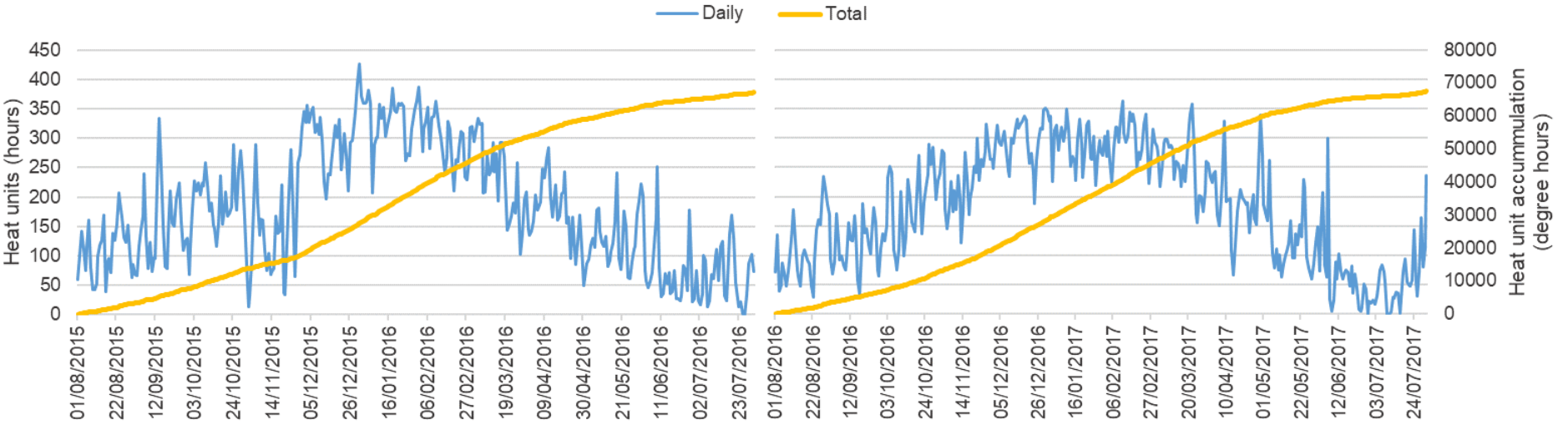

Figure 3.4: Heat unit accumulation for De Doorns, Hex River Valley from August 2015 to July 2016 and August 2016 to July 2017 (Source: Ileaf: www.ileaf.co.za).

3.1.2 Phenology

The phenology followed a similar trend in the 2015/2016 and 2016/2017 seasons, reaching bud break, full bloom, véraison and harvest all within a maximum of three days apart (Table 3.1).

Table 3.1: Phenological stages for the 2015/2016 and 2016/2017 seasons.

Phenological stage	2015/2016 Season	2016/2017 Season
Bud break	29 August	30 August
Full bloom	29 October	1 November
Véraison	20 December	23 December
Harvest	26 January	26 January

The temperature conditions and average relative humidity (RH) one day before and one day after bud break and full bloom, as well as the ripening period (véraison – harvest) for both seasons are presented in Table 3.2.

Table 3.2: Temperature conditions and average relative humidity around bud break, full bloom and ripening at De Doorns, Hex River Valley for the 2015/2016 and 2016/2017 seasons (Source: Ileaf: www.ileaf.co.za).

Phenological Stage	Date	Minimum (°C)	Maximum (°C)	Average (°C)	Average Relative Humidity (%)
Bud break 2015	28/08/15	9.8	24.7	16.3	69.0
	29/08/15	6.7	21.2	13.3	72.1
	30/08/15	11.6	13.5	12.7	87.9
Bud break 2016	29/08/16	8.1	32.1	18.9	54.3
	30/08/16	7.0	32.4	17.6	57.7
	31/08/16	8.7	28.7	17.0	48.3
Full bloom 2015	28/10/15	11.0	36.0	22.3	47.0
	29/10/15	13.3	27.8	19.7	65.1
	30/10/15	11.5	24.1	16.9	74.5
Full bloom 2016	31/10/16	7.0	28.2	19.2	50.6
	01/11/16	8.6	30.7	19.9	61.9
	02/11/16	11.3	34.0	22.2	55.2
Ripening 2015/2016	20/12/15 - 26/01/16	15.8*	32.7*	24.4*	57.3*
Ripening 2016/2017	23/12/16 - 26/01/17	13.9*	31.7*	22.9*	54.3*

*Average for the indicated period.

3.2 EXPERIMENTAL DESIGN AND LAYOUT

To investigate the optimal phenological stage for a chemical thinning application on Sunred Seedless, a split-plot design was used, with two GA formulations applied at different concentrations as the main plot factor and the phenological stage of these thinning applications, ranging from flowering to set, the subplot factor.

The main plot design consisted of a randomised complete block design, with nine treatments which were replicated four times. The layout of the experimental block (Fig. 3.5) consisted of six rows, with each row containing six experimental units. Each experimental unit contained four vines, with the two centre vines used as the experimental data unit. Field sampling was performed in the data experimental unit.

	Panel number					
Row number	**6**	**5**	**4**	**3**	**2**	**1**
	Replicate 1			**Replicate 2**		
24	T6	T7	T1	T8	T9	T5
23	T2	T5	T9	T4	T7	T3
22	T8	T4	T3	T1	T6	T2
21	T3	T2	T6	T9	T8	T7
20	T7	T1	T8	T6	T5	T4
19	T4	T9	T5	T3	T2	T1
	Replicate 3			**Replicate 4**		

Figure 3.5: Experimental layout of the Sunred Seedless GA thinning trial at De Doorns Experimental Farm.

3.3 MAIN PLOT TREATMENTS

The main plot GA thinning treatment consisted of two GA formulations, namely GA_3 (commercial product: ProGibb® 40%; supplier: Valent BioSciences™, A Division of Philagro SA (Pty) Ltd., Somerset West) and GA_{4+7} (commercial product: Novagib® 10 SL; supplier: Fine Agrochemicals Limited; distributor: Villa Crop Protection, Kempton Park), applied at different concentrations. The commercial standard concentration of GA_3 recommended for the thinning of Sunred Seedless was used. In contrast to GA_3, there is no recommended norm for applying GA_{4+7} for thinning or berry sizing of table grapes. Therefore, GA_{4+7} was applied at three different concentrations during the first season (2015/2016) and five different concentrations during the second season (2016/2017). In the second season, two additional treatments were added, a GA_{4+7} thinning and berry sizing treatment and a GA_{4+7} berry sizing only treatment.

All treatments, in combination with a wetting agent (Villa 51), were applied with a 13L Stihl mist blower by directing the spray nozzle towards the bunch zone (Fig. 3.6 A). The experimental units surrounding the treatment being applied were covered with plastic sheets to prevent contamination between treatments (Fig. 3.6 B). The application of treatments took place between 6:00 am and 8:00 am, with the temperature ranging from 11.27°C to 13.57°C (Table 3.3). Wind speed during the time of application for both seasons was low enough not to cause excessive drift (Table 3.3).

Figure 3.6: A) Application method used for applying the treatments directly at the bunch zone and B) Plastic sheets used to prevent contamination between treatments.

Table 3.3: Temperate, relative humidity and wind speed conditions during the application of treatments for both 2015/2016 and 2016/2017 seasons, for De Doorns, Hex River Valley (Source: Ileaf: www.ileaf.co.za).

Season	Date	Treatment	Time of application	Average dry temperature (°C)	Average relative humidity (%)	Average wind speed (m/s)
2015/ 2016	10/31/2015	Thinning	6:00 - 8:00	11.27	84.33	1.82
	11/04/2015	Thinning	6:00 - 8:00	12.53	48.33	2.52
2016/ 2017	11/03/2016	Thinning	6:00 - 8:00	13.57	77.20	0.40
	11/10/2016	Sizing	6:00 - 8:00	11.63	79.67	0.41

In this experiment, the efficacy of a standard GA_3 thinning treatment, recommended for Sunred Seedless, was compared to various concentrations of a GA_{4+7} thinning treatment. Along with thinning efficacy, the optimal phenological stage to apply a GA thinning treatment on Sunred Seedless was also determined. This was done by including a subplot factor, that consisted of bunches which were at different phenological stages on the day of application. The subplot factor is described in Section 3.4.

3.3.1 2015/2016 season

The main plot GA_3 and GA_{4+7} thinning treatments for the first season were applied on two different application dates, namely 31 October 2015 and 4 November 2015. Visual observations revealed that

most of the clusters were at a phenological stage of 10% berry set on 31 October 2015 and at berry set on 4 November 2015. During a commercial thinning application, the phenological stage required for thinning is determined by visual observation of the clusters. To avoid confusion between the phenological stage determined by commercial standards and the individually marked phenological stage per cluster, as described in Section 3.4, the application at 10% berry set (31 October 2015) will hereafter be referred to as the early application and the application at berry set (4 November 2015) as the late application. At each of the two application dates, the standard GA_3 concentration recommended for thinning was applied along with three different GA_{4+7} concentrations (Table 3.4).

Nine treatments were applied (Table 3.4): Treatment 1 was a control treatment and no thinning treatment was applied. Treatments 2 to 5 were applied as an early application on 31 October 2015 and Treatments 6 to 9 were applied as a late application on 4 November 2015. Treatment 2 and 6 (5 ppm GA_3), consisted of a GA_3 thinning treatment applied at the commercial concentration recommended for Sunred Seedless. Treatment 3 and 7 (7.5 ppm GA_{4+7}) consisted of a GA_{4+7} thinning treatment applied at half the concentration recommended by the supplier of the GA_{4+7} product. Treatment 4 and 8 (15 ppm GA_{4+7}) consisted of a GA_{4+7} thinning treatment applied at the concentration recommended by the supplier. Treatment 5 and 9 (30 ppm GA_{4+7}) consisted of a GA_{4+7} thinning treatment applied at double the concentration recommended by the supplier.

Table 3.4: Control and GA treatments applied on Sunred Seedless for the first season (2015/2016) of the study.

Treatment code	GA formulation	Rate	Commercial phenological stage and application date
T1	Untreated control	No thinning application	-
T2	GA3	5 ppm	**Early application** (10% Berry set on 31 October 2015)
T3	GA4+7	7.5 ppm	
T4	GA4+7	15 ppm	
T5	GA4+7	30 ppm	
T6	GA3	5 ppm	**Late application** (Berry set on 4 November 2015)
T7	GA4+7	7.5 ppm	
T8	GA4+7	15 ppm	
T9	GA4+7	30 ppm	

3.3.2 2016/2017 season

Results obtained with the treatments applied in 2015/2016 were discussed with the developer of the GA_{4+7} and the treatments to be applied in 2016/2017 were planned in consultation with them. Treatments for the second season consisted of GA_3 and GA_{4+7} thinning treatments applied on 3 November 2016 and a GA_{4+7} berry sizing treatment applied on 10 November 2016. To determine the optimal phenological stage for a GA thinning application on Sunred Seedless, the split-plot factor contained bunches marked at four different phenological stages present in the vineyard on the day of application, described in Section 3.4.2. The second application for berry sizing was applied when most of the berries reached 6-8 mm in diameter.

Nine treatments were applied in the 2016/2017 season (Table 3.5): Treatment 1 was a control treatment and no thinning treatment was applied. Treatment 2 (5 ppm GA_3) consisted of a GA_3 thinning treatment applied at the commercial concentration recommended for Sunred Seedless. Treatment 3 (7.5 ppm GA_{4+7}), Treatment 4 (15 ppm GA_{4+7}) and Treatment 5 (30 ppm GA_{4+7}) contained a GA_{4+7} thinning treatment applied at concentrations recommended by the developer of the GA_{4+7} product for the first season of the experiment. Treatment 6 (60 ppm GA_{4+7}) and Treatment 7 (120 ppm GA_{4+7}) consisted of a GA_{4+7} thinning treatment applied at concentrations recommended by the developer for the second season of the experiment. Treatment 8 (60 ppm GA_{4+7} + 60 ppm GA_{4+7}) consisted of a GA_{4+7} thinning treatment followed by a berry sizing treatment applied at concentrations recommended by the developer for the second season of the experiment. Treatment 9 (60 ppm GA_{4+7}) contained no thinning treatment, but a GA_{4+7} berry sizing treatment was applied at a concentration recommended by the developer.

Table 3.5: Control and GA treatments applied to Sunred Seedless for the second season (2016/2017) of the study.

Treatment code	GA formulation	Thinning application (3 November 2016)		Sizing application (10 November 2016)	
		Rate	Phenological stage	Rate	Phenological stage
T1	Untreated control	No thinning application	50% flowering; 80-100% flowering; 10% set and berry set.	No sizing application	
T2	GA3	5 ppm		No sizing application	
T3	GA4+7	7.5 ppm			
T4	GA4+7	15 ppm			
T5	GA4+7	30 ppm			
T6	GA4+7	60 ppm			
T7	GA4+7	120 ppm			
T8	GA4+7	60 ppm		60 ppm	6-8 mm berry size
T9	GA4+7	No thinning application			

3.4 SUBPLOT FACTORS

3.4.1 2015/2016 season

Before applying the GA thinning treatments, three different phenological stages present in the vineyard on the day were identified. Within each experimental data unit, six inflorescences per phenological stage (subplot factor) were marked using insulation tape. Different colours of insulation tape were placed around the peduncle of bunches to indicate the different phenological stages.

On the first application date, 31 October 2015, the three phenological stages identified were: 80-100% flowering (80-100%F), marked with red insulation tape around the peduncle; 10% berry set (10%BS), marked with green insulation tape, and berry set (BS), marked with blue insulation tape (Table 3.6). The treatments receiving the GA thinning application on the first application date had a total of 18 marked inflorescences per experimental data unit.

On the second application date, 4 November 2015, the three phenological stages identified were: 10%BS, marked with red insulation tape around the pedicel; BS, marked with green insulation tape, and berry set + 4 days (BS+4D), marked with blue insulation tape (Table 3.6). The treatments receiving the GA thinning application on the second application date had a total of 18 marked inflorescences per experimental data unit.

Table 3.6: Penological stages identified/subplot treatments applied on the two GA thinning application dates for the first season (2015/2016) of the study.

Application date	Phenological stage/ Subplot treatment (at thinning)	Code	Colour marker
31 October 2015	80-100% Flowering	80-100%F	Red
	10% Berry set	10%BS	Green
	Berry Set	BS	Blue
4 November 2015	10% Berry set	10%BS	Red
	Berry Set	BS	Green
	Berry Set + 4 days	BS+4	Blue

For the 2015/2016 season, a total of 648 bunches were marked for individual evaluation in order to determine possible differences in the bunch structure as influenced by the different treatments. During bunch development, these marked bunches were left in their natural state. No bunch preparations through bunch shortening, removal of laterals or the removal of individual berries were applied to the marked bunches. Only the secondary bunches were removed if present. The aim was to evaluate the bunches in their natural state to determine if any differences in bunch structure and compactness observed were related to the treatments only.

3.4.2 2016/2017 season

The day before the GA thinning treatments was applied, four different phenological stages were identified. Within each experimental data unit, five inflorescences per phenological stage were marked using insulation tape. Different colours of insulation tape were placed around the peduncle of bunches to indicate the different phenological stages (Fig. 3.7).

The four phenological stages identified were: 50% flowering (50%F), marked with white insulation tape around the peduncle; 80-100%F, marked with red insulation tape; 10%BS, marked with green insulation tape; and BS, marked with blue insulation tape (Table 3.7). Each experimental data unit had a total of 20 marked inflorescences.

Table 3.7: Four phenological stages identified/ timing subplot treatments applied to the GA thinning application date for the second season (2016/2017) of the study.

Application date	Phenological stage/ Timing subplot treatment	Code	Colour marker
3 November 2016	50% Flowering	50%F	White
	80-100% Flowering	80-100%F	Red
	10% Berry set	10%BS	Green
	Berry Set	BS	Blue

For the 2016/2017 season, a total of 720 were marked for individual evaluation in order to determine possible differences in the bunch structure as influenced by the different treatments. During bunch development, these marked bunches were left to remain in their natural state, as described in Section 3.4.1.

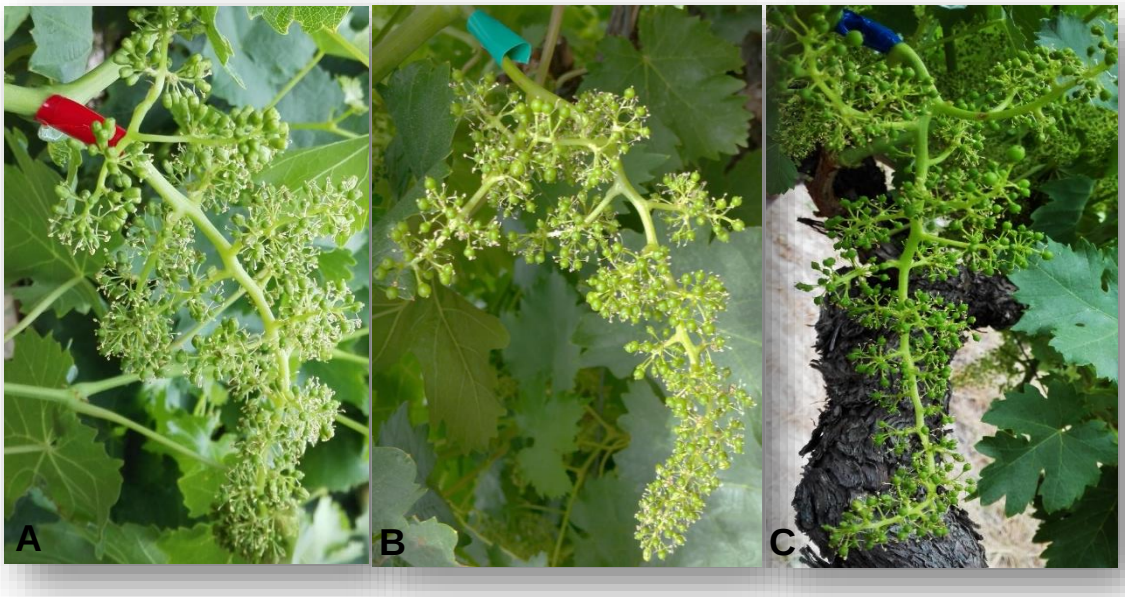

Figure 3.7: Phenological stages of Sunred Seedless during thinning application. A) 80-100% flowering; B) 10% set; and C) berry set.

3.5 STATISTICAL PROCEDURES

The experimental design was a randomised block with nine treatments replicated in four blocks, according to a Latinised 3x3 (row x column) arrangement. Analysis of variance was performed according to the experimental design, using GLM (General Linear Models) procedure of SAS software (Version 9.4; SAS Institute Inc, Cary, USA). A split-plot analysis of variance with the phenological stage as the subplot factor was also performed (Little & Hills, 1972). In order to test for deviation from normality, the Shapiro-Wilk test was performed on the standardised residuals (Shapiro & Wilk, 1965). Fisher's least significant difference was calculated at the 5% level to compare means (Ott, 1998). A probability level of 5% was considered significant for all significance tests.

3.6 PRE-HARVEST EVALUATION

3.6.1 Berry sampling

During the first season (2015/2016), berry samples were collected from 27 November 2015 to 26 January 2016 at weekly intervals. To monitor berry development by determining berry mass and measuring berry length and diameter, a weekly sample of 50 berries per experimental data unit were sampled from 27 November to 15 December 2015. From véraison until harvest (23 December 2015 to 26 January 2016), the weekly sample of 50 berries used for monitoring berry development continued and these samples were also used to monitor berry ripening by determining total soluble solids (TSS), titratable acidity (TA) and pH.

Within each experimental data unit, an even number of berries was sampled from each of the two data vines. Sampling occurred on random bunches within each experimental data unit, excluding marked bunches. To ensure a representative berry sample, sampling took place by selecting berries at the top, middle and bottom of bunches. Each berry was removed from the bunch by inserting a cut through the pedicel with a clean scissor. Berry sampling was performed during the cooler temperatures of the mid-morning. After all the samples were collected on each sampling date, they were taken back to the laboratory for berry development and ripening measurements.

Results from the first season delivered the expected berry development pattern for the cultivar. Furthermore, there were no significant differences between the treatments. Therefore, berry development and ripening were not monitored during the 2016/2017 season.

3.6.2 Berry development and ripening

To monitor berry development in weekly intervals, the length and diameter of the samples mentioned in Section 3.6.1 were measured using a digital caliper. The total sample mass of each sample was

measured to determine the average berry mass (g). A digital scale was used to determine the sample mass (Precisa, Type. 280-9826, PAG Oerlikon AG, Zurich, Switzerland).

To monitor berry ripening, the juice of each 50 berry sample was used to determine TSS, TS, and pH. The berries of each 50 berry sample were homogenized. A sieve was used to separate the juice from the homogenized grape sample and the clear juice was retained for further measurements. The TSS, expressed as degrees Brix (°Brix), was measured with a handheld digital refractometer (Atago PAL-1, Tokyo, Japan). The pH and TA (g/L) were measured by titrating 50mL of the clear juice with 0.33% sodium hydroxide (NaOH) to an endpoint of pH 7 with an automated titrator (Metrohm 785 DMP Titrino, Herisau, Switzerland). Calibration of the Metrohm at pH 7 and pH 4 took place before the samples were placed into the Metrohm.

3.6.3 Bunch length

In the first season (2015/2016), initial bunch length measurements of all marked bunches were taken in the vineyard four weeks after the thinning application. In the second season (2016/2017), this initial bunch length was measured the day before the thinning applications took place, on the same day that the bunches were marked. Bunch length was again measured at harvest in both seasons. Using a ruler, the bunch length was determined by measuring from the first lateral to the tip of the bunch (Fig. 3.8 A).

3.7 HARVEST EVALUATION

The experimental block forms part of a commercial Sunred Seedless block and therefore the grapes were harvested one day before the harvest of the commercial grapes destined for the export market to avoid the loss of experimental bunches. The harvest date for the commercial Sunred Seedless was determined when the TSS reached 17°B, or a minimum TSS of 16°B combined with a minimum sugar to acid ratio of 25:1 to comply to export standards (DAFF, 2016). The harvest date for the experimental bunches in both seasons was on 26 January.

In both seasons the marked bunches were harvested, with harvest evaluations performed on these bunches. Harvest evaluations on bunch structure, as well as rudimentary seeds, were performed in line with a protocol developed and applied by the Viticulture Division at ARC Infruitec-Nietvoorbij.

3.7.1 Bunch structure

Bunch structure measurements took place by systematically working through each experimental data unit at a time. The bunch mass of each marked bunch was determined using a digital scale (Radwag, Type, WTC 30/C1, Zakład Mechaniki Precyzyjnej, Poland). The bunch length was determined by measuring from the first lateral to the tip of the bunch.

Each bunch was visually assessed to determine the compactness of the bunch, based on a 5-point scale used by ARC Infruitec-Nietvoorbij. The 5-point compactness scale groups bunches into categories with values ranging from 1 (very compact) to 5 (very loose). The distinction between the compactness of the bunches is based on the degree of movement of the berries and the visibility of pedicels. With the scale, the value of 1 is described as a very compact bunch with no open spaces between berries, resulting in berries with a deformed shape. A value of 2 is described as a compact bunch with limited open spaces between berries and limited movement of the bunch. A value of 3 is described as the desired bunch compactness, with optimal open spaces between berries allowing for good movement of the bunch but with no pedicels visible. A value of 4 is described as a loose bunch, with more than desired open spaces between berries and with some visible pedicels. A value of 5 is described as a very loose bunch, with too many open spaces, too little berries and too many visible pedicels.

The rachis diameter was determined 1 cm above the first lateral by using a digital caliper (Fig. 3.8 B). Further measurements of the rachis included measuring the distance between the first and the fifth laterals.

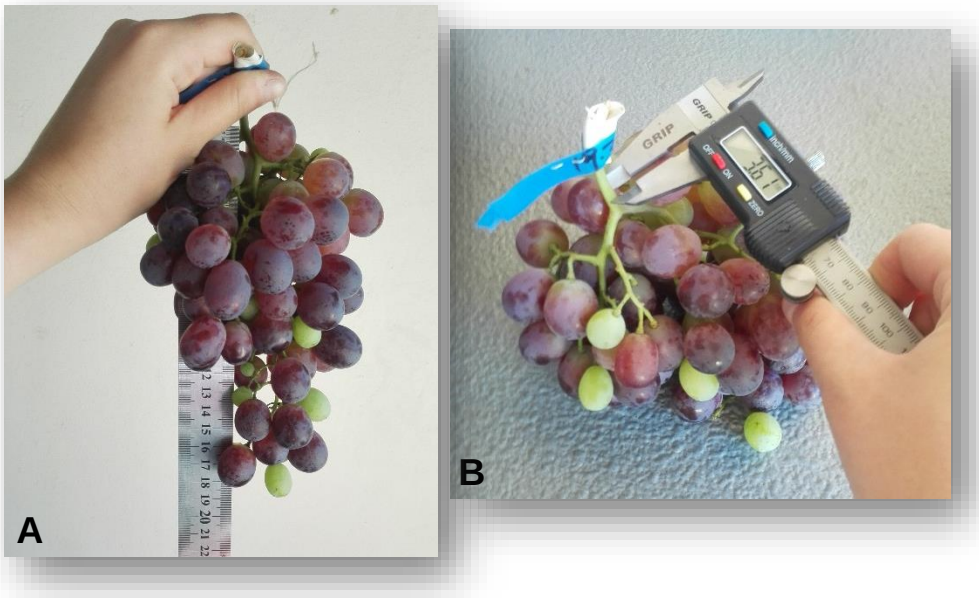

Figure 3.8: Bunch structure measurements at harvest. A) Bunch length measurements and B) Rachis diameter measurements.

From each marked bunch, the first four laterals were removed and placed in a plastic bag, marked accordingly for the rest of the measurements to be taken at the laboratory. At the laboratory, each sample, consisting of the four laterals, was subjected to various measurements.

All the berries were cut off from the four laterals and divided into three groups; normal berries, small berries and shot berries (Fig. 3.9). For Sunred Seedless, normal berries are classified as berries larger than 16 mm in diameter. Small berries are berries smaller than 16 mm in diameter and larger than 10 mm and lastly, shot berries are defined as berries smaller than 10 mm in diameter, which

are green and seedless. The number of berries in each group was counted and noted. The mass of the normal berries was determined. After that, these berries were placed back into the plastic bag to serve as sample material for determining berry length and diameter, TSS, pH and TA, separately for each colour code linked to a specific phenological stage (red, green, blue, white) within the data experimental unit, as described in Sections 3.6.1 and 3.6.2.

The total lateral length for the four laterals was measured using a piece of string to follow the outline length of the four laterals and measuring the length of the rope against a steel ruler (Fig. 3.10). The number of normal berries, along with the total lateral length were used to calculate the number of berries per cm of rachis, which is used as a parameter to assess bunch compactness (Lynn & Jensen, 1966).

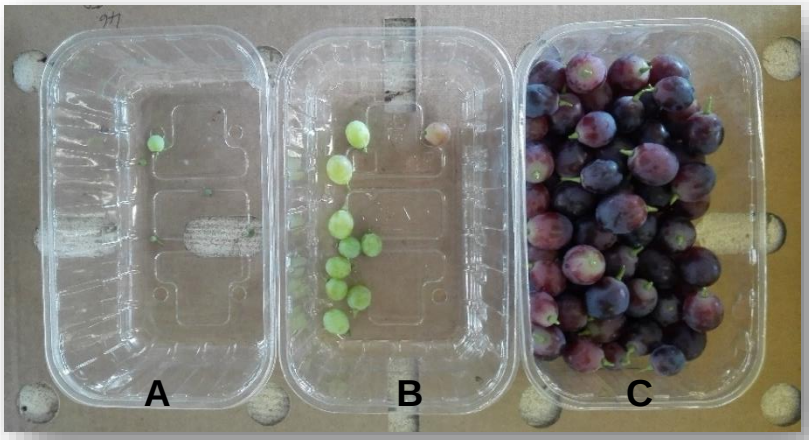

Figure 3.9: Berries from the first four laterals divided into three size groups. A) Shot berries: smaller than 10 mm; B) Small berries: smaller than 16 mm and larger than 10 mm; and C) Normal berries: larger than 16 mm.

Figure 3.10: The length of the four laterals was determined by using a string to follow the outline and measuring the rope against a steel ruler.

3.7.2 Rudimentary seeds

At harvest, a sample of ten berries per phenological stage (subplot factor) within each experimental data unit was randomly selected to evaluate the rudimentary seed occurrence and size as affected by GA treatment and application timing.

The total mass of each 10 berry sample was measured to determine the average berry mass. The rudimentary seeds of each berry were removed and divided into classes according to seed width: small (< 1 mm); medium (1-2 mm); and large (> 2 mm) as can be seen in Figure 3.11. The different classes of rudimentary seeds per berry were counted and noted. The average number of rudimentary seeds per berry was determined, along with the distribution of seed size per berry. The wet mass of the rudimentary seeds of each 10 berry sample was determined using a digital scale (Ohaus, Type. AR2140, Ohaus Corporation, Pine Brook, USA) to determine the average rudimentary seed mass and average rudimentary seed mass per berry.

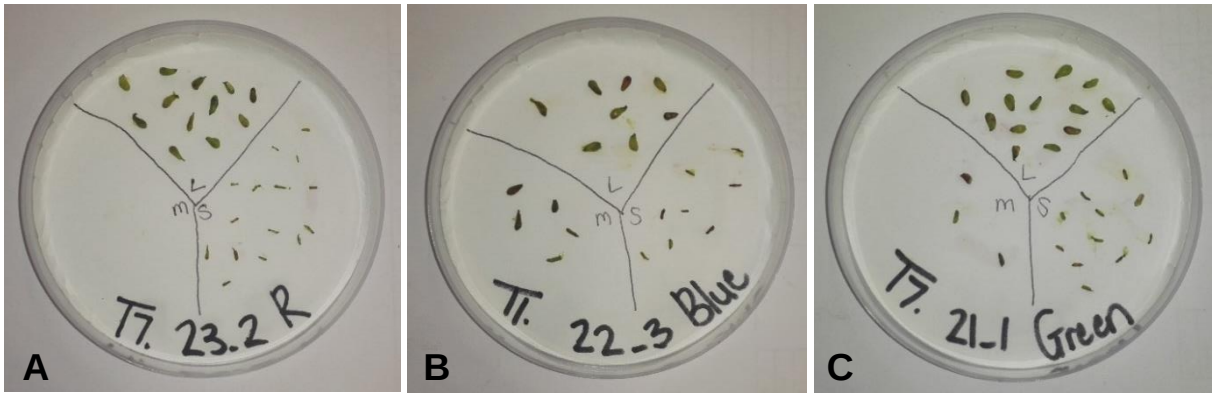

Figure 3.11 (A-C): Examples of rudimentary seeds from three of the 10-berry samples, divided into three classes according to seed width; small (<1 mm); medium (1-2 mm); and large (>2 mm), denoted by “S”, “M” and “L” respectively in each of the photos A-C.

3.8 CANE MASS

During winter pruning the one-year-old canes of the two vines per data experimental unit were collected and weighed with a hanging scale to determine the cane mass as a parameter to assess vigour.

3.9 BUD FERTILITY

3.9.1 Potential fertility

To determine the potential fertility of data vines from each treatment, two methods were used: (i) forced budding in a glasshouse and (ii) bud dissection. The pruning method used in the experimental block consisted of an average of six half long bearers (nine to ten buds per cane) and twelve spurs (two to three buds per spur) per vine. Therefore, dormant shoots with nine bud positions each were used for determining potential fertility.

3.9.1.1 Plant material

The dormant shoots used to determine the potential fertility were collected during winter pruning and labelled accordingly. In both seasons, four canes per experimental data unit were collected. Two of

these canes were used for forced budding and the other two used for bud dissections. Therefore, for each treatment consisting of four replicates, a total of 16 canes were collected, eight of which were used for forced budding and eight for bud dissections. For the 2015/2016 season, canes were collected for treatments T1 to T5, whereas canes were collected for all treatments in the 2016/2017 season.

3.9.1.2 Forced budding in a glasshouse

One the day of dormant shoot collection, the canes were cut into single-node cuttings with a clean pruning sheer and placed into water trays in a glasshouse at 25°C, as visible in Figure 3.12 (Palma & Jackson, 1989). For the 2015/2016 season, 360 single node cuttings were placed into water trays and for the 2016/2017 season, 648 single node cuttings.

The bud break date of each single node cutting was recorded at the first signs of visible green plant tissue (Shulman *et al.*, 1983; Palma & Jackson, 1989; Dunn & Martin, 2000). Twice a week, an observation of growth was recorded until the inflorescences were noticeable to be counted. After recording the number of inflorescences per single node cutting, the cuttings were removed. With each visit to the glasshouse, fresh tap water was added to the water trays to replace water lost due to evaporation.

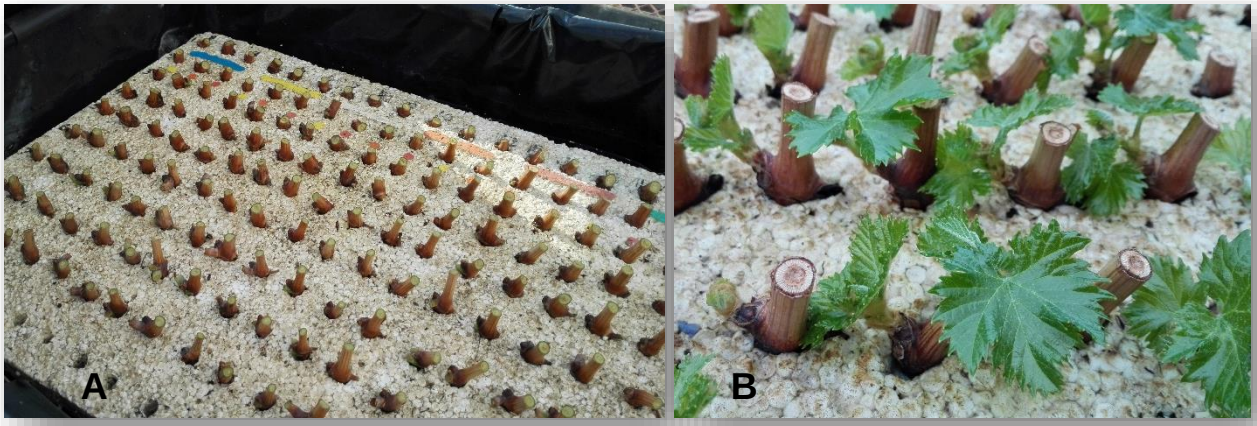

Figure 3.12: A) Single node cuttings, with dormant buds, placed in a water tray for forced budding inside a glasshouse. B) Sprouted buds after forced budding.

3.9.1.3 Bud dissections

The dormant shoots collected were stored at 0°C until bud dissections could be performed. Two dormant shoots per experimental data unit containing nine bud positions were used for bud dissection analysis. Each of the nine bud positions per cane was dissected individually.

Working under a stereomicroscope, bud dissection was performed following the procedure described by Swanepoel and Baard (1988). Srinivasan and Mullins (1981), as well as Swanepoel and Baard (1988), classified inflorescence primordia by the number of lobes present, defining

inflorescence primordia by three visible lobes present. The potential fertility was determined by counting and recording the number of inflorescence primordia per bud.

3.9.2 Actual fertility

The actual fertility was determined in the vineyard after bud break, once shoots with bunches were visible. Eight half-long bearers and eight spurs per experimental data unit were evaluated (four half-long bearers & four spurs on each of the two data vines). The half-long bearers were evaluated from bud position one to nine. Each bud position was evaluated by identifying whether bud break took place, recording the type of shoot that originated from the bud position (vegetative or reproductive shoot) and counting and recording the number of bunches per shoot. The actual fertility was only determined after the first season's treatment, *i.e.* in October 2016.

3.10 LITERATURE CITED

Avenant, E. & Avenant, J.H., 2014. Chill unit accumulation and necessity of rest breaking agents in South African table grape production regions. BIO Web Conf. Vol. 3. pp. 1017.

DAFF., 2016. Agricultural product standards act, 1990 (Act No. 119 of 1990) standards and requirements regarding control of the export of table grapes. www.daff.gov.za.

Dokoozlian, N.K., 1999. Chilling temperature and duration interact on the budbreak of “Perlette” grapevine cuttings. Hort. Sci. 34, 1-3.

Dunn, G.M. & Martin, S.R., 2000. Do temperature conditions at budburst affect flower number in *Vitis vinifera* L. cv. Cabernet Sauvignon? Aust. J. Grape Wine Res. 6, 116-124.

Linsley-Noakes, G.C., Allan, P., 1994. Comparison of 2 models for the prediction of rest completion in peaches. Sci. Hortic. 59, 107–113.

Little, T. M. & Hills, F. J., 1972. Statistical Methods in Agricultural, University of California, Davis, California 95616, 93-101.

Luedeling, E., 2012. Climate change impacts on winter chill for temperate fruit and nut production: A review. Sci. Hortic. 144, 218-229.

Lynn, C.D. & Jensen, F.L., 1966. Thinning effects of bloomtime Gibberellin sprays on Thompson Seedless table grapes. Am. J. Enol. Vitic. 17, 283-289.

Myburgh, P. & Howell, C., 2007. Evapotranspiration of *Vitis vinifera* L. cvs Sunred Seedless and Muscat Supreme in response to soil water depletion and irrigation cut off during berry ripening. S. Afr. J. Plant Soil 24, 209-213.

Ott, R.L., 1998. An Introduction to Statistical Methods and Data Analysis. Belmont, California: Duxbury Press: pp 807-837

Palma, B.A. & Jackson, D.I., 1989. Inflorescence initiation in grapes - responses to plant growth regulators. Vitis 28, 1-12.

Richardson, E.A., Seeley, S.D. & Walker, R.D., 1974. A model for estimating the completion of rest for Red Haven and Elberta peach. Hort. Sci. 9, 331-332.

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

Shulman, Y., Nir, G., Fanberstein, L. & Lavee, S., 1983. The effect of cyanamide on the release from dormancy of grapevine buds. Sci. Hortic. 19, 97-104.

Srinivasan, C. & Mullins, M.G., 1981. Physiology of flowering in the grapevine-A review. Am. J. Enol. Vitic. 32, 47-63.

Swanepoel, J.J. & Baard, F., 1988. The correlation between potential and actual fertility in the grapevine. Dec. Fruit Grow. 38, 231-235.

Winkler, A.J., Cook, J.A., Kliewer, W.M. & Lider, L.A., 1974. General Viticulture. Univ. of Calif, Press, Berkeley.

Chapter 4

Research results

The effect of GA_3 and GA_{4+7} thinning treatments on berry development and ripening of *Vitis vinifera* L. 'Sunred Seedless'

CHAPTER 4:
The effect of GA_3 and GA_{4+7} on berry development and ripening of *Vitis vinifera* L. 'Sunred Seedless'

ABSTRACT

Experiments were performed over two seasons on Sunred Seedless vines, grafted onto Ramsey, located on the ARC Infruitec-Nietvoorbij experimental farm in De Doorns. A standard GA_3 rate and various rates of GA_{4+7} was all applied at four different phenological stages to determine the effect on berry development and ripening. During the first season (2015/2016), weekly berry mass and berry length and diameter measurements occurred, along with TSS, pH and TA measurements after the onset of véraison. Berries from the different treatments followed the same trend of development and ripening throughout the growing season, indicating no plant growth regulator treatment effect on the berry development and ripening pattern. Due to no significant differences found for measurements taken throughout the first season, the berry measurements mentioned above were only taken at harvest for the second season (2016/2017). Treatments displayed a positive non-linear increase in berry mass in the absence of a lag phase, correlating with the findings of other authors where a gradual increase in berry volume is observed for seedless cultivars. The berries in this study were longer in length than in width, correlating with the natural oval berry shape of the cultivar Sunred Seedless. An increase in mean bunch mass at harvest was observed for both seasons from the earliest phenological stage up to the berry set stage, with a similar mean berry mass reported for these phenological stages. The earlier phenological stages had a better thinning effect based on the bunch and berry mass measurements of Sunred Seedless.

4.1 INTRODUCTION

The growth curve of the grape berry is defined by changes in berry mass, volume or diameter that follow a double-sigmoidal curve as a result of two rapid growth stages, separated by a lag stage (Harris *et al*., 1968; Coombe & McCarthy, 2000; Dokoozlian, 2000; Bennett, 2002; Sonnekus, 2015). A period of rapid berry growth defines Stage I, followed by a gradual decrease in berry growth, known as Stage II or the lag phase, where little to no growth occurs. The final stage, Stage III, is defined by a period of rapid berry growth and berry ripening (Harris *et al*., 1968; Coombe & Hale, 1973; Davies *et al*., 1997; Bennett, 2002), during which various physiological and biochemical changes occur within the berry (Paul *et al*., 2012).

The factors contributing to the potential berry size include the number of cells across the pericarp, the cell volume, and the translocation of organic solutes into the berry (Dokoozlian, 2000). Cell

division, responsible for the number of cells within a berry, occurs up to the first three weeks after flowering (Dokoozlian, 2000). An increase in cell volume (berry growth) occurs mainly during the two rapid growth stages, Stage I and III mentioned above (Dokoozlian, 2000). An increase in the content of organic solutes, which mainly consist of sugars, occur during the berry ripening stage, Stage III (Dokoozlian, 2000).

Endogenous factors, such as nutritional and hormonal balances, as well as exogenous factors, including climatic conditions and water availability, contribute to berry size (Ojeda *et al*., 2001; Ollat *et al*., 2002). An external application of gibberellic acid (GA_3) can result in an increase in berry size of seedless berries, with the response for each cultivar highly dependent on the phenological stage at the time of treatment and the concentration applied (Dokoozlian & Peacock, 2001; Zoffoli *et al*., 2009; Casanova *et al*., 2009).

The aim of the study was to evaluate the effect of GA_3 and GA_{4+7} treatments on berry size by quantifying berry development and ripening parameters throughout the growing season. By measuring berry length and diameter throughout the growing season, the impact of different GA_3 and GA_{4+7} treatments on berry size could be determined and quantified.

4.2 MATERIALS AND METHODS

Refer to Sections 3.3 and 3.4 for details on the experimental layout and treatments applied.

4.2.1 Berry development and ripening

Berry sampling at weekly intervals were performed to monitor berry development and ripening. The sampling date is expressed as the number of days after full bloom (DAFB), with the first sample taken at 29 DAFB and the last sample at harvest, 89 DAFB. Refer to section 3.1.2 in Chapter 3, Table 3.1, for a timeline of the phenological stages for the 2015/2016 season. A weekly 50-berry sample per data experimental unit were sampled to determine berry development by measuring berry mass, berry length and berry diameter. An additional 30 berries were sampled from véraison until harvest (52 DAFB to 89 DAFB) to monitor berry ripening by determining total soluble solids (TSS), titratable acidity (TA) and pH. Refer to sections 3.6.1 and 3.6.2 in Chapter 3, for details on berry sampling, as well as berry development and ripening measurements. The expected berry development and ripening pattern for the cultivar was found with the results of the first season and due to no significant differences between the treatments, weekly berry sampling was not repeated for the 2016/2017 season.

4.3 RESULTS AND DISCUSSION

4.3.1 Berry development and ripening

The progression of berry development and ripening for the 2015/2016 season is presented in Figures 4.1 to 4.5. None of the treatments had a significant effect on the parameters measured from 29 days after full bloom (DAFB) until harvest. Berry development and ripening data for the 2015/2016 season delivered the expected pattern for the cultivar. There were no significant differences in berry development and ripening for the 2015/2016 season, indicating that there was no plant growth regulator treatment effect on the berry development and ripening pattern.

4.3.1.1 Berry diameter and length

The development in berry diameter and length, from 29 DAFB until harvest, is given in Figures 4.1 and 4.2. Treatments followed a similar trend for change in berry diameter and length, with a weekly positive non-linear increase. The expected pattern for the treatments was met, with the untreated control positioned towards the lower end of the berry diameter and berry length spectrum.

Sunred Seedless is considered to have a large natural berry size when compared to other seedless cultivars, with an average berry mass of ~ 6 g per berry (SATI, 2016). To comply with export standards, a minimum berry diameter of 16 mm classified as a regular size is required for this particular cultivar (DAFF, 2018). Other category norms stipulated for Sunred Seedless includes 18 mm classified as large berries, 20 mm as extra-large and 22 mm as extra-extra-large. At harvest, all treatments had berry diameters ranging between 18 and 20 mm and were therefore classified as large berries according to the given export standards.

Berries of the untreated control, as well as treated berries were longer in length that in width (Figs. 4.1 & 4.2). The berries of Sunred Seedless have a natural oval shape and the expected berry shape for the cultivar was observed in this study (SATI, 2016). Ahmed Ola *et al.* (2012) reported that GA_3 increased the length of berries in contrast to forchlorfenuron (CPPU) which increased the diameter of berries. Other authors have reported similar findings (Lynn & Jensen, 1996; Casanova *et al.*, 2009; Zoffoli *et al.*, 2009; Kaplan, 2011; Ahmed Ola *et al.*, 2012; Abu-Zahra, 2013). The same occurrence of GA increasing berry length compared to the untreated control could not be found in this study.

Berry diameter and length harvest data for the 2015/2016 and 2016/2017 seasons are presented in Tables A.1 and A.2, as well as in graph format in Figures A.1 to A.4.

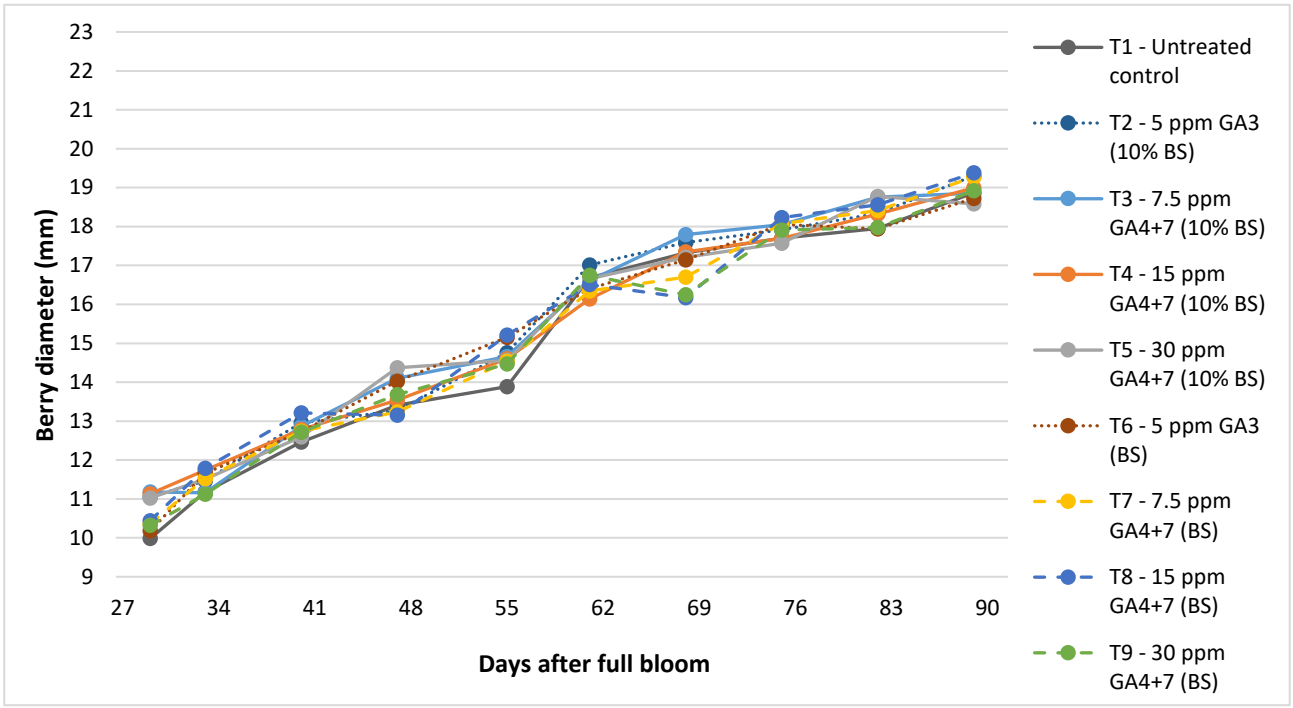

Figure 4.1: Change in berry diameter of Sunred Seedless over time, from 29 DAFB 89 DAFB (De Doorns Experimental Farm, 2015/2016 season).

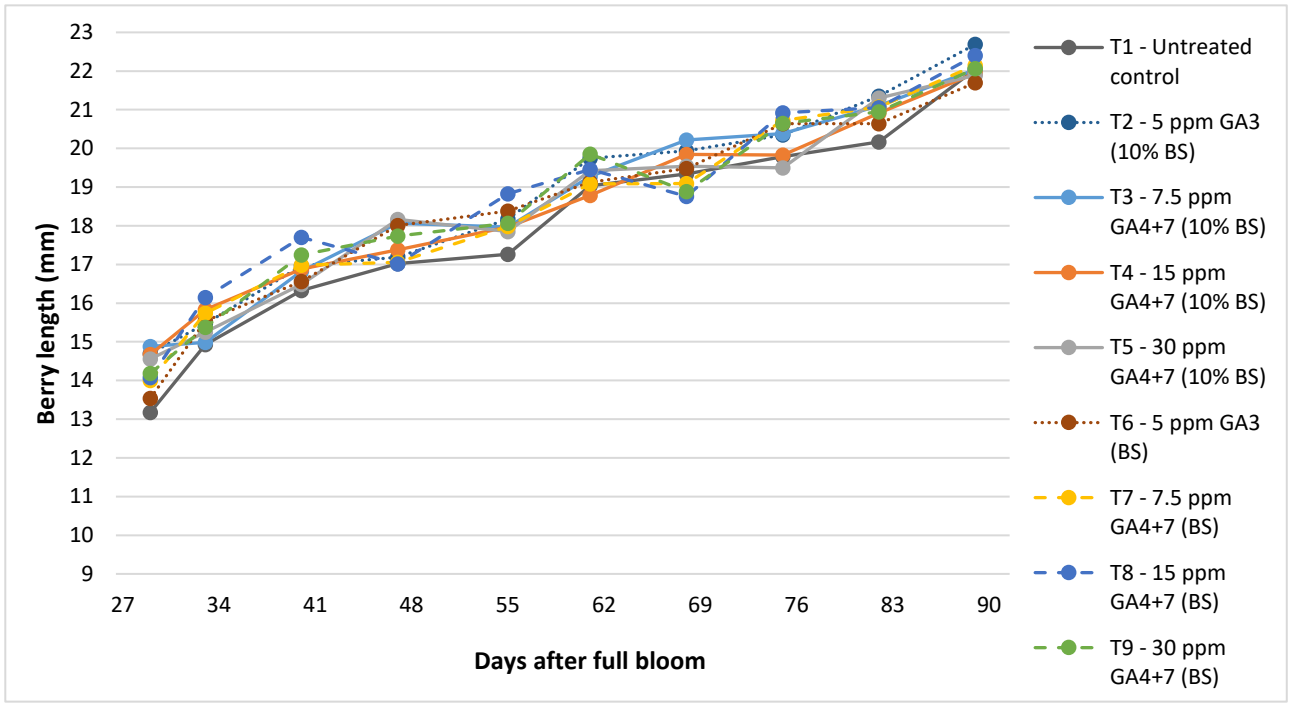

Figure 4.2: Change in berry length of Sunred Seedless over time, from 29 DAFB 89 DAFB (De Doorns Experimental Farm, 2015/2016 season).

4.3.1.2 Berry mass

The changes in berry mass, from 29 DAFB until 82 DAFB, is given in Figure 4.3. The treatments displayed a positive non-linear increase in berry mass, rather than a double sigmoidal growth curve

as expected for the development in berry mass (Coombe & Hale, 1973; Downton & Loveys, 1978; Matthews *et al.*, 1987). The formation of a double-sigmoidal curve is highly dependent on factors such as cultivar (seeded or seedless), cultivation practices and environmental conditions (Coombe & Hale, 1973; Keller, 2015). No lag phase was observed for the increase in berry mass over time. A similar trend was reported in other studies (Coombe, 1980; Raath, 2012; Sonnekus, 2015; Van der Vyver, 2016). The duration of each growth phase in the development of berry mass is influenced by the seed number per berry, therefore clearly distinguished phases are rarely observed with seedless cultivars, where a more gradual increase in berry volume is observed (Nitsch *et al.*, 1960; Iwahori *et al.*, 1968; Keller, 2015). The absence of a lag phase during berry development was ascribed by Coombe (1980) to the absence of competition between bunches, with only the primary bunch present and the secondary bunch removed. As part of standard table grape cultivation practices recommended by SATI (2016), a crop thinning action prior to flowering was applied to ensure that only one bunch per shoot remains on the vine, as well as to reduce the crop load to 28 bunches per vine.

Although no significant differences were found between treatments, the control treatment had the lowest berry mass (Fig. 4.3). The trend towards higher berry mass of treatments where GA was applied, compared to the untreated control can be explained by the increased translocation of assimilates into the berry due to a hormonal response followed by the GA application (Weaver *et al.*, 1968; Casanova *et al.*, 2009; Keller, 2015).

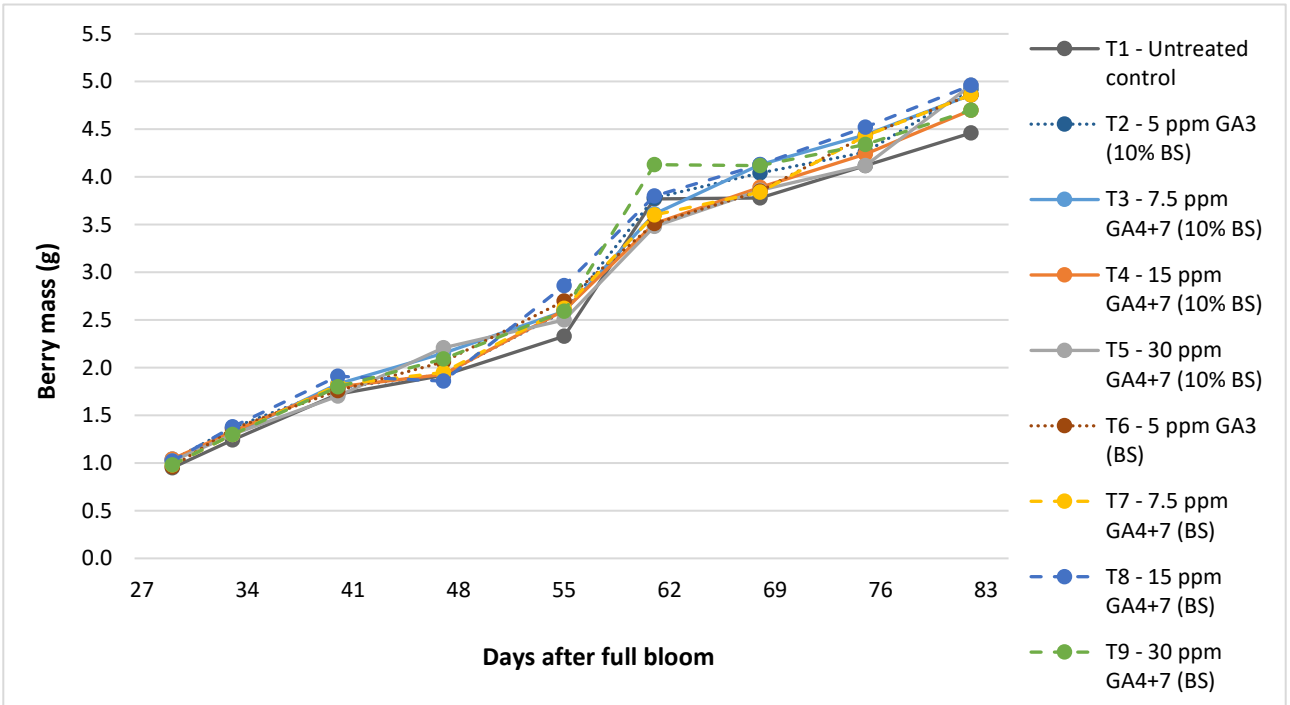

Figure 4.3: Change in berry mass of Sunred Seedless over time (De Doorns Experimental Farm, 2015/2016 season).

4.3.1.3 Total soluble solids

The change in total soluble solids (TSS), from 55 DAFB until harvest, is given in Figure 4.4. All treatments followed a similar trend, with no significant differences between treatments. The change in TSS began to reach a plateau after 82 DAFB for all the treatments.

An exponential increase in TSS was visible from 55 DAFB (3 days after the onset of véraison) to 68 DAFB (Fig. 4.4). This correlates with results found in other studies where a rapid increase in TSS accumulation was observed around the onset of véraison, indicating the start of berry ripening (Coombe, 1992; Tattersall *et al.*, 1997; Dokoozlian, 2000; Robinson & Davies, 2000; Wada *et al.*, 2008; Sonnekus, 2015).

A minimum TSS of 17°B or a minimum TSS of 16°B combined with a sugar to acid ratio of 25:1 is required for Sunred Seedless to comply with export standards (DAAF, 2016). The TSS values for the 2015/2016 season ranged from 15.03 °B to 15.90 °B (Fig. 4.4), and in the 2016/2017 season ranged from 13.50 °B to 16.65 °B (Addendum A, Table A.3). As the experimental site formed part of a commercial vineyard, grapes were harvested one day prior to the commercial harvest, therefore the TSS was generally lower than 17°B).

The objective of the experiment was to determine the effect of GA on bunch structure and ripening parameters were also evaluated to assess treatment effects. Therefore, a suboptimal TSS at harvest was acceptable within the experiment. The TSS of the 2016/2017 season indicated no significant differences between treatments (Addendum A, Table A.3).

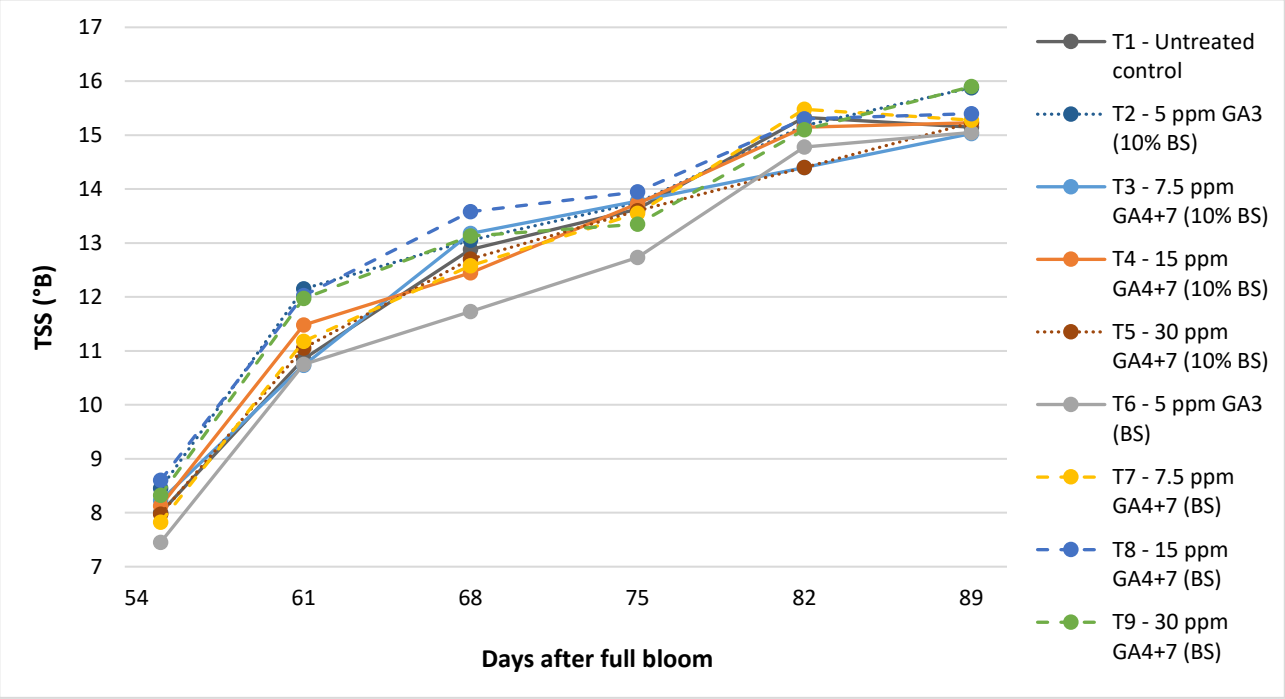

Figure 4.4: Change in berry total soluble solids (TSS) of Sunred Seedless over time (véraison to harvest) (De Doorns Experimental Farm, 2015/2016 season).

4.3.1.4 Titratable acidity

The change in titratable acidity (TA) over time, from 55 DAFB until harvest, is given in Figure 4.5. The TA decreased exponentially from 55 DAFB to 68 DAFB, with smaller changes in the rate of TA degradation thereafter. Similar results were reported for other studies (Harris *et al.*, 1968; Hrazdina *et al.*, 1984; Matthews & Anderson, 1988; Liu *et al.*, 2006; Sonnekus, 2015; Van der Vyver, 2016).

The application of GA did not affect TA of Sunred Seedless as all treatments, including the control, followed a similar trend in the decrease in TA up to harvest with no significant differences between treatments (Fig. 4.5). In contrast, Reynolds and de Savigny (2004) reported that GA_3 applied to the cultivar Sovereign Coronation decreased the TA.

All treatments followed a similar trend for the 2015/2016 season, resulting in values from 6.04 g/L to 6.59 g/L at harvest (Fig. 4.5). The TA ranged from 5.79 g/L to 8.36 g/L at harvest in the 2016/2017 season (Addendum A, Table A.3).

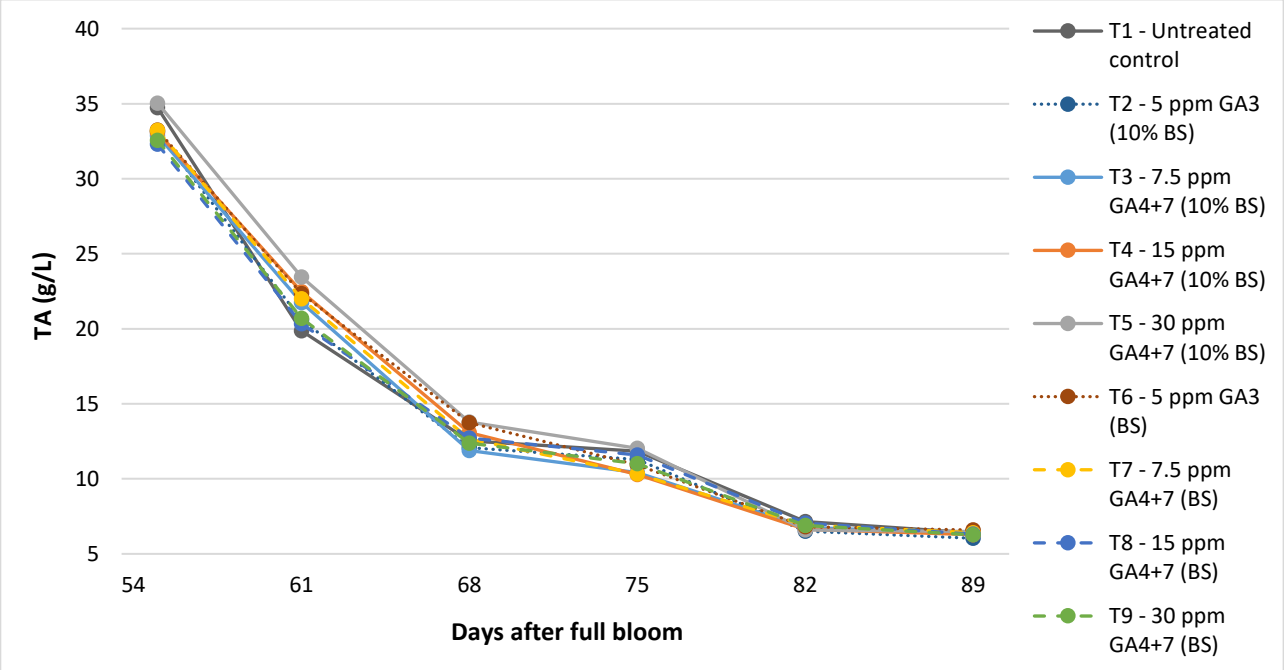

Figure 4.5: Change in berry titratable acidity (TA) of Sunred Seedless over time (véraison to harvest) (De Doorns Experimental Farm, 2015/2016 season).

4.3.2 Bunch and berry mass at harvest

Bunch and berry mass results for both the 2015/2016 and 2016/2017 seasons are presented in Tables A.4 and A.5 of Addendum A. Bunches of all treatments in both seasons reached a higher mass than the minimum of 150 g required per bunch for export of extra class berries (DAFF, 2018). From the earliest bunch phenological stage at application onwards until BS, an increase in mean bunch mass was observed for both seasons. Similarly, there was an increase in bunch mass where GA_3 was applied after flowering (Pérez & Gómez, 2000; Reynolds & de Savigny, 2004). An increase

in bunch and berry mass is expected as the GA application timing progresses, and earlier GA applications during flowering are associated with a thinning application whereas applications around berry set are used to promote berry size (Singh *et al.*, 1978; Zabadal & Dittmer, 2000; Casanova *et al*., 2009).

An increase in the concentration of GA_{4+7} used did not follow the same trend as found in cases of GA_3 mentioned above. Studies have reported an increase in bunch and berry mass as the concentration of GA_3 used, increased (Reynolds & de Savigny, 2004; Reynolds *et al.*, 2006). Coombe (1972) found GA_{4+7} to be less active in Sultana compared to GA_3 when comparing the effect on the increase in berry size.

4.3.2.1 2015/2016 season

The earlier application of 5 ppm GA_3 (10%BS) resulted in a significantly higher average bunch mass compared to the late application of 5 ppm GA_3 (BS), applied at the bunch phenological stage of BS (Table A.4 in Addendum A). This was probably due to the higher average berry mass measured for the 5 ppm GA_3 (BS) treatment (Table A.4 in Addendum A). Similarly, a post-bloom application of GA_3 on Sultana has been found to promote cell enlargement, promoting an increased berry size (Pérez & Gómez, 2000). An increase in the time available for the translocation of nutrients could be a possible explanation for higher bunch and berry mass reported for the earlier 5 ppm GA_3 treatment compared to the late 5 ppm GA_3 treatment. The application of GA_3 during flowering (an earlier application) creates an increased sink for nutrients by promoting the translocation of nutrients, such as K, towards the flowers, resulting in larger berries (Zhenming *et al.*, 2008; Casanova *et al.*, 2009).

A significantly lower average bunch mass, correlating with a lower normal berry mass, was found with the late application of 7.5 ppm GA_{4+7} (BS) during the bunch phenological stage of 10%BS. The earlier application of 7.5 ppm GA_{4+7} (10%BS) had the highest berry mass, and this was significantly higher compared to the late application of 7.5 ppm GA_{4+7} (BS), both applied at the bunch phenological stage of 10%BS.

Comparing these equal active ingredient rates applied at different timings for the 7.5 ppm GA_{4+7} treatments applied at 10%BS and the 5 ppm GA_3 treatments applied at BS, the earlier applications had significantly higher berry mass (Table A.4 in Addendum A). This was contrary to the expected result that a late application of GA would increase berry size and therefore berry mass, as reported by Coombe and Hale (1973) and Van der Vyver (2016).

The results indicate that the earlier application date for treatments mentioned above, had a better thinning effect as well as a longer available period for cell division to take place. The more pronounced thinning obtained ensures more spatial area for the naturally larger berries of Sunred Seedless to develop into. Growth hormones (GA, cytokinin & auxin) are produced by the embryos

and released into the pericarp during Stage I of berry growth where they reach high concentrations early before decreasing (Iwahori *et al.*, 1968; Scienza *et al*., 1978; Pérez *et al.*, 2000; Keller, 2015). Exogenous applications of GA therefore supplements the natural available GA and contribute to promoting cell division and enlargement.

A significantly lower mean bunch mass was reported for the bunch phenological stage of 80-100%F scompared to the BS stage, with both stages reporting the same mean berry mass. This indicates that the treatments applied at the earlier stage of flowering resulted in a better thinning effect compared to the berry set stage, which had more compact bunches.

4.3.2.2 2016/2017 season

The 60 ppm GA_{4+7} (S) treatment, applied at the bunch phenological stage of 50%F, resulted in a significantly higher average bunch mass compared to the 5 ppm GA_3 (T) treatment (Table A.5 in Addendum A). The higher bunch mass, for the 60 ppm GA_{4+7} (S), treatment along with a berry mass that tended to be lower, indicates a more compact bunch structure with more berries per bunch.

Bunch mass of the untreated control was significantly lower compared to the 15 ppm GA_{4+7} (T) and 120 ppm GA_{4+7} (T) treatments, applied at BS (Table A.5 in Addendum A). Applying a GA treatment at BS could have promoted berry sizing rather than berry thinning, which is unlikely in this case with no significant differences recorded for berry mass at BS. The significantly higher average bunch mass obtained with 15 ppm GA_{4+7} (T), applied at BS, can be ascribed to the significantly high number of normal berries per cm of lateral length (Refer to Figure 5.7 in Chapter 5).

The treatment of 7.5 ppm GA_{4+7} (T) applied at 50%F and at 10%BS resulted in a significantly higher average berry mass compared to the 60 ppm GA_{4+7} (S) and 60 ppm GA_{4+7} (T+S) treatments, respectively. The expected results would have been that the 60 ppm GA_{4+7} (T+S) treatment, that received a thinning as well as a sizing application, would have resulted in berries with the highest average berry mass.

A significant increase in the mean bunch mass is reported from 50%F to BS, with no significant differences reported for the mean berry mass between the different phenological stages. This indicates that less compact bunches are found at the earlier stages of application.

4.4 CONCLUSION

With the weekly evaluation of berry ripening parameters during the first season, the expected berry development and ripening pattern was found for Sunred Seedless growing under the prevailing conditions. Due to the lack of statistical differences between treatments, the evaluation of berry ripening parameters were not repeated in the second season. Treatments displayed a positive non-linear increase in berry mass in the absence of a lag phase, which have been ascribed to the absence of competition between bunches, with only the primary bunch present and the secondary bunch removed.

Berries were longer in length that in width, correlating with the natural oval berry shape of the cultivar Sunred Seedless. This indicated that the GA treatments did not change the natural shape of the berry.

Three days after the onset of véraison, there was an exponential increase in TSS and a decrease in TA for a period of 13 days. Treatments started reaching a plateau in the TSS seven days prior to harvest.

Bunch mass of all treatments exceeded the minimum of 150 g per bunch required for export. There was an increase in mean bunch mass for both seasons from the earliest bunch phenological stage up to BS. The mean bunch mass was lower at harvest when treatments were applied at the flowering, compared to treatments applied post-flowering, with a similar mean berry mass reported between stages. Therefore, the earlier phenological stages of flowering had a better thinning effect based on the bunch and berry mass measurements of Sunred Seedless.

Considering the applications at BS for the 2015/2016 season, the early application of 5 ppm GA_3 increased bunch mass substantially compared to late application. This was due to bigger berries. However, no consistent trend with regards to a specific treatment application timing and rate used could be observed for the bunch and berry mass measurements over two seasons.

It is recommended that other PGRs such as ABA and ethylene should be evaluated, on their own and in combination with GA, as thinning agents for Sunred Seedless in order to determine their effect on ripening parameters as well as bunch and berry mass, as discussed in this chapter. A multidisciplinary approach is recommended for further research, where parallel to the field trial evaluations of PGRs, genomic studies are also included to identify and quantify GA signalling components and availability of bioactive GAs, to contribute to understanding differences in response obtained with treatments applied in the field trial.

4.5 LITERATURE CITED

Abu-Zahra, T.R., 2013. Effect of plant hormones application methods on fruit quality of “Superior Seedless” grape. Biosci. Biotechnol. Res. Asia 10, 527-531.

Ahmed Ola, A., Rafaat, S.S., Elgendy, B. & Shaker, G.S., 2012. Effect of foliar spraying with GA_3 and/or sitofex on bud behaviour, vegetative growth, yield and cluster quality of Thompson Seedless grapevines. J. Am. Sci. 8, 21-34.

Bennett, J.S., 2002. Relationships between carbohydrate supply and reserves and the reproductive growth of grapevines (*Vitis vinifera* L.). Thesis, Lincoln University, PO Box 85084, 7647 Lincoln (Canterbury), New Zealand.

Casanova, L., Casanova, R., Moret, A. & Agustí, M., 2009. The application of Gibberellic acid increases berry size of “Emperatriz” seedless grape. Spanish J. Agric. Res. 7, 919-927.

Coombe, B.G., 1972. The regulation of set and development of the grape berry. Acta Hortic. 34, 261-274.

Coombe, B.G, 1980. Development of the grape berry. I Effects of time of flowering and competition. Crop Pasture Sci. 31, 125-131.

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

Coombe, B.G. & Hale, C.R., 1973. The hormone content of ripening grape berries and the effects of growth substance treatments. Plant Physiol. 51, 629-634.

Coombe, B.G. & McCarthy, M.G., 2000. Dynamics of grape berry growth and physiology of ripening. Aust. J. Grape Wine Res. 6, 131-135.

DAFF, 2018. Agricultural product standards act, 1990 (Act No. 119 of 1990) standards and requirements regarding control of the export of table grapes. www.daff.gov.za.

Davies, C., Boss, P.K. & Robinson, S.P., 1997. Treatment of grape berries, a non-climacteric fruit with a synthetic Auxin, retards ripening and alters the expression of developmentally regulated genes. Plant Physiol. 115, 1155-1161.

Dokoozlian, N.K., 2000. Grape berry growth and development. Raisin Production Manual. 30-37.

Dokoozlian, N.K. & Peacock, W.L., 2001. Gibberellic acid applied at bloom reduces fruit set and improves size of ‘Crimson Seedless’ table grapes. Hort. Sci. 36, 706-709.

Downton, W.J.S. & Loveys, B.R., 1978. Compositional changes during grape berry development in relation to abscisic acid and salinity. Aust. J. Plant Physiol. 5, 415-423.

Harris, J.M., Kriedemann, P.E. & Possingham, J.V., 1968. Anatomical aspects of grape berry development. Vitis 7, 106-109.

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.

Iwahori, S., Weaver, R.J. & Pool, R.M., 1968. Gibberellin-like activity in berries of seeded and seedless Tokay grapes. Plant Physiol. 43, 333-337.

Kaplan, M., 2011. The effect of the method of application of growth regulators on fruit quality of "Einset seedless" grape (*Vitis sp.* L.). Acta Agrobot. 64, give pages.

Keller, M., 2015 (2nd ed). The science of grapevines: Anatomy and physiology. Academic Press, London.

Liu, H.F., Wu, B.H., Fan, P.G., Li, S.H. & Li, L.S., 2006. Sugar and acid concentrations in 98 grape cultivars analyzed by principal component analysis. J. Sci. Food Agric. 86, 1526-1536.

Lynn, C.D. & Jensen, F.L., 1966. Thinning effects of bloom time Gibberellin sprays on Thompson Seedless table grapes. Am. J. Enol. Vitic. 17, 283-289.

Matthews, M.A. & Anderson, M.M., 1988. Fruit ripening in *Vitis vinifera* L.: Responses to seasonal water deficits. Am. J. Enol. Vitic. 39, 313-320.

Matthews, M.A., Cheng, G. & Weinbaum, S.A., 1987. Changes in water potential and dermal extensibility during grape berry development. J. Am. Soc. Hort. Sci. 112, 314-319.

Nitsch, J.P., Pratt, C., Nitsch, C. & Shaulis, N.J., 1960. Natural growth substances in Concord and Concord seedless grapes in relation to berry development. Am. J. Bot. 47, 566-576.

Ojeda, H., Deloire, A. & Carbonneau, A., 2001. Influence of water deficits on grape berry growth. Vitis 40, 141-145.

Ollat, N., Diakou-verdin, P., Carde, J.P., Barrieu, F., Gaudillere, J.P. & Moing, A., 2002. Grape berry development: A review. J. Int. Sci. Vigne. Vin. 36, 109-131.

Paul, V., Pandey, R. & Srivastava, G.C., 2012. The fading distinctions between classical patterns of ripening in climacteric and non-climacteric fruit and the ubiquity of ethylene-An overview. J. Food Sci. Technol. 49, 1-21.

Pérez, F.J. & Gómez, M., 2000. Possible role of soluble invertase in the gibberellic acid berry-sizing effect in Sultana grape. Plant Growth Regul. 3, 111-116.

Pérez, F. J., Viani, C. & Retamales, J., 2000. Bioactive gibberellins in seeded and seedless grapes: identification and changes in content during berry development. Am. J. Enol. Vitic. 51, 315-318.

Raath, P.J., 2012. Effect of varying levels of nitrogen, potassium and calcium nutrition on table grape vine physiology and berry quality. Thesis, Stellenbosch University, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Reynolds, A.G. & de Savigny, C., 2004. Influence of girdling and gibberellic acid on yield components, fruit composition, and vestigial seed formation of 'Sovereign Coronation' table grapes. Hort. Sci. 39, 541-544.

Reynolds, A.G., Roller, J.N., Forgione, A. & de Savigny, C., 2006. Gibberellic acid and basal leaf removal: Implications for fruit maturity, vestigial seed development, and sensory attributes of Sovereign Coronation table grapes. Am. J. Enol. Vitic. 57, 41-53.

Robinson, S.P. & Davies, C., 2000. Molecular biology of grape berry ripening. Aust. J. Grape Wine Res. 6, 175-188.

SATI., 2016. Guidelines for the preparation of table grapes for export 2016/17. Van der Merwe, G.G. (ed). South African Table Grape Industry, PO Box 2932, Paarl, 7620.

Scienza, A., Miravalle, R., Visai, C. & Fregdni, M., 1978. Relationships between seed number, gibberellin and abscisic acid levels and ripening in 'Cabernet Sauvignon' grape berries. Vitis 17, 361-368.

Singh, K., Weaver, R.J. & Johnson, J.O., 1978. Effect of applications of gibberellic acid on berry size, shatter, and texture of Thompson Seedless grapes. Am. J. Enol. Vitic. 29, 258-262.

Sonnekus, N., 2015. Development and change that occurs in table grape berry composition during growth. Thesis, Stellenbosch University, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Tattersall, D.B., Van Heeswijck, R. & Hoj, P.B., 1997. Identification and characterization of a fruit-specific, thaumatin-like protein that accumulates at very high levels in conjunction with the onset of sugar accumulation and berry softening in grapes. Plant Physiol. 114, 759-769.

Van der Vyver, L., 2016. The influence of gibberellic acid (GA_3) for berry thinning and berry sizing on table grape production, quality and fertility of Prime. Thesis, Stellenbosch University, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Wada, H., Shackel, K.A. & Matthews, M.A., 2008. Fruit ripening in *Vitis vinifera*: apoplastic solute accumulation accounts for pre-veraison turgor loss in berries. Planta 227, 1351-1361.

Weaver, R.J., Shindy, W.W. & Kliewer, W.M., 1968. Growth regulator induced movement of photosynthetic products into fruits of 'Black Corinth' grapes. Plant Physiol. 44, 183-189.

Zabadal, T.J. & Dittmer, T.W., 2000. Influence of gibberellic acid sprays on berry size and shot berry on 'Vanessa' grapevines. Acta Hortic. 527, 153-157.

Zhenming, N., Xuefeng, X., Yi, W., Tianzhong, L., Jin, K. & Zhenhai. H., 2008. Effects of leaf-applied potassium, gibberellin and source–sink ratio on potassium absorption and distribution in grape fruits. Sci. Hortic. 115, 164-167.

Zoffoli, J.P., Latorre, B.A. & Naranjo, P., 2009. Preharvest applications of growth regulators and their effect on postharvest quality of table grapes during cold storage. Postharvest Biol. Technol. 51, 183-192.

Chapter 5

Research results

The effect of GA_3 and GA_{4+7} thinning treatments on bunch structure, compactness and berry size distribution of *Vitis vinifera* L. 'Sunred Seedless'

CHAPTER 5:

The effect of GA_3 and GA_{4+7} thinning treatments on bunch structure, compactness and berry size distribution of *Vitis vinifera* L. 'Sunred Seedless'

ABSTRACT

Experiments were performed over two growing seasons on Sunred Seedless vines, grafted onto Ramsey, located on the ARC Infruitec-Nietvoorbij experimental farm in De Doorns. A standard GA_3 rate and various rates of GA_{4+7} were applied at four different bunch phenological stages in order to determine the optimal rate and timing to reduce bunch compactness and improve bunch structure. Bunch compactness was evaluated through a subjective visual assessment, by awarding a bunch compactness score to evaluated bunches, as well as an objective assessment method. The latter entailed the determination of the number of normal, small and shot berries per cm of lateral length. Berry size classification (%) was determined from the number of berries per cm of lateral length. Additional parameters such as bunch length, rachis diameter, and lateral length were evaluated to determine the effect of GA treatments on bunch structure. A sensitivity towards early GA applications was observed for Sunred Seedless, through increased rachis diameter and increased shot berry occurrence with GA applications applied during flowering. An increased shot berry occurrence was also observed with the use of higher GA_{4+7} rates. The study resulted in more reoccurring trends relating to the bunch phenological stage at the time of application rather than a specific GA treatment. These results confirm that the timing of a GA application plays a fundamental role in the treatment outcome for a specific cultivar.

5.1 INTRODUCTION

Rachis length, number of berries, as well as the size of the berries, are all factors that contribute to the compactness of a bunch (Tello & Ibáñez, 2018). Flower number per inflorescence, as well as fruit set success rate, determines the number of berries per bunch (Carmona *et al*., 2008), whereas Stage I and Stage III of berry development mainly determine berry size (Coombe & McCarthy, 2000; Robinson & Davies, 2000).

Bunch structure and compactness plays a fundamental role in grape quality, as berries of compact bunches are often smaller in size and deformed due to less spatial area to develop into. These berries are also prone to an uneven ripening period and are more susceptible to bunch rot diseases (Dokoozlian & Peacock, 2001; Molitor *et al.*, 2012b; Domingos *et al.*, 2016; Tello & Ibáñez, 2018).

Bunch structure alterations with the use of manual as well as chemical practices to deliver the desired bunch quality, have become a common practice among producers (Roper & Williams, 1989).

Chaturvedi and Khanduja (1979) evaluated bunch compactness on a scale of 1 to 20, based on the looseness of the bunch. Hanni *et al.* (2012) evaluated bunch compactness with a different approach by awarding a percentage to the matter of movement allowed by each bunch. Chaturvedi and Khanduja (1979), and Dokoozlian and Peacock (2001) evaluated the number of berries per cm of lateral length. Pérez and Gómez (2000), Casanova *et al.* (2009) and Zoffoli *et al.* (2009) studied pedicel diameter, with Casanova *et al.* (2009) including pedicel length and Zoffoli *et al.* (2009) including rachis thickness in their study. The increase in bunch length as leaves unfolded until the end of flowering was documented by Molitor *et al.* (2012a).

Taking the many different approaches to quantify or evaluate bunch compactness into consideration, the study aimed to evaluate and quantify the impact of different GA_3 and GA_{4+7} treatments on bunch structure, compactness and berry size distribution of Sunred Seedless. Detailed bunch structure measurements were quantified rather than using the more subjective measurements previously performed by other researchers.

5.2 MATERIALS AND METHODS

Refer to Sections 3.3 and 3.4 in Chapter 3, for details on the experimental layout and treatments applied.

5.2.1 Bunch length

Pre-harvest bunch length measurements were taken four weeks after the thinning applications in the 2015/2016 season and a day before the thinning applications in the 2016/2017 season. Pre-harvest and post-harvest bunch length was determined by measuring from the first lateral to the bunch tip using a ruler. More details are given in Sections 3.6.3 and 3.7.1 in Chapter 3.

5.2.2 Rachis measurements

An increased rachis diameter can be observed as a reaction to GA_3, due to the cell division properties of GA_3 (Raban *et al.*, 2013). The measurement of rachis diameter was done to determine whether the same phenomenon is observed in the cultivar Sunred Seedless as a reaction to GA_3, as well as GA_{4+7}. The rachis diameter was determined by using a digital calliper placed 1 cm above the first lateral (Nakamura *et al.*, 1974). Further measurements of the rachis included measuring the distance between the first and fifth laterals, taken from the start of the first lateral to the start of the fifth lateral. These assessments were to determine if any of the GA treatments applied at a specific phenological

stage promoted rachis elongation. The total length of the first four laterals was also determined and is discussed in further detail in Section 5.2.3 below.

5.2.3 Bunch compactness and berry size distribution

Measurements at harvest were performed to determine the efficacy of the GA_3 and GA_{4+7} treatments in reducing bunch compactness and improving bunch structure. Bunch compactness was assessed using two methods, namely a subjective visual assessment, as well as objective measurements, to determine the number of berries per cm of lateral length.

A visual assessment of bunch compactness was performed, based on a 5-point scale used by ARC Infruitec-Nietvoorbij. The 5-point compactness scale assigns bunches with values ranging from 1 (very compact) to 5 (very loose), with 3 described as the ideal bunch compactness. The distinction between the compactness of the bunches is based on the degree of movement of the berries and the visibility of pedicels.

Berries from the first four laterals of each bunch were removed and categorised into three groups according to their diameter (normal berries >16 mm; small berries 16-10 mm; shot berries <10 mm). The total lateral length for the four laterals was measured. The number of berries per group (normal, small and shot berries), along with the total lateral length was used to calculate the number of berries per cm of rachis, which is used as an objective parameter to assess bunch compactness (Lynn & Jensen, 1966). The mass of the normal berries was determined. The number of berries per cm of lateral length was used to determine the berry size classification, expressed as a percentage normal, small and shot berries. More details regarding bunch structure measurements are given in Section 3.7.1 in Chapter 3.

5.3 RESULTS AND DISCUSSION

Bunch structure data for the 2015/2016 season is presented in Table 5.1 and results for the 2016/2017 season in Table 5.2. The bunch length results affected by application timing of both seasons are given in Tables A.6 to A.7 of Addendum A.

5.3.1 2015/2016 season

5.3.1.1 Bunch length

Measurements performed four weeks after treatment application indicated that the earlier applications (10%BS) of 5 ppm GA_3, 15 ppm GA_{4+7} and 30 ppm GA_{4+7} had a significantly increased

pre-harvest bunch length compared to the late (BS), 30 ppm GA_{4+7} treatment applied at the bunch phenological stage of 10%BS (Table A.6, Addendum A).

At harvest, the earlier applications (10%BS) of 7.5 ppm GA_{4+7} and 30 ppm GA_{4+7}, applied at 10%BS, had a significantly higher bunch length compared to the late application (BS) of 5 ppm GA_3 and 7.5 ppm GA_{4+7} (Table A.6, Addendum A). The earlier applications, applied at the predominant vineyard phenological stage of 10%BS, also applied at the bunch phenological stage of 10%BS, resulted in increased bunch lengths compared to the late applications (BS).

Thinning applications applied at the bunch phenological stage of BS resulted in a significantly higher bunch length with the earlier 5 ppm GA_3 (10%BS) treatment compared to the untreated control (Table A.6, Addendum A). Bunch length plays a vital role in the structure of a bunch, because an increased bunch length can contribute to obtaining a less compact bunch structure (Korkutal *et al.*, 2008; Molitor *et al.*, 2012a).

Pre-harvest bunch length data for treatments applied at 80-100%F and BS indicated no significant differences between treatments (Table A.6, Addendum A). Bunch length between treatments at harvest did not differ significantly between thinning applications applied at 80-100%F and BS+4D.

5.3.1.2 Rachis diameter

The earlier 5 ppm GA_3 (10%BS) treatment, applied at BS, significantly increased rachis diameter especially compared to the late application of 5 ppm GA_3 (BS) (Table 5.1). Comparing equal active ingredient concentrations used, with a difference of four days between the two treatments, earlier application thereof showed a higher response towards the GA_3 application. The increased response with the earlier application could be ascribed to increased time available for cell division to take place. These results correspond with the findings of other authors (Raban *et al.*, 2013; Van der Vyver, 2016), where an increase in the rachis diameter was observed when GA was applied at 10%BS, and authors who reported an increase in rachis diameter with the use of GA_3 (Nakamura & Hori, 1984). The same increased response of the earlier, compared to the late GA_3 treatment, can be seen in Table A.4 of Addendum A, where the earlier treatment had a significantly higher berry mass.

An increase in rachis diameter can be observed from 80-100%F to BS+4D when comparing the mean rachis diameter per phenological stage (Table 5.1). The BS and BS+4D stages had significantly higher rachis diameters compared to 80-100%F and 10%BS stages. The 80-100%F stage resulted in the lowest rachis diameter compared to the rest of the stages. These results are similar to the findings of Nakamura *et al.* (1974) who reported an increase in rachis diameter with GA_{4+7} and GA_3 applied after full flowering, resulting in a thicker and harder rachis. Furthermore, the

increased rachis diameter was ascribed to an increased lignin content as a result of increased phenylalanine ammonia-lyase activity in the rachis of Kyoho grapes.

The rachis diameter for the late application of 15 ppm GA_{4+7} (BS), applied at the bunch phenological stage of 10%BS, had a significantly increased rachis diameter (Table 5.1). Thinning applications applied at 80-100%F and BS+4D had no significant difference between treatments in rachis diameter.

5.3.1.3 Distance from lateral 1 to 5

The earlier 5 ppm GA_3 (10%BS) treatment had a significantly decreased distance from lateral 1 to 5 compared to 30 ppm GA_{4+7} (10%BS) applied at 10%BS (Table 5.1). The earlier 5 ppm GA_3 (10%BS) treatment also had a significantly decreased distance from lateral 1 to 5 compared to treatments 7.5 ppm GA_{4+7} (10%BS), 15 ppm GA_{4+7} (10%BS), 5 ppm GA_3 (BS), 15 ppm GA_{4+7} (BS) and the untreated control applied at BS (Table 5.1). Several authors have reported an elongation of the rachis with the use of a GA_3 prior to flowering (Weaver & McCune, 1962; Miele *et al.*, 1978; Molitor *et al.*, 2012a), therefore the application of GA_3 at 10%BS and BS in the current study was probably too late to have an effect on rachis elongation. Thinning applications applied at 80-100%FB had no significant difference between treatments (Table 5.1).

5.3.1.4 Total length of laterals 1 to 4

For the 2015/2016 season, the total length of laterals 1 to 4 was not significantly affected by the treatments and thinning application timing (Table 5.1).

Table 5.1: Rachis diameter, lateral distance and total lateral length of marked Sunred Seedless bunches at harvest (2015/2016 season).

Treatment		Rachis diameter (mm)				Lateral distance 1-5 (mm)				Total length of laterals 1-4 (mm)			
		Bunch phenological stage at application											
		80-100% Flowering	10% Berry Set	Berry Set	Berry Set + 4 Days	80-100% Flowering	10% Berry Set	Berry Set	Berry Set + 4 Days	80-100% Flowering	10% Berry Set	Berry Set	Berry Set + 4 Days
T1	Untreated control	4.28 a	4.43 abc	4.87 ab	.	43.29 a	46.88 ab	46.92 a	.	227.89 a	253.63 a	209.48 a	.
T2	5 ppm GA3 (10%BS)	4.06 a	4.15 bc	**5.48 a**	.	41.38 a	**42.25 b**	**36.72 b**	.	221.75 a	244.97 a	220.44 a	.
T3	7.5 ppm GA4+7 (10%BS)	3.95 a	4.63 ab	4.44 cb	.	45.86 a	45.31 ab	46.00 a	.	227.85 a	227.96 a	233.96 a	.
T4	15 ppm GA4+7 (10%BS)	4.01 a	4.32 abc	4.53 cb	.	41.45 a	46.68 ab	48.17 a	.	234.35 a	235.16 a	242.39 a	.
T5	30 ppm GA4+7 (10%BS)	3.93 a	4.28 bc	4.84 ab	.	43.25 a	**47.88 a**	44.58 ab	.	231.08 a	237.52 a	231.78 a	.
T6	5 ppm GA3 (BS)	.	4.18 bc	**3.94 c**	4.94 a	.	45.78 ab	49.59 a	49.56 ab	.	223.82 a	227.09 a	225.15 a
T7	7.5 ppm GA4+7 (BS)	.	**3.85 c**	4.56 cb	4.48 a	.	45.42 ab	44.04 ab	41.58 c	.	238.25 a	229.17 a	212.31 a
T8	15 ppm GA4+7 (BS)	.	**4.89 a**	4.78 ab	4.98 a	.	47.08 ab	49.08 a	52.83 a	.	227.17 a	228.88 a	252.33 a
T9	30 ppm GA4+7 (BS)	.	4.36 abc	4.71 abc	4.38 a	.	44.17 ab	43.00 ab	45.05 bc	.	234.42 a	237.53 a	253.75 a
Mean		**4.04 z**	**4.34 y**	**4.68 x**	**4.69 x**	**43.05 y**	45.72 x	45.34 x	47.26 x	228.58 x	235.88 x	228.97 x	235.89 x
LSD p=0.05		0.54	0.58	0.78	1.06	4.51	5.53	8.59	4.57	36.54	42.46	49.26	48.27

Values designated by the same letters within a column or row do not differ significantly ($p \leq 0.05$).

5.3.1.5 Bunch compactness

i. Bunch compactness score

The untreated control had a significantly lower compactness score (CS) value (more compact), compared to the late 7.5 ppm GA_{4+7} (BS) and 30 ppm GA_{4+7} (BS) treatments, applied at 10%BS, as well as the late 5 ppm GA_3 (BS) and 7.5 ppm GA_{4+7} (BS) treatments, applied at BS (Table 5.2).

A value of 3 is awarded to bunches with the desired bunch compactness that requires little to no hand thinning in the packhouse before being packed for the export market. During the first season of the study, bunches of all the treatments had a bunch CS lower than 3 (Table 5.2). The earlier bunch phenological stages at the time of the thinning application, namely 80-100%F and 10%BS, had significantly higher mean CS values (less compact) compared to BS and BS+4D. The 80-100%F stage resulted in bunches with a mean CS of 2.2 which was significantly higher compared to the rest of the phenological stages and the closest to the desired bunch CS of 3. These results indicate that GA treatments applied at the earliest phenological stage of this experiment (80-100%F) resulted in less compact bunches. Similar results were reported by other authors where the use of GA_3 applications during flowering (Lynn & Jensen, 1966; Weaver & Pool, 1971; Dokoozlian & Peacock, 2001) and at full-flowering (Christodoulou *et al.*, 1966; Lynn & Jensen, 1966; Miele *et al.*, 1978; Dokoozlian & Peacock, 2001) was shown to be effective in reducing the fruit set rate, resulting in less berries per bunch and therefore less compact bunches.

ii. Total number of berries per cm of lateral length

The late 5 ppm GA_3 (BS) treatment, applied at 10%BS, had a significantly lower number of berries per cm of lateral resulting in less compact bunches (Table 5.2). No significant differences were found for treatments applied at 80-100%F, BS and BS+4D.

The 80-100%F as well as 10%BS stage had a significantly lower mean total number of berries per cm of lateral, compared to the BS and BS+4D stages (Table 5.2). These results correlate with increased bunch CS (less compact) also found for 80-100%F and 10%BS compared to BS and BS+4D. The GA treatments applied at the earlier phenological stages resulted in less compact bunches, with less berries per cm of lateral length compared to GA treatments applied onwards from berry set. Increasing the rate of GA_{4+7} treatments did not reduce the number of berries per bunch (Table 5.2). In contrast, an increase in GA_3 reduced the number of berries per bunch (Weaver, 1958; Reynolds *et al.*, 2016).

Table 5.2: Bunch compactness score and berries per cm of lateral length of marked Sunred Seedless bunches at harvest (2015/2016 season).

Treatment		Bunch compactness score (1-5)				Total berries per cm of lateral			
		Bunch phenological stage at application							
		80-100% Flowering	10% Berry Set	Berry Set	Berry Set + 4 Days	80-100% Flowering	10% Berry Set	Berry Set	Berry Set + 4 Days
T1	Untreated control	1.82 a	**1.38 d**	**1.04 b**	.	3.54 a	3.52 ab	4.18 a	.
T2	5 ppm GA3 (10%BS)	2.46 a	1.71 bcd	1.22 ab	.	3.49 a	4.25 a	4.43 a	.
T3	7.5 ppm GA4+7 (10%BS)	2.14 a	1.71 bcd	1.33 ab	.	4.24 a	4.37 a	5.02 a	.
T4	15 ppm GA4+7 (10%BS)	2.45 a	1.64 cd	1.28 ab	.	3.64 a	4.37 a	4.43 a	.
T5	30 ppm GA4+7 (10%BS)	2.15 a	1.50 cd	1.42 ab	.	3.64 a	4.46 a	5.10 a	.
T6	5 ppm GA3 (BS)	.	2.28 abc	1.75 a	1.41 a	.	**2.98 b**	3.97 a	3.80 a
T7	7.5 ppm GA4+7 (BS)	.	2.46 ab	1.67 a	1.66 a	.	3.77 ab	4.28 a	4.57 a
T8	15 ppm GA4+7 (BS)	.	1.67 bcd	1.43 ab	1.17 a	.	4.15 a	5.10 a	4.71 a
T9	30 ppm GA4+7 (BS)	.	2.58 a	1.57 ab	1.45 a	.	4.15 a	4.49 a	4.37 a
Mean		**2.20 x**	**1.88 y**	**1.41 z**	**1.42 z**	**3.71 y**	**4.00 y**	**4.55 x**	**4.36 x**
LSD p=0.05		0.94	0.79	0.55	0.85	1.20	1.08	1.63	3.40

Values designated by the same letters within a column or row do not differ significantly ($p \leq 0.05$).

5.3.1.6 Berry size distribution

i. Normal berries per cm of lateral length

The number of normal berries per cm of lateral length, for the late 5 ppm GA_3 (BS) treatment, applied at 10%BS, had a significantly lower number of normal berries compared to the untreated control, 5 ppm GA_3 (10%BS), 15 ppm GA_{4+7} (10%BS), 30 ppm GA_{4+7} (10%BS) and 15 ppm GA_{4+7} (BS) treatments (Table 5.2). This correlated with the significantly lower total berries per cm of lateral (Tables 5.2 & 5.3). The earlier 30 ppm GA_{4+7} (10%BS) treatment had a significantly higher number of normal berries compared to the late 5 ppm GA_3 (BS) and 7.5 ppm GA_{4+7} (BS) treatments. For thinning treatments applied at BS, the untreated control had a significantly higher number of normal berries compared to the earlier 15 ppm GA_{4+7} (10%BS) treatment (Table 5.3).

GA treatments applied at the earlier phenological stages (80-100%F & 10%BS) resulted in a significantly lower mean number of normal berries per cm of lateral length compared to GA treatments applied onwards from berry set (Table 5.3). These results correlate with the significantly lower mean total berries per cm of later found with the earlier phenological stages (80-100%F & 10%BS).

ii. Small berries per cm of lateral length

An undesired high number of small berries per cm of lateral length can result in an increased amount of labour required to prepare bunches according to export standards, as even berry size throughout the bunch is an important contributing factor to bunch quality. The number of small berries per cm of lateral length for the earlier 5 ppm GA_3 (10%BS) treatment, applied at BS, was significantly lower than for the earlier 7.5 ppm GA_{4+7} (10%BS) treatment (Table 5.3). No significant differences reported for treatments applied at 80-100%F, 10%BS and BS+4D.

iii. Shot berries per cm of lateral length

Shot berries per cm of lateral length, ranging from 0.04 to 0.34, were recorded for the 2015/2016 season (Table 5.3). The untreated control had a significantly lower number of shot berries per cm of lateral length recorded at all bunch phenological stages. The earlier 5 ppm GA_3 (10%BS) treatment also had a significantly lower number of shot berries per cm of lateral for treatments applied at 80-100%F, 10%BS and BS. The number of shot berries for the earlier 7.5 ppm GA_{4+7} (10%BS) treatment, applied at 80-100%F and 10%BS, was significantly higher per cm of lateral length.

Table 5.3: Berry size distribution per cm of lateral length of marked Sunred Seedless bunches at harvest (2015/2016 season).

Treatment		Normal berries per cm of lateral				Small berries per cm of lateral				Shot berries per cm of lateral			
		Bunch phenological stage at application											
		80-100% Flowering	10% Berry Set	Berry Set	Berry Set + 4 Days	80-100% Flowering	10% Berry Set	Berry Set	Berry Set + 4 Days	80-100% Flowering	10% Berry Set	Berry Set	Berry Set + 4 Days
T1	Untreated control	3.68 a	3.78 ab	**4.34 a**	.	0.52 a	0.45 a	0.53 ab	.	**0.10 b**	**0.04 c**	**0.10 c**	.
T2	5 ppm GA3 (10%BS)	2.98 a	3.74 ab	3.99 ab	.	0.42 a	0.41 a	**0.35 b**	.	**0.09 b**	**0.11 bc**	**0.10 c**	.
T3	7.5 ppm GA4+7 (10%BS)	3.32 a	3.42 abc	4.05 ab	.	0.68 a	0.70 a	0.77 a	.	0.24 a	0.26 a	0.19 abc	.
T4	15 ppm GA4+7 (10%BS)	2.93 a	3.78 ab	**3.39 b**	.	0.70 a	0.41 a	0.70 ab	.	0.21 ab	0.18 ab	0.34 a	.
T5	30 ppm GA4+7 (10%BS)	3.32 a	**3.91 a**	4.24 ab	.	0.45 a	0.41 a	0.61 ab	.	0.14 ab	0.14 abc	0.25 ab	.
T6	5 ppm GA3 (BS)	.	**2.88 c**	4.06 ab	4.37 a	.	0.61 a	0.53 ab	0.36 a	.	0.08 bc	0.16 bc	0.07 a
T7	7.5 ppm GA4+7 (BS)	.	3.03 bc	3.65 ab	3.58 a	.	0.56 a	0.43 ab	0.78 a	.	0.18 ab	0.20 abc	0.21 a
T8	15 ppm GA4+7 (BS)	.	3.66 ab	4.26 ab	4.21 a	.	0.38 a	0.67 ab	0.36 a	.	0.11 bc	0.17 bc	0.14 a
T9	30 ppm GA4+7 (BS)	.	3.26 abc	3.69 ab	3.25 a	.	0.69 a	0.65 ab	0.87 a	.	0.20 ab	0.15 bc	0.24 a
Mean		**3.25 y**	**3.49 y**	**3.96 x**	**3.85 x**	0.55 x	0.51 x	0.58 x	0.59 x	0.15 x	0.15 x	0.18 x	0.17 x
LSD p=0.05		0.83	0.76	0.92	1.73	0.45	0.41	0.36	1.33	0.14	0.13	0.15	0.44

Values designated by the same letters within a column or row do not differ significantly ($p \leq 0.05$).

iv. Berry size classification (%)

The ideal berry size distribution within a bunch would consist of a high percentage of normal berries, with the lowest possible percentage of small- and shot berries. Lower small and shot berry occurrence directly correlates with decreased labour requirements for the preparation of a bunch of export standard. The earlier 5 ppm GA_3 (10%BS) treatment resulted in a significantly higher percentage normal berries as well as significantly lower percentage small berries for treatments applied at BS, compared to the earlier GA_{4+7} (10%BS) treatments of 7.5 ppm and 15 ppm (Table 5.4). The 5 ppm GA_3 (10%BS) treatment, therefore, delivered the desired berry size distribution, with the highest percentage normal berries and the lowest percentage small and shot berries compared to the 7.5 ppm and 15 ppm GA_{4+7} (10%BS) treatments. The untreated control resulted in a significantly lower percentage shot berries compared to the earlier 7.5 ppm and 15 ppm GA_{4+7} (10%BS) treatments as well as the late 7.5 ppm and 30 ppm GA_{4+7} (BS) treatments, for treatments applied at 10%BS (Table 5.4).

Table 5.4: Berry size classification (%) of marked Sunred Seedless bunches at harvest (2015/2016 season).

Treatment		% Normal berries				% Small berries				% Shot berries			
		Bunch phenological stage at application											
		80-100% Flowering	10% Berry Set	Berry Set	Berry Set + 4 Days	80-100% Flowering	10% Berry Set	Berry Set	Berry Set + 4 Days	80-100% Flowering	10% Berry Set	Berry Set	Berry Set + 4 Days
T1	Untreated control	85.50 a	88.32 a	87.45 ab	.	12.20 a	10.75 a	10.49 ab	.	2.30 a	**0.93 c**	2.06 b	.
T2	5 ppm GA3 (10%BS)	85.31 a	88.18 a	**90.11 a**	.	12.03 a	9.27 a	**7.66 b**	.	2.66 a	2.56 bc	2.24 b	.
T3	7.5 ppm GA4+7 (10%BS)	78.07 a	78.54 a	80.62 bc	.	16.13 a	15.78 a	15.46 a	.	5.81 a	5.69 a	3.92 b	.
T4	15 ppm GA4+7 (10%BS)	75.95 a	86.43 a	76.63 c	.	18.62 a	9.43 a	15.92 a	.	5.43 a	4.15 ab	**7.46 a**	.
T5	30 ppm GA4+7 (10%BS)	84.54 a	87.41 a	82.91 abc	.	11.85 a	9.33 a	12.12 ab	.	3.61 a	3.26 abc	4.97 ab	.
T6	5 ppm GA3 (BS)	.	81.10 a	85.42 ab	90.95 a	.	16.57 a	11.23 ab	7.57 a	.	2.33 bc	3.35 b	1.48 a
T7	7.5 ppm GA4+7 (BS)	.	80.17 a	85.59 ab	78.51 a	.	15.14 a	9.98 ab	16.83 a	.	4.69 ab	4.44 ab	4.67 a
T8	15 ppm GA4+7 (BS)	.	88.00 a	83.28 abc	89.13 a	.	9.21 a	13.22 ab	7.84 a	.	2.79 bc	3.51 b	3.03 a
T9	30 ppm GA4+7 (BS)	.	78.53 a	82.73 abc	76.24 a	.	16.49 a	13.97 ab	18.65 a	.	4.99 ab	3.30 b	5.11 a
Mean		81.87 x	84.07 x	83.86 x	83.71 x	14.17 x	12.44 x	12.23 x	12.72 x	3.96 x	3.49 x	3.92 x	3.57 x
LSD p=0.05		14.56	11.78	8.48	35.90	12.20	10.26	6.58	27.22	3.51	2.77	3.22	9.12

Values designated by the same letters within a column or row do not differ significantly ($p \leq 0.05$).

5.3.2 2016/2017 season

5.3.2.1 Bunch length

The only application timing that had significant differences in bunch lengths at harvest was the 50%F phenological stage (Table A.7 of Addendum A), indicating a higher response at the 50%F stage towards the different GA treatments. The lowest GA_{4+7} rate of 7.5 ppm resulted in the shortest bunch length at harvest, bearing in mind that the pre-harvest measurement did not differ significantly from the rest of the treatments. The 15 ppm GA_{4+7} (T) and 60 ppm GA_{4+7} (S) treatments resulted in significantly higher bunch lengths compared to the 7.5 ppm GA_{4+7} (T) treatment (Table A.7 of Addendum A). The 15 ppm GA_{4+7} (T) and 60 ppm GA_{4+7} (S) treatments led to an increase of 4.53 cm and 4.78 cm, respectively, from pre-harvest to harvest.

Comparing the mean between phenological stages, the 10%BS stage resulted in significantly higher mean bunch lengths at harvest (23.29 cm), while 10%BS also resulted in the largest difference between pre-harvest and harvest with a difference of 2.95 cm (Table A.7 of Addendum A).

5.3.2.2 Rachis diameter

The 5 ppm GA_3 (T) treatment had a significantly lower rachis diameter compared to the 120 ppm GA_{4+7} (T) treatment applied at 50%F as well as the 60 ppm GA_{4+7} (T+S) treatment applied at 80-100%F (Table 5.5). The increased concentration of 120 ppm GA_{4+7} (T) used at 50%F and the double application of 60 ppm GA_{4+7} (T+S) at 80-100%F, resulted in an increased rachis diameter compared to the low rate and single application of 5 ppm GA_3. These results indicate a response towards the high rate, as well as double application of GA_{4+7} compared to GA_3 applied during flowering.

An increase in rachis diameter can be observed from 50%F to BS when comparing the mean rachis diameter per phenological stage (Table 5.5). The 10%BS and BS stages resulted in significantly higher rachis diameters compared to the 50%F and 80-100%F stages, correlating with the findings of Nakamura *et al.* (1974) who recorded an increase in rachis diameter with GA_{4+7} and GA_3 applied after full flowering.

5.3.2.3 Distance from lateral 1 to 5

The distance between lateral 1 to 5 relates to the possible elongation effect associated with the use of GA at certain phenological stages. Similar to the bunch length results, only the 50%F application timing resulted in significant differences between treatments (Table 5.5). The GA_3 treatment induced a significantly longer distance between laterals 1 to 5 compared to the GA_{4+7} treatments of 7.5 ppm (T), 60 ppm (T) and 120 ppm (T) (Table 5.5). Compared to the 60 ppm GA_{4+7} (T) treatment, the GA_3

treatment increased the distance between laterals 1 to 5 by 12.88 mm. Application of treatments at the 50%F phenological stage led to significantly lower mean lateral distance compared to the other phenological stages.

5.3.2.4 Total length of laterals 1 to 4

Higher rates of GA_{4+7} used, such as 30 ppm (T), 60 ppm (T), 120 ppm (T) and 60 ppm (S), resulted in significantly higher total lateral lengths compared to the lower rate 7.5 ppm (T) GA_{4+7} treatment, applied at 50%F (Table 5.5). The results obtained with the treatments applied at the 50%F stage indicate that an increased GA_{4+7} rate improves total lateral length compared to lower GA_{4+7} rates, if applied early during flowering. Similar to the mean distance between laterals 1 to 5, the 50%F phenological stage had a significantly lower mean total lateral lengths compared to the other phenological stages.

At 10%BS the 60 ppm GA_{4+7} (T) treatment resulted in a significant increase of 39.05 mm in total lateral length compared to the untreated control (Table 5.5).

Table 5.5: Rachis diameter, lateral distance and total lateral length of marked Sunred Seedless bunches at harvest (2016/2017 season).

Treatment		Rachis diameter (mm)				Lateral distance 1-5 (mm)				Total length of laterals 1-4 (mm)			
		Bunch phenological stage at application											
		50% Flowering	80-100% Flowering	10% Berry Set	Berry Set	50% Flowering	80-100% Flowering	10% Berry Set	Berry Set	50% Flowering	80-100% Flowering	10% Berry Set	Berry Set
T1	Untreated control	3.32 ab	4.12 ab	4.70 a	4.76 ab	45.75 ab	47.31 a	45.90 a	48.10 a	214.12 ab	266.35 a	**240.80 b**	279.62 ab
T2	5 ppm GA3 (T)	**3.19 b**	**3.56 b**	4.65 a	4.81 ab	**50.78 a**	44.80 a	49.40 a	47.25 a	225.63 ab	235.70 a	250.40 ab	250.45 ab
T3	7.5 ppm GA4+7 (T)	3.38 ab	3.84 ab	5.13 a	4.67 b	42.58 bc	51.10 a	49.25 a	47.67 a	**191.19 b**	265.40 a	268.55 ab	280.02 a
T4	15 ppm GA4+7 (T)	3.57 ab	4.19 a	4.73 a	5.18 ab	47.10 ab	46.76 a	47.05 a	49.00 a	224.43 ab	280.75 a	271.25 ab	252.10 ab
T5	30 ppm GA4+7 (T)	3.59 ab	4.03 ab	4.74 a	4.76 ab	45.83 ab	48.20 a	47.25 a	47.55 a	251.79 a	250.90 a	260.74 ab	257.15 ab
T6	60 ppm GA4+7 (T)	3.20 b	3.88 ab	4.73 a	4.99 ab	**37.90 c**	44.80 a	46.95 a	47.40 a	244.73 a	233.31 a	**279.85 a**	248.10 b
T7	120 ppm GA4+7 (T)	**3.96 a**	4.09 ab	4.79 a	**5.30 a**	41.93 bc	48.65 a	46.50 a	46.65 a	242.30 a	270.80 a	267.45 ab	266.95 ab
T8	60 ppm GA4+7 (T+S)	3.46 ab	4.27 a	4.81 a	5.07 ab	46.83 ab	45.95 a	50.25 a	47.65 a	224.10 ab	267.45 a	267.25 ab	260.35 ab
T9	60 ppm GA4+7 (S)	3.74 ab	3.95 ab	4.89 a	5.17 ab	45.72 ab	50.30 a	45.05 a	46.09 a	251.40 a	260.35 a	267.95 ab	252.10 ab
Mean		**3.49 z**	**3.99 y**	**4.80 x**	**4.97 x**	**44.94 y**	47.54 x	47.51 x	47.48 x	**229.97 y**	259.00 x	263.80 x	260.76 x
LSD p=0.05		0.66	0.60	0.75	0.61	6.31	8.47	6.01	4.96	47.04	53.63	36.59	31.91

Values designated by the same letters within a column or row do not differ significantly ($p \leq 0.05$).

5.3.2.5 Bunch compactness

i. Bunch compactness score

Compared to the 2015/2016 season, where none of the treatments reached a CS of 3 (Table 5.2), some of the bunches of the 2016/2017 season obtained CS ranging from 3.20 to 4.48 (Table 5.6). Consequently, bunches were less compact compared to the previous season.

Treatments applied at 50%F resulted in CS values ranging from 3.56 to 4.48 (Table 5.6). The only significant difference was found between the 5 ppm GA_3 treatment (with a CS of 4.48) and the 7.5 ppm GA_{4+7} treatment (with a CS of 3.56) (Table 5.6). The GA_3 treatment at 50%F delivered undesired straggly bunches.

The GA_3 treatment had a significantly higher CS compared to the 60 ppm GA_{4+7} (T) treatment applied at 80-100%F (Table 5.6). Treatments applied at the 80-100%F phenological stage resulted in CS values ranging from 3.2 to 3.9 (Table 5.6). These value are considered slightly too high, a quick hand thinning action in the vineyard or the removal of uneven berries in the packhouse will result in bunches with a straggly appearance. If the berries are even in size with no thinning action required to remove uneven berries, these CS values could deliver bunches with a desirable appearance.

Application of treatments at 10%BS delivered better CS results in the 2016/17 season (Table 5.6) compared to the 2015/2016 season (Table 5.6), with values ranging from 1.85 to 2.65. The 60 ppm GA_{4+7} (T+S) delivered significantly better results with a CS of 2.65 compared to the 7.5 ppm GA_{4+7} (T), 30 ppm GA_{4+7} (T) and 60 ppm GA_{4+7} (S) treatments, applied at 10%BS (Table 5.6).

Taking into consideration that no hand thinning was performed on the bunches, a bunch CS with a value close to 3 will result in the most desired bunch compactness for bunches receiving no hand thinning. Bunches with this compactness could still receive a quick hand thinning action to remove smaller berries from the bunch in order to ensure an even berry size distribution throughout the bunch to comply with export standards.

There was a significant decrease in the mean compactness score from the 50%F to BS phenological stage (Table 5.6). The 50%F phenological stage resulted in a mean bunch CS of 4.07. These results indicate that applying a thinning treatment at 50%F is too early, as CS values of 4 indicate loose bunches. The 80-100%F stage had a significantly higher mean CS compared to the 10%BS and BS stages. The application of GA treatments during flowering resulted in less compact bunches, agreeing with the results of other authors (Christodoulou *et al*., 1966; Lynn & Jensen, 1966; Weaver & Pool, 1971; Miele *et al*., 1978; Dokoozlian & Peacock, 2001).

ii. Total number of berries per cm of lateral length

Within the phenological stages of 50%F and 80-100%F, the total berries per cm of lateral and bunch CS, followed a similar trend in the significant differences found between treatments (Table 5.6). At 50%F, the 5ppm GA_3 (T) treatment had a significantly lower number of berries per cm of lateral compared to the 7.5 ppm GA_{4+7} (T) treatment, following the same trend as the CS. The 5 ppm GA_3 (T) treatment, applied at 80-100%F, resulted in a significantly lower number of berries per cm of lateral compared to the 60 ppm GA_{4+7} (T) treatment as well as the untreated control. The significantly reduced number of berries per cm of lateral found for the 5 ppm GA_3 (T) treatment at 50%F and 80-100%F correlate with the significantly higher CS, indicating a looser bunch structure.

At the BS stage, the GA_3 treatment had a significantly lower total number of berries per cm of lateral compared to the 7.5 ppm GA_{4+7} (T), 15 ppm GA_{4+7} (T) and 60 ppm GA_{4+7} (S) treatments (Table 5.6). The total number of berries per cm of lateral for the GA_3 treatment also tended to be the lowest of all the treatments for the 50%F, 80-100%F and BS phenological stages.

Comparing the mean number of berries per cm of lateral between the different phenological stages, the 2016/2017 season had considerably less berries per cm compared to the 2015/2016 season, indicating a positive change with the adjustment of treatments from the first to the second season. The 50%F and 80-100%F resulted in a significantly lower mean number of berries per cm of lateral, compared to 10%BS and BS stage (Table 5.6).

Table 5.6: Bunch compactness score and berries per cm of lateral length of marked Sunred Seedless bunches at harvest (2016/2017 season).

Treatment		Bunch compactness score (1-5)				Total berries per cm of lateral			
		Bunch phenological stage at application							
		50% Flowering	80-100% Flowering	10% Berry Set	Berry Set	50% Flowering	80-100% Flowering	10% Berry Set	Berry Set
T1	Untreated control	4.09 ab	3.36 ab	2.50 ab	1.72 a	2.48 ab	3.11 a	3.60 b	3.79 abc
T2	5 ppm GA3 (T)	**4.48 a**	**3.90 a**	2.50 ab	1.90 a	**1.94 b**	**2.41 b**	3.80 ab	**3.35 c**
T3	7.5 ppm GA4+7 (T)	3.56 b	3.50 ab	1.90 b	1.45 a	2.72 a	2.93 ab	4.31 a	4.02 ab
T4	15 ppm GA4+7 (T)	4.18 ab	3.80 ab	2.40 ab	1.45 a	2.36 ab	2.84 ab	4.17 ab	**4.29 a**
T5	30 ppm GA4+7 (T)	4.27 ab	3.50 ab	1.89 b	1.75 a	2.41 ab	2.96 ab	4.16 ab	3.67 bc
T6	60 ppm GA4+7 (T)	3.97 ab	**3.20 b**	2.40 ab	1.60 a	2.37 ab	**3.01 a**	3.65 ab	3.81 abc
T7	120 ppm GA4+7 (T)	3.98 ab	3.70 ab	2.40 ab	1.50 a	2.60 a	2.78 ab	3.81 ab	3.82 abc
T8	60 ppm GA4+7 (T+S)	4.23 ab	3.60 ab	**2.65 a**	1.75 a	2.14 ab	2.86 ab	3.82 ab	3.58 bc
T9	60 ppm GA4+7 (S)	3.85 ab	3.75 ab	1.85 b	1.53 a	2.53 ab	2.58 ab	3.85 ab	4.02 ab
Mean		**4.07 w**	**3.59 x**	**2.28 y**	**1.63 z**	**2.39 z**	**2.83 y**	**3.91 x**	**3.82 x**
LSD p=0.05		0.78	0.69	0.75	0.55	0.63	0.58	0.67	0.58

Values designated by the same letters within a column or row do not differ significantly ($p \leq 0.05$).

5.3.2.6 Berry size distribution

i. Normal berries per cm of lateral length

The untreated control had a significantly higher number of normal berries per cm of lateral compared to the 120 ppm GA_{4+7} (T) treatment, applied at 80-100%F (Table 5.7). These results are to be expected due to a positive correlation between increased GA rates and the number of shot berries (Christodoulou *et al.*, 1966; Dokoozlian & Peacock 2001), which would reduce the number of normal berries per cm of lateral. The 50%F and 80-100%F had a significantly lower mean number of normal berries per cm of lateral compared to the 10%BS and BS stages (Table 5.7), indicating that GA treatments applied during flowering reduced the number of normal berries.

ii. Small berries per cm of lateral length

Hardly any significant differences were observed concerning small berries per cm of lateral length, with the only significant difference observed at 10%BS (Table 5.7). The 15 ppm GA_{4+7} (T) treatment had significantly more small berries per cm of lateral compared to the 30 ppm GA_{4+7} (T) and 60 ppm GA_{4+7} (T) treatments (Table 5.7). There were no significant differences in the total, normal or shot berries per cm of lateral length for treatments applied at 10%BS, therefore the significant differences found within the small berries per cm of lateral at 10%BS cannot easily be explained (Tables 5.6 & 5.7).

iii. Shot berries per cm of lateral length

Considering the higher GA_{4+7} rates used in the 2016/2017 season, a similar number of shot berries per cm of lateral were reported compared to the 2015/2016 season. Shot berries per cm of lateral length, ranging from 0.01 to 0.33, were recorded for the 2016/2017 season (Table 5.7). The untreated control and 5 ppm GA_3 (T) treatment resulted in a significantly lower number of shot berries per cm of lateral compared to the high rate 120 ppm GA_{4+7} (T) treatment, applied at 50%F and 80-100%F (Table 5.7). Similar results were reported by other authors, where an increase in shot berries was observed with increased GA rates applied during flowering (Christodoulou *et al.*, 1966; Dokoozlian & Peacock, 2001). The 30 ppm GA_{4+7} (T), applied at 50%F, 80-100%F and BS, had a significantly higher number of shot berries per cm of lateral length compared to the untreated control and 5 ppm GA_3 (T). No significant differences were found for treatments applied at 10%BS. The mean shot berries per cm of lateral was significantly lower at BS, compared to 50%F, 80-100%F and 10%BS.

Table 5.7: Berry size distribution per cm of lateral length of marked Sunred Seedless bunches at harvest (2016/2017 season).

Treatment		Normal berries per cm of lateral				Small berries per cm of lateral				Shot berries per cm of lateral			
		Bunch phenological stage at application											
		50% Flowering	80-100% Flowering	10% Berry Set	Berry Set	50% Flowering	80-100% Flowering	10% Berry Set	Berry Set	50% Flowering	80-100% Flowering	10% Berry Set	Berry Set
T1	Untreated control	1.58 a	**2.32 a**	2.79 a	3.22 ab	0.90 a	0.70 a	0.72 ab	0.53 a	**0.01 e**	**0.09 b**	0.88 a	0.04 bc
T2	5 ppm GA3 (T)	1.19 a	1.81 ab	3.14 a	3.09 ab	0.67 a	0.53 a	0.55 ab	0.25 a	0.08 de	**0.06 b**	0.11 a	**0.01 c**
T3	7.5 ppm GA4+7 (T)	1.51 a	2.14 ab	3.54 a	3.41 ab	1.16 a	0.67 a	0.65 ab	0.52 a	0.05 de	0.12 ab	0.12 a	0.09 abc
T4	15 ppm GA4+7 (T)	1.31 a	1.79 ab	3.14 a	3.71 a	0.91 a	0.83 a	**0.84 a**	0.46 a	0.14 bcd	0.22 ab	0.18 a	0.12 ab
T5	30 ppm GA4+7 (T)	1.27 a	1.91 ab	3.53 a	2.98 b	0.91 a	0.74 a	0.48 b	0.55 a	**0.23 ab**	**0.30 a**	0.16 a	**0.14 a**
T6	60 ppm GA4+7 (T)	1.51 a	2.21 ab	2.99 a	3.21 ab	0.79 a	0.57 a	0.46 b	0.49 a	0.09 cde	0.23 ab	0.19 a	0.10 abc
T7	120 ppm GA4+7 (T)	1.28 a	**1.74 b**	2.94 a	3.25 ab	0.99 a	0.73 a	0.64 ab	0.48 a	**0.33 a**	**0.30 a**	0.23 a	0.09 abc
T8	60 ppm GA4+7 (T+S)	1.10 a	1.82 ab	3.03 a	3.14 ab	0.83 a	0.87 a	0.68 ab	0.39 a	**0.20 bc**	0.17 ab	0.11 a	0.05 bc
T9	60 ppm GA4+7 (S)	1.66 a	1.85 ab	3.11 a	3.52 ab	0.79 a	0.63 a	0.63 ab	0.46 a	0.09 cde	0.10 ab	0.11 a	0.05 abc
Mean		**1.38 z**	**1.95 y**	**3.14 x**	**3.28 x**	**0.88 x**	**0.70 y**	**0.63 y**	**0.46 z**	**0.13 y**	**0.18 x**	**0.23 xy**	**0.08 z**
LSD p=0.05		0.85	0.54	0.86	0.68	0.70	0.40	0.33	0.32	0.12	0.21	0.15	0.09

Values designated by the same letters within a column or row do not differ significantly ($p \leq 0.05$).

iv. Berry size classification (%)

Results of treatments applied in the 2016/2017 season indicated little variation in the percentage of normal and small berries, but treatments influenced the percentage shot berries significantly (Table 5.8). No significant differences between the untreated control and treatments were observed for the percentage of normal and small berries.

For treatments applied at BS, the 5 ppm GA_3 (T) treatment resulted in a significantly higher percentage of normal berries compared to the 30 ppm GA_{4+7} (T) treatment (Table 5.8). These findings correlate with the significant differences found for the percentage of shot berries at BS, where the 30 ppm GA_{4+7} (T) treatment resulted in a significantly higher percentage shot berries compared to the 5 ppm GA_3 (T) treatment, as well as the untreated control, 60 ppm GA_{4+7} (T+S) and 60 ppm GA_{4+7} (S) treatments.

Similary to the 2015/2016 season, the 5 ppm GA_3 (T) treatment applied at BS delivered the most desirable berry size distribution, with the highest percentage of normal berries and the lowest percentage of shot berries (Table 5.8). For treatments applied at BS, the 5 ppm GA_3 (T) treatment resulted in a significantly higher percentage of normal berries compared to the 30 ppm GA_{4+7} (T) treatment (Table 5.8). These findings correlate with the significant differences found for the percentage of shot berries at BS, where the 30 ppm GA_{4+7} (T) treatment resulted in a significantly higher percentage of shot berries compared to the 5 ppm GA_3 (T) treatment, as well as the untreated control, 60 ppm GA_{4+7} (T+S) and 60 ppm GA_{4+7} (S) treatments.

A significantly lower percentage of shot berries was obtained with the untreated control and the 5 ppm GA_3 (T) treatment, for thinning treatments applied at 50%F and 80-100%F compared to the 120 ppm GA_{4+7} (T) treatment (Table 5.8). The highest rate treatment of 120 ppm GA_{4+7} (T), applied during flowering, resulted in a significantly higher percentage of shot berries compared to the untreated control and the 5 ppm GA_3 (T) treatment. These results indicate a positive correlation between an increased GA_{4+7} rate and the number of shot berries, similar results with the use of GA_3 have been reported (Christodoulou *et al.*, 1966; Dokoozlian & Peacock, 2001).

There was a significant increase in the mean percentage of normal berries from 50%F to BS (Table 5.8). Treatments applied at 50%F and 80-100%F had a significantly higher mean normal berry percentage compared to treatments applied at 10%BS and BS. Applying GA treatments at the earlier phenological stages resulted in reduced mean normal berry percentages, indicating a higher sensitivity of Sunred Seedless to the formation of small- and shot berries with GA treatments applied during flowering.

A decrease in the mean percentage of small berries can be observed from 50%F to BS (Table 5.8). The 50%F and 80-100%F stages resulted in a significantly higher percentage of small berries compared to the 10%BS and BS stages. Similarly, the 50%F and 80-100%F had a significantly higher percentage of shot berries compared to the 10%BS and BS stages. These findings correlate with the significant differences found for the percentage normal berries, indicating a higher sensitivity of the earlier phenological stages to GA treatments with regard to the formation of small and shot berries. The same trend was not observed during the 2015/2016 season.

Table 5.8: Berry size classification (%) of marked Sunred Seedless bunches at harvest (2016/2017 season).

Treatment		% Normal berries				% Small berries				% Shot berries			
		Bunch phenological stage at application											
		50% Flowering	80-100% Flowering	10% Berry Set	Berry Set	50% Flowering	80-100% Flowering	10% Berry Set	Berry Set	50% Flowering	80-100% Flowering	10% Berry Set	Berry Set
T1	Untreated control	63.25 a	75.16 a	77.24 a	84.45 ab	36.47 a	22.06 a	20.37 ab	14.48 a	**0.27 d**	**2.78 b**	2.40 a	**1.07 b**
T2	5 ppm GA3 (T)	61.82 a	75.32 a	82.84 a	**92.29 a**	34.50 a	21.62 a	14.36 ab	7.30 a	**3.68 cd**	**3.06 b**	2.80 a	**0.41 b**
T3	7.5 ppm GA4+7 (T)	55.04 a	72.99 a	81.46 a	83.72 ab	43.13 a	22.97 a	15.83 ab	13.68 a	1.82 cd	4.04 ab	2.71 a	2.61 ab
T4	15 ppm GA4+7 (T)	55.66 a	62.08 a	73.68 a	86.39 ab	38.19 a	29.90 a	**21.50 a**	10.92 a	6.15 bc	8.02 ab	4.82 a	2.69 ab
T5	30 ppm GA4+7 (T)	52.20 a	65.13 a	84.59 a	81.02 b	38.25 a	24.99 a	11.55 b	15.14 a	9.55 ab	9.87 ab	3.86 a	3.85 a
T6	60 ppm GA4+7 (T)	63.24 a	73.54 a	82.00 a	84.65 ab	32.83 a	18.86 a	12.80 ab	12.82 a	3.93 cd	7.60 ab	5.20 a	2.53 ab
T7	120 ppm GA4+7 (T)	48.78 a	63.00 a	77.41 a	85.35 ab	38.73 a	26.43 a	16.81 ab	12.42 a	**12.49 a**	10.57 a	5.78 a	2.23 ab
T8	60 ppm GA4+7 (T+S)	51.57 a	64.20 a	79.23 a	87.57 ab	39.07 a	29.95 a	17.94 ab	11.14 a	9.37 ab	5.86 ab	2.83 a	**1.29 b**
T9	60 ppm GA4+7 (S)	65.28 a	71.72 a	80.97 a	87.85 ab	31.34 a	24.17 a	16.08 ab	10.92 a	3.38 cd	4.12 ab	2.80 a	**1.22 b**
Mean		**57.43 z**	**69.24 y**	**79.93 x**	**85.92 w**	**36.95 x**	**24.55 y**	**16.36 z**	**12.09 z**	**5.63 x**	**6.21 x**	**3.69 y**	**1.99 z**
LSD p=0.05		28.56	14.51	12.16	10.04	27.32	12.24	9.45	8.62	5.13	7.10	3.87	2.47

Values designated by the same letters within a column or row do not differ significantly ($p \leq 0.05$).

5.4 CONCLUSION

A significant increase in mean rachis diameter from the earliest to the latest phenological stage was observed for both seasons. Results showed that GA treatments applied after flowering resulted in significantly increased rachis diameters. An increased sensitivity towards high rates and double applications of GA_{4+7} was observed for treatments applied at 50%F, with significantly increased rachis diameters compared to the low rate and single application of 5 ppm GA_3 (T).

Although none of the treatments during the 2015/2016 season induced bunches of the desired CS of 3, bunches in the 2016/2017 season for treatments applied at 50%F and 80-100%F exceeded this recommended norm. Results indicate that the application of a thinning treatment at 50%F is most likely too early for Sunred Seedless as bunches were straggly. The mean bunch CS decreased significantly, *i.e.* bunches became more compact, from the earliest to the latest phenological stage. The application of GA treatments during flowering resulted in less compact bunches.

The 5 ppm GA_3 (T) treatment (2016/2017 season) resulted in the least number of total berries per cm of lateral length for the 50%F, 80-100%F and BS stages, indicating a looser bunch structure. The 5 ppm GA_3 treatment had a significantly reduced number of total berries per cm of lateral compared to the untreated control at 80-100%F. Treatments applied at the two earlier phenological stages resulted in a significantly reduced mean number of total berries per cm of lateral, as well as a significantly reduced mean normal berries per cm of lateral, compared to the two later phenological stages. This indicates that applying a GA treatment during flowering reduced fruit set, by significantly reducing the number of berries per cm of lateral. Applying a GA thinning treatment too early during flowering should result in overthinning and thus straggly bunches. The untreated control had a significantly higher number of normal berries per cm of lateral compared to the 120 ppm GA_{4+7} (T) treatment, applied at 80-100%F, as well as a significantly lower number of shot berries per cm of lateral compared to the 120 ppm GA_{4+7} (T) treatment, applied at 50%F and 80-100%F.

Application of 5 ppm GA_3 (10%BS) at BS generally produced grapes with the most desirable berry size classification in terms of the highest percentage of normal berries and the lowest percentage shot berries. The high rate treatment of 120 ppm GA_{4+7} (T) (2016/2017 season), applied at 50%F and 80-100%F, resulted in a significantly higher percentage of shot berries compared to the untreated control and the 5 ppm GA_3 (T) treatment. A significant increase in the mean percentage of normal berries, as well as a significant decrease in the mean percentage small berries, is observed from 50%F to BS, for the 2016/2017 season. Given that treatments applied at 50%F and 80-100%F had a significantly higher mean percentage of shot berries compared to the 10%BS and BS stages, results indicate that Sunred Seedless has a higher sensitivity for the formation of shot berries when

GA is applied during flowering. Instead of increasing the abscission of weaker flowers (berries), applications during flowering increased the formation of shot berries. An increase in shot berry occurrence can also be observed with the use of higher GA_{4+7} rates.

The sensitivity of Sunred Seedless towards early GA applications (during flowering) observed in this study, confirms why the early application of GA thinning treatments for this particular cultivar do not give economically acceptable results. The study resulted in more reoccurring trends regarding the bunch phenological stage at the time of application, rather than trends regarding a specific GA treatment and treatment rates. These results confirm that the timing of a GA applications play a fundamental role in the treatment outcome for a specific cultivar.

It is recommended that other PGRs such as ABA and ethylene should be evaluated on their own and in combination with GA, as thinning agents for Sunred Seedless. A multidisciplinary approach is recommended for further research, where parallel to the field trial evaluations of PGRs, genomic studies are also included to identify and quantify GA signalling components and availability of bioactive GAs, to contribute to understanding differences in response obtained with treatments applied in the field trial.

Some of the observations have only been recorded during one of the two seasons. Repeating the trial for a third season could have provided a better indication regarding trends observed during the first two seasons.

5.5 LITERATURE CITED

Carmona, M.J., Chaïb, J., Martínez-Zapater, J.M. & Thomas, M.R., 2008. A molecular genetic perspective of reproductive development in grapevine. J. Exp. Bot. 59, 2579-2596.

Casanova, L., Casanova, R., Moret, A. & Agustí, M., 2009. The application of Gibberellic acid increases berry size of “Emperatriz” seedless grape. Spanish J. Agric. Res. 7, 919-927.

Chaturvedi, K.N. & Khanduja, S.D., 2016. Response of Perlette clusters to gibberellic acid applied at different stages of bloom. Vitis 18, 10.

Christodoulou, A., Pool, R. & Weaver, R., 1966. Prebloom thinning of Thompson Seedless grapes is feasible when followed by bloom spraying with gibberellin. Calif. Agric. 20, 8-10.

Coombe, B.G. & McCarthy, M.G., 2000. Dynamics of grape berry growth and physiology of ripening. Aust. J. Grape Wine Res. 6, 131-135.

Dokoozlian, N.K. & Peacock, W.L., 2001. Gibberellic acid applied at bloom reduces fruit set and improves size of “Crimson Seedless” table grapes. Hort. Sci. 36, 706-709.

Domingos, S., Nobrega, H., Raposo, A., Cardoso, V., Soares, I., Ramalho, J.C., Leitão, A.E., Oliveira, C.M. & Goulao, L.F., 2016. Light management and gibberellic acid spraying as thinning methods in seedless table grapes (*Vitis vinifera* L.): Cultivar responses and effects on the fruit quality. Sci. Hortic. 201, 68-77.

Hanni, E., Lardschneider, E. & Kelderer, M., 2012. Alternatives to the use of gibberellins for bunch thinning and bunch compactness reduction on grapevine. Acta Hortic. 978, 335-345.

Korkutal, I., Bahar, E. & Gökhan, Ö., 2008. The characteristics of substances regulating growth and development of plants and the utilization of Gibberellic Acid (GA_3) in viticulture. World J. Agric. Sci. 4, 321-325.

Lynn, C.D. & Jensen, F.L., 1966. Thinning effects of bloom time Gibberellin sprays on Thompson Seedless table grapes. Am. J. Enol. Vitic. 17, 283-289.

Miele, A., Weaver, R.J. & Johnson, J., 1978. Effect of potassium gibberellate on fruit-set and development of Thompson Seedless and Zinfandel grapes. Am. J. Enol. Vitic. 29, 79-82.

Molitor, D., Behr, M., Hoffmann, L. & Evers, D., 2012a. Research note: Benefits and drawbacks of pre-bloom applications of gibberellic acid (GA_3) for stem elongation in Sauvignon blanc. S. Afr. J. Enol. Vitic. 33, 198-202.

Molitor, D., Behr, M., Hoffmann, L. & Evers, D., 2012b. Impact of grape cluster division on cluster morphology and bunch rot epidemic. Am. J. Enol. Vitic. 63, 508-514.

Nakamura, M. & Hori, Y., 1984. Postharvest berry drop of seedless berries produced by GA treatment in grape cultivar Kyoho. Tohoku J. Agric. Res. 34.

Nakamura, M., Takahashi, E. & Noda, T., 1974. Rachis lignification and seedless berry production in seeded grape cultivar “Kyoho” as influenced by gibberellin and quercetin. Technical Bulletin of the Faculty of Horticulture, Chiba University 22, 7-12.

Pérez, F.J. & Gómez, M., 2000. Possible role of soluble invertase in the gibberellic acid berry-sizing effect in Sultana grape. Plant Growth Regul. 30, 111-116.

Raban, E., Kaplunov, T., Zutahy, Y., Daus, A., Alchanatis, V., Ostrovsky, V., Lurie, S. & Lichter, A., 2013. Rachis browning in four table grape cultivars as affected by growth regulators or packaging. Postharvest Biol. Technol. Elsevier B.V. 84, 88-95.

Reynolds, A., Robbins, N., Lee, H.S. & Kotsaki, E., 2016. Impacts and interactions of abscisic acid and gibberellic acid on Sovereign Coronation and Skookum seedless table grapes. Am. J. Enol. Vitic. 67, 327-338.

Robinson, S.P. & Davies, C., 2000. Molecular biology of grape berry ripening. Aust. J. Grape Wine Res. 6, 175-188.

Roper, T.R. & Williams, L.E., 1989. Net CO_2 assimilation and carbohydrate partitioning of grapevine leaves in response to trunk girdling and gibberellic acid application. Plant Physiol. 89, 1136-1140.

Tello, J. & Ibáñez, J., 2018. What do we know about grapevine bunch compactness? A state-of-the-art review. Aust. J. Grape Wine Res. 24, 6-23.

Van der Vyver, L., 2016. The influence of gibberellic acid (GA_3) for berry thinning and berry sizing on table grape production, quality and fertility of Prime. Thesis, Stellenbosch University, Stellenbosch University, Private Bag X1, 7602 Matieland (Stellenbosch), South Africa.

Weaver, RJ., 1958. Effect of gibberellic acid on fruit set and berry enlargement in seedless grapes of *Vitis vinifera*. Nature 181, 851-852.

Weaver, R.J. & McCune, S.B., 1962. Studies on prebloom sprays of gibberellin to elongate and loosen clusters of Thompson Seedless grapes. Am. J. Enol. Vitic. 13, 15-19.

Weaver, R.J. & Pool, R.M., 1971. Chemical thinning of grape clusters (*Vitis vinifera* L.). Vitis 10, 201-209.

Zoffoli, J.P., Latorre, B.A. & Naranjo, P., 2009. Preharvest applications of growth regulators and their effect on postharvest quality of table grapes during cold storage. Postharvest Biol. Technol. 51, 183-192.

Chapter 6

Research results

The effect of GA_3 and GA_{4+7} thinning treatments on rudimentary seed size of *Vitis vinifera* L. 'Sunred Seedless'

CHAPTER 6:

The effect of GA_3 and GA_{4+7} thinning treatments on rudimentary seed size of *Vitis vinifera* L. 'Sunred Seedless'

ABSTRACT

Experiments were performed over two growing seasons on Sunred Seedless vines, grafted onto Ramsey, located on the ARC Infruitec-Nietvoorbij experimental farm in De Doorns. A standard GA_3 rate and various rates of GA_{4+7} were all applied at four different phenological stages to determine the optimal rate and timing to decrease rudimentary seed size compared to an untreated control. Rudimentary seed size distribution was determined by collecting the rudimentary seeds from grape berries and classing them into three classes according to their diameter (small <1 mm; medium =1-2 mm; large >2 mm). The number of rudimentary seeds per berry was counted. After that, they were weighed to obtain their fresh mass to calculate the average rudimentary seed mass per berry and average rudimentary seed mass per seed. Seasonal differences were observed with reduced average seed masses recorded for the 2016/2017 season compared to the 2015/2016 season. Although not consistent over the two seasons, a trend was observed that applying a thinning treatment during flowering instead of during the early stages of berry development resulted in a decreased mean average rudimentary seed mass per berry, as well as an improved rudimentary seed size distribution with an increased percentage of small rudimentary seeds. Results indicated that there were no consistent trend regarding the effect of different GA_3 or GA_{4+7} application timing and rates on rudimentary seed size.

6.1 INTRODUCTION

The table grape export market defines grape quality by the following attributes: seedlessness, firmness, even bunch and berry size, consistent bunch shape and skin colour. Table grape production in South Africa has to adapt to meet these market requirements in order to produce grapes that can be exported.

During the evaluation of cultivars and/ or selections, grapes are regarded as seedless when rudimentary seeds are soft, green and not perceptible organoleptically (Burger *et al.*, 2003). The international consumer market defines seedless grapes with detectable rudimentary seeds as a negative characteristic, decreasing the marketability of such grapes. Sunred Seedless is an example of a cultivar that tends to develop larger rudimentary seeds, increasing the noticeability thereof when

consumed. With increased consumer demands for seedless grapes, viticultural manipulations that reduce rudimentary seed size could have a valuable contribution from a marketing perspective.

Rudimentary seeds or seed traces are the result of stenospermocarpy, a fruit set mechanism characterized by the abortion of the embryo two to four weeks after fertilization and termination of further seed development (Stout, 1936; Coombe, 1960; Nitsch *et al.*, 1960; Winkler *et al.*, 1962; Mullins *et al.*, 1992; Dokoozlian, 2000; Perl *et al.*, 2000; Roller, 2003; Iland *et al.*, 2011). The inherent rudimentary seed size of a cultivar is linked to the timing of embryo abortion, which can be delayed in cultivars with larger rudimentary seeds (Dokoozlian, 2000). Korkutal (2005) reported that embryo abortion occurs between five and ten days after full flowering.

Increased endogenous GA levels of seeded cultivars contribute to their naturally large berry size (Lavee, 1960; Kato *et al*., 1998; Agüero *et al*., 2000; Perez *et al*., 2000). Seed number per berry also contributes to berry size, and a higher number of seeds per berry generally promotes the development of larger berries (Keller, 2015). This leads to a reduced rate in berry development and an initial delay in ripening. The lack of seeds in seedless table grape cultivars is a limiting factor of berry growth (Keller, 2015), posing a challenge in meeting the requirements of consumers for large grapes. An external gibberellic acid (GA) application is therefore used to stimulate the sink strength of seedless berries, increasing berry size (Keller, 2015). GA_3 applications during flowering have been reported to decrease rudimentary seed size in specific table grape cultivars and inducing parthenocarpy, due to ovule sensitivity towards increased phytohormone levels or a reduction in pollen germination and pollen tube growth (Motomura & Ito, 1972; Fukunaga & Kurooka, 1987; Kimura *et al.*, 1996; Okamoto & Miura, 2005). Other factors influencing rudimentary seed size include climatic conditions, the age of the grapevine and the choice of rootstock, as different rootstocks contribute to different levels or hormone production (Christensen *et al.*, 1983; Dokoozlian, 2000).

The outcome of a GA_3 application to promote seedlessness is time-sensitive, temperature and cultivar dependent and an unsuccessful application could result in an increased shot berries, thickening of the peduncles and pedicels and over thinning of berries (Motomura & Ito, 1972). There are also reports of GA_3 spray applications having a negative effect on the return fertility of grapevines (Jawanda *et al.*, 1974; Palma & Jackson, 1989; Orth, 1990; Christensen, 2000; Dokoozlian & Peacock, 2001; Molitor *et al.*, 2012).

Although the use of GA_3, applied during or before flowering, to promote seedlessness in table grapes has been reported by numerous authors (Coombe, 1960; Sugiura & Inaba, 1966; Iwahori *et al.*, 1968; Agüero *et al*., 2000; Cheng *et al.*, 2013), limited published research articles are available containing detailed rudimentary seed measurements linked to application timing and rate for GA_3 promoting seedlessness. Also, there are hardly any articles on the use of GA_{4+7} as an alternative to GA_3. GA_{4+7}

is registered in South Africa as a calyx end russet control agent in apples, but is not registered for use on table grapes. The study aimed to determine whether GA_3 or GA_{4+7}, applied at variable rates and phenological stages, could reduce the occurrence and size of rudimentary seeds in Sunred Seedless in the Hex River Valley.

6.2 MATERIALS AND METHODS

Refer to Sections 3.3 and 3.4 in Chapter 3, for details on the experimental layout and treatments applied.

6.2.1 Rudimentary seeds

A representative sample of 40 berries per treatment replicate, consisting of 10 berries per bunch phenological stage, were selected at harvest. The average berry mass of each 10 berry sample per subplot treatment was determined. For each berry the rudimentary seeds were removed and divided into classes according to the seed diameter (small <1 mm; medium =1-2 mm; large >2 mm). The number of rudimentary seeds per berry was counted and the distribution of rudimentary seed size per berry also noted. The fresh mass of the rudimentary seeds of each 10-berry sample was weighed to calculate the average rudimentary seed mass per berry and average rudimentary seed mass per seed.

6.3 RESULTS AND DISCUSSION

6.3.1 2015/2016 season

6.3.1.1 Number of rudimentary seeds per berry

There were no significant differences between treatments when they were applied earlier in the season at the phenological stage of 80-100%F and 10%BS (Table 6.1). The late 5 ppm GA_3 (BS) treatment, applied at BS, resulted in a significantly higher number of seeds per berry, with 2.63 seeds per berry, compared to other treatments ranging between 2.05 and 2.15 (Table 6.1). The late 7.5 ppm GA_{4+7} (BS) treatment had a significantly lower number of seeds per berry compared to the 15 ppm GA_{4+7} (BS) treatment, applied at BS+4D. A grape berry can have up to four seeds, but most have less than four (Dokoozlian, 2000). Although limited literature regarding the number of rudimentary seeds per berry and their mass is available, a study performed by Christensen *et al.* (1983) found that there was an average of 2.27 rudimentary seeds per berry for Thompson Seedless.

6.3.1.2 Total rudimentary seed mass per berry

The untreated control had a significantly higher total seed mass per berry compared to all other treatments, indicating a possible treatment effect for treatments receiving a GA thinning application at BS (Table 6.1). The late 7.5 ppm GA_{4+7} (BS) treatment had a significantly lower total seed mass per berry compared to other treatments receiving a GA thinning application at BS+4D, which can be explained by the significantly lower number of seeds recorded. Treatments applied at BS+4D resulted in a significantly higher mean total seed mass compared to the treatments applied at the earlier bunch phenological stages. These results indicate a possible response to GA applied from 80-100%F to BS, with a reduction in the mean total seed mass per berry. These results are similar to findings of other authors, where a GA_3 application during flowering (Avenant, 2000) as well as post-flowering (Halbrooks & Mortensen, 1988; Reynolds & de Savigny, 2004) resulted in a reduced rudimentary seed mass.

6.3.1.3 Average rudimentary seed mass

The average seed mass found for the 2015/2016 season (Table 6.1), is comparable to the average rudimentary seed size of Sunred Seedless. The average rudimentary seed mass of Sunred Seedless is 9.4 mg, but it can be as large as 22.1 mg, which is large compared to that of Sultanina which can vary between 5.3 mg and 6.7 mg per rudimentary seed (Avenant, 2000).

The untreated control resulted in a significantly higher average seed mass compared to the earlier 5 ppm GA_3 (10%BS) treatment, applied at 80-100%F and BS (Table 6.1). Likewise, there was also found to be a decrease in rudimentary seed size when grapevines were treated with GA_3 during flowering or during the early stages of seed development (Coombe, 1960; Sugiura & Inaba, 1966; Iwahori *et al.*, 1968; Agüero *et al*., 2000; Cheng *et al.*, 2013). The untreated control had a significantly higher average seed mass compared to the late 5 ppm GA_3 (BS) and 30 ppm GA_{4+7} (BS) treatments, applied at BS (Table 6.1). The 7.5 ppm GA_{4+7} (BS) treatment, applied at BS+4D, had a significantly lower average seed mass, correlating with the significantly lower total seed mass and number of seeds per berry found for this particular treatment. Treatments applied at BS+4D, resulted in a significantly higher mean total seed mass per berry, as well as mean average seed mass, compared to the earlier phenological stages. This indicates the possibility that a thinning application applied at BS+4D could be too late to have an impact on rudimentary seed size and mass.

The 2015/2016 season produced results comparable to other studies reporting a decrease in rudimentary seed size when treated with GA_3 during flowering or early berry development (Coombe, 1960; Sugiura & Inaba, 1966; Iwahori *et al*., 1968; Halbrooks & Mortensen, 1988; Agüero *et al*., 2000; Avenant, 2000; Reynolds & de Savigny, 2004; Cheng *et al*., 2013).

Table 6.1: Number of seeds per berry, total seed mass per berry (mg) and average seed mass (mg) of treatment samples from marked Sunred Seedless bunches (2015/2016 season).

Treatment		Number of seeds per berry				Total seed mass per berry (mg)				Average seed mass (mg)			
		Bunch phenological stage at application											
		80-100% Flowering	10% Berry Set	Berry Set	Berry Set + 4 Days	80-100% Flowering	10% Berry Set	Berry Set	Berry Set + 4 Days	80-100% Flowering	10% Berry Set	Berry Set	Berry Set + 4 Days
T1	Untreated control	2.00 a	1.93 a	2.48 ab	.	20.02 a	16.55 a	**29.96 a**	.	**12.07 a**	8.30 a	**12.04 a**	.
T2	5 ppm GA3 (10%BS)	2.18 a	2.28 a	2.23 ab	.	13.11 a	19.22 a	15.53 b	.	**6.01 b**	8.26 a	**7.06 b**	.
T3	7.5 ppm GA4+7 (10%BS)	2.55 a	2.38 a	2.20 ab	.	19.44 a	19.16 a	18.38 b	.	9.16 ab	8.00 a	8.88 ab	.
T4	15 ppm GA4+7 (10%BS)	2.43 a	2.25 a	2.13 b	.	22.51 a	23.76 a	20.05 b	.	7.73 ab	11.82 a	9.38 ab	.
T5	30 ppm GA4+7 (10%BS)	2.20 a	2.30 a	2.13 b	.	18.66 a	23.03 a	17.65 b	.	9.84 ab	9.79 a	7.76 ab	.
T6	5 ppm GA3 (BS)	.	2.50 a	**2.63 a**	2.13 ab	.	22.71 a	17.55 b	27.12 a	.	9.34 a	6.73 b	12.50 a
T7	7.5 ppm GA4+7 (BS)	.	2.38 a	2.05 b	**2.03 b**	.	24.86 a	17.82 b	**18.39 b**	.	10.43 a	8.29 ab	**8.76 b**
T8	15 ppm GA4+7 (BS)	.	1.98 a	2.15 b	2.37 a	.	15.73 a	17.47 b	28.96 a	.	7.65 a	7.79 ab	12.07 a
T9	30 ppm GA4+7 (BS)	.	2.18 a	2.30 ab	2.20 ab	.	14.72 a	16.68 b	24.99 a	.	7.11 a	7.14 b	11.56 ab
Mean		2.27 x	2.24 x	2.26 x	2.18 x	18.75 y	19.97 y	19.01 y	**24.87 x**	8.96 y	8.97 y	8.34 y	**11.22 x**
LSD p=0.05		0.61	0.68	0.46	0.27	13.96	10.93	8.71	5.02	5.67	5.42	4.30	3.14

Values designated by the same letters within a column or row do not differ significantly ($p \leq 0.05$).

6.3.1.4 Rudimentary seed size distribution

No significant differences in the percentage small, medium or large rudimentary seeds were observed for treatments applied at the bunch phenological stage of 80-100%F (Table 6.2). The desired rudimentary seed size distribution would be to have the highest possible percentage of small seeds, along with the lowest possible percentage of large seeds, reducing the noticeability of the rudimentary seeds during consumption.

For treatments applied at the phenological stage of 10%BS, the untreated control and the late 15 ppm GA_{4+7} (BS) treatment resulted in a significantly higher percentage of medium-sized rudimentary seeds compared to the late application of 7.5 ppm GA_{4+7} (BS) (Table 6.2). The high percentage of medium rudimentary seeds associated with the late application of 15 ppm GA_{4+7} (BS) can be explained by the reduction in the percentage of large rudimentary seeds recorded at 10% BS.

Berries from the untreated control had a significantly higher percentage of large seeds compared to all GA_{4+7} treatments, excluding the late 30 ppm GA_{4+7} (BS) treatment applied at BS (Table 6.2). The two GA_3 treatments did not differ significantly from the untreated control. The higher percentage of large seeds for the untreated control correlates with the higher average seed mass (Table 6.1). During the 2015/2016 season, the majority of the GA_{4+7} treatments applied at the phenological stage of BS, reduced the occurrence of large rudimentary seeds compared to the untreated control.

The higher rate application of 30 ppm GA_{4+7} (BS) was associated with a significantly higher percentage of large seeds compared to the lower rate application of 7.5 ppm GA_{4+7} (BS), for treatments applied at BS+4D, indicating a possible dose rate response for GA_{4+7} (Table 6.2).

Treatments applied at the earliest phenological stage, 80-100%F, resulted in a significantly higher mean percentage of small seeds compared to the BS+4D stage as well as a significantly lower percentage medium seeds compared to the BS stage (Table 6.2). Although the 80-100%F stage resulted in no significant differences between treatments, the mean value of the treatments delivered desirable results, suggesting that a thinning application during flowering could result in reduced rudimentary seed size. Similar results of reduced rudimentary seeds for Sunred Seedless have been reported with the use of GA_3 during flowering (Avenant, 2000).

Table 6.2: Rudimentary seed size distribution of treatment samples from marked Sunred Seedless bunches (2015/2016 season).

Treatment		Size distribution of rudimentary seeds (%)											
		% Small				% Medium				% Large			
		Bunch phenological stage at application											
		80-100% Flowering	10% Berry Set	Berry Set	Berry Set + 4 Days	80-100% Flowering	10% Berry Set	Berry Set	Berry Set + 4 Days	80-100% Flowering	10% Berry Set	Berry Set	Berry Set + 4 Days
T1	Untreated control	30.59 a	30.42 a	25.31 ab	.	25.76 a	**39.17 a**	19.39 b	.	43.65 a	30.42 ab	**55.30 a**	.
T2	5 ppm GA3 (10%BS)	48.03 a	43.11 a	35.16 ab	.	22.04 a	25.18 ab	30.63 b	.	29.94 a	31.71 ab	34.21 ab	.
T3	7.5 ppm GA4+7 (10%BS)	51.93 a	39.45 a	38.90 a	.	16.24 a	21.99 ab	29.66 b	.	31.83 a	38.56 ab	31.45 b	.
T4	15 ppm GA4+7 (10%BS)	41.78 a	34.75 a	**15.18 b**	.	22.17 a	21.21 ab	**52.02 a**	.	36.06 a	**44.04 a**	32.80 b	.
T5	30 ppm GA4+7 (10%BS)	39.69 a	40.23 a	34.31 ab	.	14.15 a	20.96 ab	36.57 ab	.	46.16 a	38.81 ab	29.12 b	.
T6	5 ppm GA3 (BS)	.	31.70 a	38.85 a	26.87 a	.	24.68 ab	22.13 b	27.40 a	.	**43.62 a**	39.02 ab	45.73 ab
T7	7.5 ppm GA4+7 (BS)	.	42.12 a	40.91 a	49.19 a	.	17.61 b	31.33 b	25.96 a	.	40.27 ab	27.76 b	**24.85 b**
T8	15 ppm GA4+7 (BS)	.	43.68 a	44.64 a	26.87 a	.	**37.47 a**	29.78 b	26.99 a	.	18.85 b	25.58 b	46.15 ab
T9	30 ppm GA4+7 (BS)	.	38.31 a	44.09 a	22.36 a	.	20.95 ab	19.72 b	17.81 a	.	40.74 ab	36.19 ab	**59.83 a**
Mean		**42.40 x**	38.20 xy	35.26 xy	**31.32 y**	**20.07 y**	25.47 xy	**30.14 x**	24.54 xy	37.53 x	36.34 x	34.60 x	44.14 x
LSD p=0.05		23.81	26.43	20.83	45.06	18.86	19.58	19.33	34.15	23.99	23.67	21.66	31.04

Values designated by the same letters within a column or row do not differ significantly ($p \leq 0.05$).

6.3.2 2016/2017 season

6.3.2.1 Number of rudimentary seeds per berry

The 60 ppm GA_{4+7} (T+S) treatment had a significantly higher number of rudimentary seeds per berry, with 2.35 seeds per berry compared to the 7.5 ppm GA_{4+7} (T) treatment with 1.10 seeds per berry, at 50%F (Table 6.3). For treatments applied at 10%BS, the 30 ppm GA_{4+7} (T) had a significantly higher number of rudimentary seeds per berry compared to the 5 ppm GA_3 (T), 15 ppm GA_{4+7} (T) and 60 ppm GA_{4+7} (S) treatments.

6.3.2.2 Total rudimentary seed mass per berry

A comparable mean number of seeds per berry with noticeably lower seed masses was recorded in the 2016/2017 season (Table 6.3) compared to the 2015/2016 season (Table 6.1). Reynolds and de Savigny (2004) and Reynolds *et al.* (2006) also reported vast seasonal differences concerning rudimentary seed number and especially with regard to their seed mass.

At the earliest phenological stage of application, 50%F, the 7.5 ppm GA_{4+7} (T) treatment resulted in significantly higher total seed mass per berry compared to the untreated control and 60 ppm GA_{4+7} (T+S) treatment (Table 6.3). The 7.5 ppm GA_{4+7} (T) treatment had the highest total seed mass per berry, although it had the lowest number of seeds per berry, meaning fewer, but larger seeds. The 5 ppm GA_3 (T) treatment had a significantly higher total seed mass per berry compared to all other treatments, including the untreated control, applied at 80-100%F. At this stage, there is no plausible reason for the unexpected results. No significant differences in the rudimentary seed mass were found for treatments applied at the bunch phenological stage of BS. Comparing the mean total seed mass per berry for each bunch phenological stage, 50%F was associated with a significantly lower total seed mass compared to the 10%BS stage.

6.3.2.3 Average rudimentary seed mass

The untreated control of GA treatments applied at 50%F had a significantly lower average seed mass compared to the 7.5 ppm GA_{4+7} (T), 30 ppm GA_{4+7} (T) and 60 ppm GA_{4+7} (S) treatments (Table 6.3). This correlated with the significantly lower total seed mass per berry of the untreated control. Also for application at 50%F, the untreated control, along with the 60 ppm GA_{4+7} (T) and 60 ppm GA_{4+7} (T+S) treatments, had significantly lower average seed mass compared to the 30 ppm GA_{4+7} (T) treatment (Table 6.3).

Similar to the significantly higher total seed mass, the average seed mass of the 5 ppm GA_3 (T) treatment applied at 80-100%F, was significantly higher than 7.5 ppm GA_{4+7} (T), 120 ppm GA_{4+7} (T),

60 ppm GA_{4+7} (T+S) and 60 ppm GA_{4+7} (S) treatments (Table 6.3). The average seed mass was significantly higher compared to the untreated control, 7.5 ppm GA_{4+7} (T), 120 ppm GA_{4+7} (T), 60 ppm GA_{4+7} (T+S) and 60 ppm GA_{4+7} (S) treatments. At 10%BS, the 60 ppm GA_{4+7} (S) treatment had a significantly higher average seed mass compared to the 7.5 ppm GA_{4+7} (T), 60 ppm GA_{4+7} (T) and 120 ppm GA_{4+7} (T) treatments.

Noticeably higher average seed masses were observed for the 2015/2016 season (Table 6.1) compared to the 2016/2017 season (Table 6.3). Temperatures below average during flowering and early fruit development, could delay embryo abortion and result in an increased noticeability of rudimentary seeds (Dokoozlian, 2000). Flowering generally occurs when the average daily temperature is higher than 18°C (Keller, 2015). Optimal temperatures for pollen tube growth range from 25°C to 30°C, with an inhibiting effect observed for temperatures below 10°C and above 35°C (Keller, 2015). In the current study, for the period of 1 October to 30 November (pre-flowering to early fruit development), the average temperature for the 2015/2016 season was 1.7°C lower at 16.76°C compared to the 2016/2017 season at 18.46°C (Table 6.4). The lower average temperature during flowering and early fruit development could have contributed to the higher rudimentary seed mass in the 2015/2016 season.

No consistent trend regarding the effect of different GA_3 or GA_{4+7} application timing and rates on rudimentary seed size could have been concluded over two seasons. Similar results regarding rudimentary seed mass have been found by Christensen *et al.* (1983), where dry seed mass varied substantially between different seasons. Reynolds & de Savigny (2004) and Reynolds *et al.* (2006) also recorded vast seasonal differences concerning rudimentary seed mass.

Table 6.3: Number of seeds per berry, total seed mass per berry (mg) and average seed mass (mg) of treatment samples from marked Sunred Seedless bunches (2016/2017 season).

Treatment		Number of seeds per berry				Total seed mass per berry (mg)				Average seed mass (mg)			
		Bunch phenological stage at application											
		50% Flowering	80-100% Flowering	10% Berry Set	Berry Set	50% Flowering	80-100% Flowering	10% Berry Set	Berry Set	50% Flowering	80-100% Flowering	10% Berry Set	Berry Set
T1	Untreated control	2.25 ab	2.33 a	2.15 ab	2.03 a	3.84 b	6.16 b	7.58 a	6.83 a	**1.77 c**	2.61 b	3.50 ab	3.69 a
T2	5 ppm GA_3 (T)	2.30 ab	2.40 a	1.80 b	1.90 a	6.47 ab	**12.55 a**	6.08 a	4.50 a	2.85 abc	**5.44 a**	3.37 ab	2.83 a
T3	7.5 ppm GA_{4+7} (T)	1.10 b	2.05 a	2.18 ab	2.15 a	**9.03 a**	4.49 b	6.83 a	6.11 a	4.05 ab	2.24 b	3.10 b	2.79 a
T4	15 ppm GA_{4+7} (T)	2.05 ab	2.13 a	1.70 b	1.60 a	5.56 ab	6.99 b	5.04 a	7.16 a	2.91 abc	3.60 ab	3.48 ab	4.27 a
T5	30 ppm GA_{4+7} (T)	2.10 ab	1.60 a	**2.60 a**	2.00 a	7.59 ab	5.92 b	6.85 a	7.27 a	**4.34 a**	3.08 ab	2.68 b	3.70 a
T6	60 ppm GA_{4+7} (T)	1.98 ab	1.68 a	2.35 ab	1.73 a	5.23 ab	7.17 b	6.60 a	6.08 a	1.97 bc	4.24 ab	2.98 b	3.53 a
T7	120 ppm GA_{4+7} (T)	2.23 ab	2.45 a	2.20 ab	2.15 a	6.01 ab	6.20 b	9.98 a	6.92 a	2.73 abc	2.51 b	4.48 ab	3.24 a
T8	60 ppm GA_{4+7} (T+S)	**2.35 a**	2.03 a	2.40 ab	2.25 a	4.08 b	4.78 b	7.63 a	5.43 a	1.85 bc	2.41 b	3.25 ab	2.34 a
T9	60 ppm GA_{4+7} (S)	1.28 ab	2.25 a	1.68 b	1.83 a	5.39 ab	4.65 b	8.93 a	5.82 a	3.22 ab	2.29 b	**5.18 a**	3.39 a
Mean		1.96 x	2.10 x	2.12 x	1.96 x	**5.91 y**	6.55 xy	**7.28 x**	6.23 xy	**2.85 y**	3.16 xy	**3.56 x**	3.31 xy
LSD p=0.05		1.24	0.96	0.75	0.83	3.84	4.77	5.03	3.79	2.25	2.52	2.04	2.06

Values designated by the same letters within a column or row do not differ significantly ($p \leq 0.05$).

Table 6.4: Average minimum and maximum temperatures for De Doorns, Hex River Valley, for the period of 1 October to 30 November 2015/2016 and 2016/2017 seasons (Source: Ileaf: www.ileaf.co.za).

Period	Average minimum temperature (°C)	Average maximum temperature (°C)	Average temperature (°C)
1 Oct – 30 Nov 2015	9.18	25.06	16.76
1 Oct – 30 Nov 2016	8.90	27.31	18.46

6.3.2.4 Rudimentary seed size distribution

At 10%BS, the untreated control had a significantly higher percentage of large seeds, along with a significantly lower percentage of medium seeds compared to the 15 ppm GA_{4+7} (T) treatment (Table 6.5). The 5 ppm GA_3 (T) treatment, applied at BS, had a significantly higher percentage medium berries compared to the 7.5 ppm GA_{4+7} (T), 15 ppm GA_{4+7} (T), 60 ppm GA_{4+7} (T), 120 ppm GA_{4+7} (T) and 60 ppm GA_{4+7} (T+S) treatments (Table 6.5). No significant differences were reported between the untreated control and treatments in the percentage of small seeds. No consistent trend regarding a specific GA_3 or GA_{4+7} application rate and timing combination was reported for the 2016/2017 season, with regards to the ideal rudimentary seed size distribution.

Similar to the 2015/2016 season, application at the earlier phenological stages, 50%F and 80-100%F, resulted in a significantly higher mean percentage of small berries compared to the later stage of BS (Tables 6.2 & 6.5). Application of GA treatments at the latest bunch phenological stage, BS, also had a significantly higher percentage of medium rudimentary seeds compared to application at earlier stages of development.

Table 6.5: Rudimentary seed size distribution of treatment samples from marked Sunred Seedless bunches (2016/2017 season).

Treatment		Size distribution of rudimentary seeds (%)											
		% Small				% Medium				% Large			
		Bunch phenological stage at application											
		50% Flowering	80-100% Flowering	10% Berry Set	Berry Set	50% Flowering	80-100% Flowering	10% Berry Set	Berry Set	50% Flowering	80-100% Flowering	10% Berry Set	Berry Set
T1	Untreated control	55.14 abc	44.42 ab	29.90 a	36.02 a	27.93 a	25.25 ab	**18.12 c**	36.03 ab	16.93 b	30.33 a	**51.98 a**	27.92 a
T2	5 ppm GA_3 (T)	46.39 abc	33.05 b	33.80 a	22.97 a	27.63 a	25.69 ab	26.20 bc	**57.81 a**	25.98 ab	41.26 a	40.00 ab	19.22 a
T3	7.5 ppm GA_{4+7} (T)	45.83 abc	55.43 ab	38.60 a	28.57 a	27.08 a	21.14 ab	36.45 ab	32.23 b	27.08 ab	23.43 a	24.95 ab	39.20 a
T4	15 ppm GA_{4+7} (T)	**28.92 c**	55.53 ab	42.41 a	37.75 a	24.73 a	22.32 ab	**45.16 a**	33.13 b	**46.34 a**	22.15 a	**12.43 b**	29.12 a
T5	30 ppm GA_{4+7} (T)	34.17 bc	**68.68 a**	41.77 a	37.20 a	18.33 a	10.98 b	19.13 bc	40.22 ab	**47.50 a**	20.37 a	39.10 ab	22.58 a
T6	60 ppm GA_{4+7} (T)	64.76 ab	48.95 ab	49.35 a	29.29 a	19.37 a	**38.55 a**	24.99 bc	24.48 b	15.87 b	12.50 a	25.66 ab	42.24 a
T7	120 ppm GA_{4+7} (T)	62.45 ab	55.93 ab	42.90 a	40.83 a	10.67 a	13.51 b	26.15 bc	33.33 b	26.87 ab	30.56 a	30.95 ab	25.83 a
T8	60 ppm GA_{4+7} (T+S)	**65.05 a**	**66.46 a**	56.34 a	47.90 a	15.61 a	13.63 b	24.75 bc	27.83 b	19.34 b	19.91 a	**18.91 b**	24.27 a
T9	60 ppm GA_{4+7} (S)	**26.86 c**	42.98 ab	31.12 a	31.54 a	26.76 a	26.17 ab	31.88 abc	35.69 ab	**46.38 a**	30.86 a	37.00 ab	32.77 a
Mean		**47.73 xy**	**52.38 x**	**40.69 yz**	**34.67 z**	22.01 y	21.92 y	28.09 y	**35.64 x**	30.25 x	25.71 x	31.22 x	29.24 x
LSD p=0.05		30.68	30.15	32.23	25.78	22.54	22.59	18.09	22.56	23.86	29.35	31.47	30.98

Values designated by the same letters within a column or row do not differ significantly ($p \leq 0.05$).

6.4 CONCLUSION

The 5 ppm GA_3 (10%BS) treatment, applied at 80-100%F and BS, had a significantly lower average seed mass compared to the untreated control. At the bunch phenological stage of BS, for the 2015/2016 season, a treatment effect was visible through a reduction in the total seed mass per berry of all treatments, compared to the untreated control. Unfortunately, the same results as mentioned above, could not be reproduced during the 2016/2017 season.

Comparing the timing of treatments per phenological stage for the 2015/2016 season, the 80-100%F stage had a significantly lower mean average seed mass per berry, along with significantly higher percentage small seeds, compared to the BS+4D phenological stage. For the 2016/2017, the 50%F stage had a significantly lower mean average seed mass per berry, along with significantly lower percentage medium seeds, compared to the 10%BS phenological stage. Although not consistent over the two seasons, a trend was observed that applying a thinning treatment during flowering instead of during the early stages of berry development, resulted in a decreased average seed mass per berry, as well as an improved rudimentary seed size distribution with an increased percentage small rudimentary seeds.

The application of GA at different phenological stages had no significant effect on the number of rudimentary seeds in Sunred Seedless grapes. No consistent trends could be observed with regard to the effect of different rates of application on the number of rudimentary seeds. Results showed that there are seasonal differences in average mass of rudimentary seeds. This is most likely due to differences in prevailing temperatures during pre-flowering and early fruit development. The application of a thinning treatment during flowering instead of during the early stages of berry development as with average seed mass per berry, reduced the mean average seed mass per berry as well. This indicated a possible response of Sunred Seedless to GA applications during flowering or during the early stages of seed development regardless of the concentration. There was also an improved rudimentary seed size distribution with an increased percentage of small rudimentary seeds with earlier applications of GA.

The 5 ppm GA_3 treatment applied at 80-100%F were the most promising, as it resulted in smaller rudimentary seeds. The 5 ppm GA_3 treatment applied at 80-100%F should be included for further evaluation in future experiments, to increase seedlessness of table grapes.

6.5 LITERATURE CITED

Agüero, C., Vigliocco, A., Abdala, G. & Tizio, R., 2000. Effect of gibberellic acid and uniconazole on embryo abortion in the stenospermocarpic grape cultivars Emperatriz and Perlon. Plant Growth Regul. 30, 9-16.

Avenant, J.H., 2000. Effect of different cultivation practices on seed trace size of Sunred Seedless-preliminary results. Dec. Fruit Grower 50, 16.

Burger, P., Gerber, C., Gerber, A. & Ellis, P.J.L., 2003. Breeding seedless grapes in South Africa by means of embryo rescue. Acta Hortic. 603, 565-569.

Cheng, C., Xu, X., Singer, S.D., Li, J., Zhang, H., Gao, M., Wang, L., Song, J. & Wang, X., 2013. Effect of GA_3 treatment on seed development and seed-related gene expression in grape. PLoS One 8, 1-2.

Christensen, L.P., 2000. Growth Regulators. Raisin Production Manual. pp. 139–141.

Christensen, P., Ramming, D. & Andris, H., 1983. Seed trace content of Fiesta grapes. Am. J. Enol. Vitic. 34, 257-259.

Coombe, B.G., 1960. Relationship of growth and development to changes in sugars, auxins, and gibberellins in fruit of seeded and seedless varieties of *Vitis Vinifera*. Plant Physiol. 35, 241-250.

Dokoozlian, N.K., 2000. Grape berry growth and development. In: Christensen, L.P. (ed). Raisin production manual, vol. 3393. UCANR Publications, California. pp. 30-37.

Dokoozlian, N.K. & Peacock, W.L., 2001. Gibberellic acid applied at bloom reduces fruit set and improves size of “Crimson Seedless” table grapes. Hort. Sci. 36, 706-709.

Fukunaga, S. & Kurooka, H., 1987. Studies of seedlessness of 'Kyoho' grapes induced by gibberellin in combination with Streptomycin. Bul. Univ. Osaka Prefec. 40, 1-10.

Halbrooks, M.C. & Mortensen, J.A., 1988. Influence of gibberellic acid and various management practices on berry, seed and cluster development in 'Orlando Seedless' grape. Proc. Flo. State Hort. Soc. 100, 312-315.

Iland, P., Dry, P., Proffitt, T. & Tyerman, S.D., 2011. The Grapevine: from the Science to the Practice of Growing Vines for Wine. Patrick Iland Wine Promotions Pty Ltd, Adelaide, South Australia.

Iwahori, S., Weaver, R.J. & Pool, R.M., 1968. Gibberellin-like activity in berries of seeded and seedless Tokay grapes. Plant Physiol. 43, 333-337.

Jawanda, J.S., Singh, R. & Pal, R.N., 1974. Effect of growth regulators on floral bud drop, fruit characters and quality of Thompson Seedless grape (*Vitis vinifera* L.). Vitis 13, 215-221.

Kato, K., Ohara, H., Takahashi, E., Matsui, H. & Nakayama, M., 1998. Endogenous gibberellin-induced parthenocarpy in grape berries. Acta Hortic. 514, 69-74.

Keller, M., 2015 (2nd ed). The science of grapevines: Anatomy and physiology. Academic Press, London.

Kimura, P.H., Okamoto, G., & Hirano, K., 1996. Effects of gibberellic acid and streptomycin on pollen germination and ovule and seed development in Muscat Bailey A. Am. J. Enol. Vitic. 47, 152-156.

Korkutal, I., 2005. Embryo abortion in some new seedless table grape (*Vitis vinifera* L.) varieties. Int. J. Bot. 1, 168-171.

Lavee, S., 1960. Effect of gibberellic acid on seeded grapes. Nature 185, 395.

Molitor, D., Behr, M., Hoffinann, L. & Evers, D., 2012. Research note: Benefits and drawbacks of pre-bloom applications of gibberellic acid (GA_3) for stem elongation in Sauvignon blanc. S. Afr. J. Enol. Vitic. 33, 198-202.

Motomura, Y. & Ito, H., 1972. Exogenous gibberellin as responsible for the seedless development of grapes. Tohoku J. Agr. Res. 23, 15-31.

Mullins, M.G., Bouquet, A. & Williams, L.E., 1992. Biology of Horticultural Crops: Biology of the grapevine. Cambridge University Press, Cambridge.

Nitsch, J.P., Pratt, C., Nitsch, C. & Shaulis, N.J., 1960. Natural growth substances in Concord and Concord seedless grapes in relation to berry development. Am. J. Bot. 47, 566-576.

Okamoto, G., & Miura, K., 2005. Effect of pre-bloom GA application on pollen tube growth in cv. Delaware grape pistils. Vitis 44, 157-159.

Orth, C.H.F., 1990. Effect of spraying or dipping with gibberellic acid on bud fertility of Muscat Seedless. Dec. Fruit Grow. 40, 289-292.

Palma, B.A. & Jackson, D.I., 1989. Inflorescence initiation in grapes - responses to plant growth regulators. Vitis 28, 1-12.

Perez, F. J., Viani, C., & Retamales, J., 2000. Bioactive gibberellins in seeded and seedless grapes: identification and changes in content during berry development. Am. J. Enol. Vitic. 51, 315-318.

Perl, A., Sahar, N. & Spiegel-Roy, P., 2000. Conventional and biotechnological approaches in breeding seedless table grapes. Acta Hortic. 528, 607-612.

Reynolds, A.G. & de Savigny, C., 2004. Influence of girdling and gibberellic acid on yield components, fruit composition, and vestigial seed formation of ‘Sovereign Coronation' table grapes. Hort. Sci. 39, 541-544.

Reynolds A.G., Roller J.N., Forgione A. & de Savigny C., 2006. Gibberellic acid and basal leaf removal: Implications for fruit maturity, vestigial seed development, and sensory attributes of Sovereign Coronation table grapes. Am. J. Enol. Vitic. 57, 41-53.

Roller, J.N., 2003. Gibberellic acid and basal leaf removal: Implications for fruit maturity, vestigial seed development, and sensory attributes of Sovereign Coronation grapes. Thesis, Brock University, 812 Sir Isaac Brock Way, St. Catharines, Ontario, L2S 3A1.

Sugiura, A. & Inaba, A., 1966. Studies on the mechanism of gibberellin-induced seedlessness of Delaware grapes. I. J. Jpn. Soc. Hortic. Sci. 35, 233-241.

Stout, A., 1936. Seedlessness in grapes. NY Agr. Exp. Stn. Tech. Bull. 238.

Winkler, A.J., Cook, J.A., Kliewer, W.M. & Lider, L.A., 1962. General Viticulture. University of California Press, California.

Chapter 7

Research results

The effect of GA_3 and GA_{4+7} thinning treatments on the return fertility of *Vitis vinifera* L. 'Sunred Seedless'

CHAPTER 7:

The effect of GA_3 and GA_{4+7} thinning treatments on the return fertility of *Vitis vinifera* L. 'Sunred Seedless'

ABSTRACT

Experiments were performed over two growing seasons on Sunred Seedless vines, grafted onto Ramsey, located on the ARC Infruitec-Nietvoorbij experimental farm in De Doorns. A standard GA_3 rate and various rates of GA_{4+7} were applied at four different bunch phenological stages in order to determine the effect on the return fertility of Sunred Seedless. Potential fertility was determined during the winter with the use of two methods, namely bud dissection and forced budding in a glasshouse, with the actual fertility determined in the vineyard after bud break once bunches were clearly visible. All treatments, over both seasons, reached commercially acceptable bud break percentages. The mean number of bunches per sprouted bud, assessed through forced budding, decreased from June 2016 to June 2017, possibly due to the adjustment in treatments, containing increased rates of GA_{4+7}. Compared to the untreated control, the 5 ppm GA_3 (10%BS) treatment resulted in a decreased potential fertility as determined through bud dissection (June 2016), as well as the actual fertility assessments (October 2016). A poor correlation between the potential fertility determined through bud dissection and forced budding was reported, compared to the actual fertility determined in the vineyard. Potential fertility assessments should therefore not be used for crop estimations, but rather used to verify the pruning system used for a specific cultivar.

7.1 INTRODUCTION

The use of plant growth regulators (PGRs) in table grape production has become a common practice for producers in South Africa, with the use of gibberellic acid (GA_3), forchlorfenuron (CPPU), ethephon and s-abscisic acid (s-ABA). Various published research articles are available on the effect of GA_3 on bunch elongation, berry thinning and berry sizing, but minimal research information is available regarding the effect on return fertility of the grapevine.

Bunch induction and initiation of inflorescence primordia, within the buds, commences during flowering and lasts until the period between véraison and harvest. (Williams, 2000). The number of flower primordia per vine of the current season is therefore determined during the previous growing season (Ahmed Ola *et al*., 2012). During bunch induction and initiation of inflorescence primordia, various factors can influence the outcome of grapevine fertility the following year, such as

environmental factors (temperature, light & water) and physiological factors (plant hormones, carbohydrates & nutrients).

From the limited literature available, authors have reported a negative effect on return fertility following the use of GA_3, which include decreased bud fertility (Jawanda *et al*., 1974), decreased fertility with full cover applications (Orth, 1990) as well as with applications applied during flowering (Mullins *et al*., 1992; Dokoozlian, 1999; Dokoozlian & Peacock, 2001; Weyand & Schultz, 2005), during berry set (Peacock, 1998; Ahmed Ola *et al*., 2012) and with applications containing high rates of GA_3 (Peacock, 1998; Dokoozlian *et al*., 2000; Korkutal *et al*., 2008; Ahmed Ola *et al*., 2012; Elgendy *et al*., 2012). The application of 50 ppm GA_3 during the 50-80% flowering stage resulted in a decreased return fertility in Riesling (Weyand & Schultz, 2005). The effect of GA_{4+7} on the return fertility of the grapevine has not yet been reported, but the use of GA_{4+7} reduced apple flower bud formation (Davis, 2002) as well as delayed and prolonged apple flower bud initiation when the fruit was treated with both GA_3 and GA_{4+7} (Bertelsen *et al*., 2002).

Considering all the above-mentioned, the aim of the study was to evaluate the effect of GA_3 and GA_{4+7} applied at variable rates and phenological stages on the return fertility of Sunred Seedless in the Hex River valley.

7.2 MATERIALS AND METHODS

7.2.1 Potential fertility

The potential fertility of the experimental vineyard was determined using two methods, namely bud dissection and forced budding in a glasshouse during the winter of the 2015/2016 and 2016/2017 seasons. The pruning method used for the experimental vineyard contained an average of six half long bearers (nine to ten buds per cane) and twelve spurs (two to three buds per spur) per vine. The potential fertility was determined using canes with nine bud positions each.

Four canes per experimental data unit were collected, two canes were used for forced budding and two for bud dissections. A total of 16 canes per treatment were collected. Canes were only collected for treatments T1 to T5 in the 2015/2016 season, whereas canes for all treatments (T1 to T9) were collected in the 2016/2017 season.

To determine potential fertility through forced budding, the canes were cut into single-node cuttings and placed into water trays in a glasshouse at 25°C (Palma & Jackson, 1989). The bud break date of each single node cutting was recorded at the first signs of visible green plant tissue (Shulman *et*

al., 1983; Palma & Jackson, 1989; Dunn & Martin, 2000). Growth observations were recorded twice a week, until the inflorescences were clearly noticeable in order to be counted and recorded.

For the bud dissection investigation, dormant canes collected were stored at 0°C until bud dissections could be performed following the procedure described by Swanepoel and Baard (1988), working under a stereomicroscope. The potential fertility was determined by counting and recording the number of inflorescence primordia per bud. Bud mite damage was also recorded during bud dissections, with hardly any presence in the buds of the assessed dormant canes of Sunred Seedless in both seasons.

7.2.2 Actual fertility

The actual fertility was determined in the vineyard after bud break, once shoots with bunches were visible and before crop control was applied in the vineyard. Eight half-long bearers (nine bud positions) and eight spurs per experimental data unit were evaluated. Each bud position was evaluated by identifying whether bud break took place, recording the type of shoot that originated from the bud position (vegetative or reproductive shoot), as well as counting and recording the number of bunches per shoot. The actual fertility was only determined in October 2016, after the first season's treatments were applied on 31 October and 4 November 2015.

7.2.3 Bud break percentage

The bud break percentage was determined during forced budding in June 2016 and 2017, as well as during the actual fertility assessments in October 2016. The bud break percentage was calculated to determine whether it was affected by the GA_3 or GA_{4+7} treatments, as well as to determine if bud break percentages above 80% can be reached in order to comply with levels of a commercial standard (Dokoozlian & Williams, 1995; Dokoozlian, 1999).

7.3 RESULTS AND DISCUSSION

7.3.1 Potential fertility

7.3.1.1 Forced budding

No significant differences between treatments were recorded for forced budding assessments of bud break percentages and bunches per sprouted bud, performed in June 2016 or June 2017 (Tables 7.1 & 7.2). The mean number of bunches per sprouted bud, over all treatments, for forced budding, was lower in June 2017 than in June 2016. This potential fertility decreased from 1.34 bunches per sprouted bud in June 2016 to 0.79 in June 2017. Williams (2000) reported that the potential fertility

from one season to the following season could differ by as much as 25%. Alternatively, the number of bunches recorded for forced budding in June 2017 could have been impacted by conditions in the glasshouse, such as radiation and temperature fluctuations, resulting in the decreased potential fertility, compared to June 2016. A further explanation for the decrease in fertility could be due to the treatment adjustments in the 2016/2017 season where much higher dosage rates of GA_{4+7} (up to 120 ppm) were applied compared to the 2015/2016 season (up to 30 ppm). Similar to these findings, authors have reported that high rates of GA_3 decrease grapevine fertility (Peacock, 1998; Dokoozlian *et al*., 2000; Korkutal *et al*., 2008; Ahmed Ola *et al*., 2012; Elgendy *et al*., 2012).

7.3.1.2 Bud dissection

In June 2016, significant differences were found between treatments with the bud dissection assessments (Table 7.1). The untreated control had a significantly higher potential fertility compared to the 5 ppm GA_3 (10%BS), 7.5 ppm GA_{4+7} (10%BS) and the 15 ppm GA_{4+7} (10%BS) treatments. No significant differences were recorded between treatments assessed in June 2017 (Table 7.2).

The mean number of inflorescence primordia per bud determined through bud dissections was similar in June 2017 and June 2016, with an average of 0.90 and 0.85 respectively (Tables 7.1 & 7.2). These results did not follow the same pattern as the forced budding results, where the mean potential fertility over all treatments was lower in June 2017 compared to June 2016. Given the intricate nature of bud dissections, it could be possible that inflorescence primordia were miscounted or misinterpreted as leaf primordia during the bud dissection during June 2016.

Table 7.1: Potential fertility of Sunred Seedless in De Doorns, after the first season's treatments.

Treatments applied in Oct-Nov 2015		Potential fertility (Jun-16)		
		Forced budding		Bud dissection
		Bud break (%)	No. of bunches per sprouted bud	No. of inflorescence primordia per bud*
T1	Untreated Control	94.44 a	1.41 a	**1.04 a**
T2	5 ppm GA3 (10%BS)	98.61 a	1.23 a	**0.78 b**
T3	7.5 ppm GA4+7 (10%BS)	95.83 a	1.43 a	**0.78 b**
T4	15 ppm GA4+7 (10%BS)	95.83 a	1.45 a	**0.77 b**
T5	30 ppm GA4+7 (10%BS)	100.00 a	1.19 a	0.88 ab
Mean		96.94	1.34	0.85
LSD p=0.05		8.01	0.31	0.24

*Average number of node positions 1 to 9.
Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

Table 7.2: Potential fertility of Sunred Seedless in De Doorns, after the second season's treatments.

Treatments applied in Nov 2016		Potential fertility (June-17)		
		Forced budding		Bud dissection
		Bud break (%)	No. of bunches per sprouted bud	No. of inflorescence primordia per bud*
T1	Untreated Control	93.06 a	0.91 a	0.89 a
T2	5 ppm GA3 (T)	95.83 a	0.71 a	0.89 a
T3	7.5 ppm GA4+7 (T)	97.22 a	0.76 a	0.84 a
T4	15 ppm GA4+7 (T)	98.61 a	0.83 a	0.95 a
T5	30 ppm GA4+7 (T)	97.22 a	0.75 a	0.94 a
T6	60 ppm GA4+7 (T)	95.83 a	0.79 a	0.95 a
T7	120 ppm GA4+7 (T)	94.44 a	0.79 a	0.94 a
T8	60 ppm GA4+7 (T+S)	98.61 a	0.81 a	0.97 a
T9	60 ppm GA4+7 (S)	100.00 a	0.88 a	0.91 a
Mean		96.39	0.79	0.90
LSD p=0.05		7.54	0.28	0.15

*Average number of node positions 1 to 9.
Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

7.3.1.3 Seasonal comparison of similar treatments

With the adjustment of treatments from the 2015/2016 to the 2016/2017 season, the first five treatments (T1-T5) remained the same concerning the GA concentration used as thinning treatments. In order to establish whether there were differences in the potential fertility between similar treatments from one season to the other, statistically, treatments T1 to T5 were evaluated separately (Table 7.3).

There were no significant differences in terms of the % bud break, determined through forced budding between season or between treatments (Tables 7.3). For all treatments, including the untreated control, a decrease in potential fertility, determined through forced budding, was reported from June 2016 to June 2017 (Table 7.3). However, within a season, no significant differences in the number of bunches per sprouted bud were reported between the untreated control and treatments, but differences between seasons were observed (Tables 7.3). The mean number of bunches per sprouted bud were significantly lower in June 2017, compared to June 2016 (Table 7.3). Due to no significant differences recorded, the decrease in potential fertility indicates towards a seasonal effect, rather than a treatment effect. It is therefore important to repeat a trial over a few seasons, in order to establish whether results recorded were due to seasonal effects or due to treatments effects.

The number of inflorescence primordia per bud determined through bud dissections for the untreated control in June 2016 was significantly higher compared to the 5 ppm GA_3, 7.5 ppm GA_{4+7} and 15

ppm GA_{4+7} (Table 7.3). The untreated control had a significantly higher number of inflorescence primordia per bud when comparing the mean between treatments over two seasons (Table 7.3). There were no significant differences for the potential fertility determined by bud dissection in June 2016 compared to June 2017 (Table 7.3).

Table 7.3: Potential fertility comparison between seasons (2015/2016 & 2016/2017) for similar treatments from both seasons (T1-T5) of treated Sunred Seedless in De Doorns.

Treatments		Potential fertility								
		Forced budding						Bud dissection		
		Bud break (%)			No. of bunches per sprouted bud			No. of inflorescence primordia per bud*		
		Jun-16	Jun-17	Mean	Jun-16	Jun-17	Mean	Jun-16	Jun-17	Mean
T1	Untreated Control	94.44 a	93.06 a	93.75 a	1.41 a	0.91 a	1.16 a	**1.04 a**	0.89 a	**0.96 a**
T2	5 ppm GA3	93.06 a	95.83 a	97.22 a	1.23 a	0.71 a	0.97 a	0.78 b	0.89 a	0.84 ab
T3	7.5 ppm GA4+7	98.61 a	97.22 a	96.53 a	1.43 a	0.76 a	1.10 a	0.78 b	0.84 a	0.81 b
T4	15 ppm GA4+7	95.83 a	98.61 a	97.22 a	1.45 a	0.83 a	1.14 a	0.77 b	0.95 a	0.86 ab
T5	30 ppm GA4+7	100.00 a	97.22 a	98.61 a	1.19 a	0.75 a	0.97 a	0.88 ab	0.94 a	0.91 ab
Mean		96.94 x	96.39 x	96.67	**1.34 x**	**0.79 y**	1.07	0.85 x	0.90 x	0.88
LSD p=0.05		8.01	6.68	5.14	0.31	0.31	0.23	0.24	0.15	0.15

*Average number of node positions 1 to 9.

Values designated by the same letters within a column or row do not differ significantly ($p \leq 0.05$).

7.3.2 Actual Fertility (October 2016)

7.3.2.1 Assessment in vineyard

The untreated control had a significantly higher number of bunches per sprouted bud on a 9 node half-long bearer compared to the 5 ppm GA_3 (10%BS) and 15 ppm GA_{4+7} (10%BS) treatments (Table 7.4). Similar to the number of bunches sprouted per bud on a half-long bearer, the untreated control had a significantly higher average number of bunches per vine compared to the 5 ppm GA_3 (10%BS) treatment. These results correlate with the findings of Dokoozlian (1998), who reported that GA_3 applied at berry set resulted in a significant decrease in the return fertility, with a decrease of 77% in cane pruned vines and 43% in spur pruned vines of Crimson Seedless. Similarly, Peacock (1998) reported a 29% decrease in return fertility with GA_3 applied during berry set. In a study performed over two seasons on the cultivar Thompson Seedless, Ahmed Ola *et al.* (2012) found that GA_3 applied during berry set resulted in the lowest percentage of fertile buds.

The 7.5 ppm GA_{4+7} (BS) treatment had a significantly lower number of bunches per vines compared to the untreated control, although the number of bunches per spouted bud did not differ significantly (Table 7.4). This was most likely due to the significantly lower bud break percentage of the 7.5 ppm GA_{4+7} (BS) treatment, resulting in a reduced number of shoots and bunches per vine.

Comparing the mean potential fertility over all treatments, determined in June 2016, to the actual fertility determined in October 2016, the mean number of bunches per node of the half-long bearer evaluation (1.43) was slightly higher than the mean potential fertility determined through forced budding (1.34), but much higher than the mean potential fertility determined through bud dissection (0.85). The mean actual fertility and mean potential fertility determined through the forced budding assessment are comparable to one another, indicating a possible experimental error in the determination of the potential fertility through bud dissection, as previously mentioned in Section 7.3.2.1.

After two seasons of treatment applications, the fertility of the experimental vineyard decreased from 2016 to 2017 when comparing the mean actual fertility (1.43) determined in October 2016 to the mean potential fertility determined through forced budding (0.79) and bud dissection (0.90) in June 2017 (Tables 7.2 & 7.4). This corresponds with the findings of authors who also recorded a decrease in the return fertility following a GA application (Jawanda *et al*., 1974; Orth, 1990; Mullins *et al*., 1992; Dokoozlian, 1999; Dokoozlian *et al*., 2000; Dokoozlian & Peacock, 2001; Weyand & Schultz, 2005; Korkutal *et al*., 2008; Ahmed Ola *et al*., 2012; Elgendy *et al*., 2012).

Table 7.4: Actual fertility of Sunred Seedless in De Doorns, after the first season's treatments, determined in October 2016.

Treatments applied in Oct-Nov 2015		Bud break (Oct-16) (%)	Actual fertility (Oct-16)		
			Average no. of bunches per node		No. of bunches per vine
			Half-long bearer (9 nodes)	Spur (3 nodes)	
T1	Untreated Control	97.05 ab	**1.54 a**	1.38 a	**133.00 a**
T2	5 ppm GA3 (10%BS)	100.00 a	**1.34 b**	1.17 a	**114.88 b**
T3	7.5 ppm GA4+7 (10%BS)	98.61 ab	1.41 ab	1.28 a	122.38 ab
T4	15 ppm GA4+7 (10%BS)	94.44 ab	1.34 b	1.26 a	117.75 ab
T5	30 ppm GA4+7 (10%BS)	94.44 ab	1.38 ab	1.42 a	125.50 ab
T6	5 ppm GA3 (BS)	100.00 a	1.47 ab	1.36 a	128.13 ab
T7	7.5 ppm GA4+7 (BS)	91.67 b	1.37 ab	1.18 a	116.56 b
T8	15 ppm GA4+7 (BS)	100.00 a	1.47 ab	1.35 a	128.13 ab
T9	30 ppm GA4+7 (BS)	97.22 ab	1.50 ab	1.34 a	129.38 ab
Mean		97.05	1.43	1.30	123.97
LSD p=0.05		6.92	0.19	0.30	15.81

Values designated by the same letters within a column do not differ significantly ($p \leq 0.05$).

7.3.2.2 No. of bunches per node position

The first node position (P1) for both the half-long bearer as well as the spur had a significantly lower mean number of bunches. This indicates that the first node of Sunred Seedless had the lowest fertility compared to the other node positions. Comparing the mean number of bunches per node position for the half-long bearer, positions one to three (P1 – P3) had a significantly lower actual fertility compared to node positions four to nine (P4 – P9) (Table 7.5). These results serve as verification for the half-long bearer pruning method used in the experimental vineyard, as node positions four to nine has higher fertility.

7.3.3 Bud break percentage

Commercially acceptable bud break percentages of higher than 80% were obtained with forced budding (June 2016 & 2017) and actual fertility (October 2016) assessments (Tables 7.1, 7.2 & 7.4). There were no significant differences in the bud break percentages of the different treatments, including the untreated control. In contrast, Ahmed Ola *et al*. (2012) reported that higher concentrations of GA_3, such as 40 ppm, resulted in a decreased bud break percentage. The same correlation could not be found in this study with the higher rates of GA_{4+7}.

Table 7.5: Actual fertility of Sunred Seedless in De Doorns, after the first season's treatments, expressed as the number of bunches per node position.

Treatments applied in Oct-Nov 2015		Actual fertility (Oct 2016)											
		Half-long bearer (No. of bunches per node position)									Spur (No. of bunches per node position)		
		P1	P2	P3	P4	P5	P6	P7	P8	P9	P1	P2	P3
T1	Untreated Control	1.19 ab	1.21 a	1.44 abc	1.59 ab	1.77 a	1.74 abc	1.82 ab	1.60 a	1.89 a	1.28 a	1.49 a	1.75 ab
T2	5 ppm GA3 (10%BS)	1.06 ab	1.10 a	0.74 d	1.51 ab	1.62 a	1.75 abc	1.66 ab	1.49 a	1.26 b	1.07 a	1.53 a	1.17 c
T3	7.5 ppm GA4+7 (10%BS)	1.08 ab	1.31 a	1.23 bc	1.07 b	1.65 a	1.43 c	1.76 ab	1.72 a	1.60 ab	1.20 a	1.47 a	1.33 bc
T4	15 ppm GA4+7 (10%BS)	0.67 b	1.27 a	1.59 ab	1.48 ab	1.43 a	1.46 bc	1.54 ab	1.69 a	1.46 ab	1.20 a	1.53 a	1.44 abc
T5	30 ppm GA4+7 (10%BS)	0.83 ab	1.33 a	1.23 bc	1.32 ab	1.52 a	1.78 ab	1.74 ab	1.57 a	1.71 ab	1.33 a	1.59 a	1.79 ab
T6	5 ppm GA3 (BS)	1.06 ab	1.46 a	1.32 abc	1.61 ab	1.51 a	1.70 abc	1.61 ab	1.67 a	1.44 ab	1.33 a	1.47 a	1.31 bc
T7	7.5 ppm GA4+7 (BS)	1.00 ab	1.29 a	1.17 c	1.29 ab	1.42 a	1.83 a	1.96 a	1.63 a	1.69 ab	1.35 a	1.34 a	1.23 c
T8	15 ppm GA4+7 (BS)	1.46 a	1.33 a	1.49 abc	1.79 a	1.73 a	1.58 abc	1.32 b	1.28 a	1.66 ab	1.34 a	1.57 a	1.88 a
T9	30 ppm GA4+7 (BS)	0.98 ab	1.42 a	1.67 a	1.86 a	1.72 a	1.45 bc	1.91 ab	1.25 a	1.67 ab	1.31 a	1.58 a	1.83 a
Mean		**1.04 d**	**1.30 c**	**1.32 c**	1.50 b	1.60 ab	1.64 ab	1.70 a	1.54 ab	1.60 ab	**1.27 b**	1.51 a	1.53 a
LSD p=0.05		0.69	0.67	0.42	0.59	0.44	0.35	0.59	0.49	0.58	0.48	0.33	0.49

Values designated by the same letters within a column or row do not differ significantly ($p \leq 0.05$).

7.3.4 Correlation between fertility assessment methods

The correlation between bud dissections and forced budding used for determining the potential fertility was very low, with R^2 = 0.039 (Table 7.6). The correlation between the actual fertility determined in the vineyard and the potential fertility determined through bud dissections (R^2 = 0.039) and forced budding (R^2 = 0.029) respectively were also very low (Table 7.6). These results indicate that the potential fertility determined through forced budding and bud dissections were poorly correlated to the actual fertility. The use of bud dissections and forced budding should therefore not be used by producers for crop estimations, as it might correlate poorly to the actual fertility. Potential fertility assessments should instead be used only to verify the pruning system used for a specific cultivar.

Table 7.6: Correlation between potential fertility determined through forced budding and bud dissections and the actual fertility of the 2015/2016 season, assessed through coefficients of determination (R^2).

Fertility assessment correlation (2015/2016)			
Variables	**Bud dissection**	**Forced budding**	**Actual fertility**
Bud dissection	**1.000**	0.039	0.337
Forced budding	0.039	**1.000**	0.029
Actual fertility	0.337	0.029	**1.000**

7.4 CONCLUSION

Results showed that all treatments reached commercially acceptable bud break percentages of ≥ 80% in forced budding assessments in June of 2016 and 2017, as well as during actual fertility assessments in October 2016. The GA_3 and GA_{4+7} treatments applied did not affect the bud break of the experimental vineyard. For similar treatments of the 2015/2016 season and 2016/2017 season, a decreased mean number of bunches per sprouted bud were recorded from June 2016 to June 2017, assessed through forced budding. The decrease in potential fertility recorded was ascribed to a seasonal effect, due to no significant differences recorded between the untreated control and treatments within a season.

The untreated control had a significantly higher number of inflorescence primordia per bud, when compared to the 5 ppm GA_3 (10%BS), 7.5 ppm GA_{4+7} (10%BS) and the 15 ppm GA_{4+7} (10%BS) treatments, for potential fertility assessed through bud dissection in June 2016. This indicates that the GA treatments applied possibly decreased the potential fertility compared to the untreated control, after one season of treatments applied. Given that the actual fertility (October 2016) of the untreated control had a significantly higher number of bunches per half-long bearer compared to the 5 ppm GA_3 (10%BS) and 30 ppm GA_{4+7} (10%BS) treatments, as well as having significantly higher

average number of bunches per vine compared to the 5 ppm GA_3 (10%BS) treatment, it appeared that GA_3 resulted in a decrease in return fertility. Two seasons of GA treatments resulted in a decreased fertility, when comparing the mean actual fertility (October 2016) to the mean potential fertility determined through forced budding (June 2017) and bud dissection (June 2017). For future studies, the initial potential and actual fertility, before the treatment commences, should be included as well as the actual fertility of the season following the last season's treatments, when investigating the effect of GA treatments on return fertility.

The use of 5 ppm GA_3 reduced the actual fertility of Sunred Seedless in this study, after one season of treatment application, compared to the untreated control. In further evaluations of the 5 ppm GA_3 treatment for thinning of Sunred Seedless and other cultivars or selections, this negative impact on the actual fertility must be considered when producers and/or researchers are considering higher dosages or multiple applications of GA_3.

The poor correlation between the potential fertility, assessed through bud dissection and forced budding, compared to the actual fertility results determined in the vineyard could be ascribed to experimental errors and/or the occurrence of primary bud necrosis (PBN) in dissected buds. Potential fertility assessments should therefore not be used for crop estimations, but rather only used to verify the pruning system recommended for a specific cultivar.

7.5 LITERATURE CITED

Ahmed Ola, A., Rafaat, S.S., Elgendy, B. & Shaker, G.S., 2012. Effect of foliar spraying with GA_3 and/or sitofex on bud behaviour, vegetative growth, yield and cluster quality of Thompson Seedless grapevines. J. Am. Sci. 8, 21-34.

Bertelsen, M.G., Tustin, D.S. & Waagepetersen, R.P., 2002. Effects of GA_3 and GA_{4+7} on early bud development of apple. J. Hortic. Sci. Biotech. 77, 83-90.

Davis, D.E., 2002. Inhibition of flower bud initiation and development in apple by defoliation, gibberellic acid and crop load manipulation. Thesis, Virginia Polytechnic Institute and State University, 800 Washington Street, SW, Suite 150, Blacksburg, VA 24061, United States.

Dokoozlian, N.K, 1998. Influence of gibberellic acid berry sizing sprays on Crimson seedless table grape. University of California Cooperative Extension, Tulare County.

Dokoozlian, N.K., 1999. Chilling temperature and duration interact on the budbreak of “Perlette” grapevine cuttings. Hort. Sci. 34, 1-3.

Dokoozlian, N.K. & Peacock, W.L., 2001. Gibberellic acid applied at bloom reduces fruit set and improves size of “Crimson Seedless” table grapes. Hort. Sci. 36, 706-709.

Dokoozlian, N.K, Peacock, B., Luvisi, D. & Vasquez, S., 2000. Cultural practices for Crimson Seedless table grapes. Pub. TB, pp.16

Dokoozlian, N.K. & Williams, L.E., 1995. Chilling exposure and hydrogen cyanamide interact in breaking dormancy of grape buds. Hort. Sci. 30, 1224-1247.

Dunn, G.M. & Martin, S.R., 2000. Do temperature conditions at budburst affect flower number in Vitis vinifera L. cv. Cabernet Sauvignon? Aust. J. Grape Wine Res. 6, 116-124.

Elgendy, R.S.S., Shaker, G.S.H. & Ahmed, O.A., 2012. Effect of foliar spraying with GA_3 and/or CPPU on bud behavior, vegetative growth, yield and cluster quality of Thompson seedless grapevines. J. Am. Sci. 8, 21-34.

Jawanda, J.S., Singh, R. & Pal, R.N., 1974. Effect of growth regulators on floral bud drop, fruit characters and quality of Thompson Seedless grape (*Vitis vinifera* L.). Vitis 13, 215-221.

Korkutal, I., Bahar, E. & Gökhan, Ö., 2008. The characteristics of substances regulating growth and development of plants and the utilization of gibberellic acid (GA_3) in viticulture. World J. Agric. Sci. 4, 321-325.

Mullins, M.G., Bouquet, A. & Williams, L.E., 1992. Biology of Horticultural Crops: Biology of the grapevine. Cambridge University Press, Cambridge.

Orth, C.H.F., 1990. Effect of spraying or dipping with gibberellic acid on bud fertility of Muscat Seedless. Dec. Fruit Grow. 40, 289-292.

Palma, B.A. & Jackson, D.I., 1989. Inflorescence initiation in grapes - responses to plant growth regulators. Vitis 28, 1-12.

Peacock, W., 1998. Influence of GA_3 sizing sprays on Ruby Seedless. Univ. Calif. Coop. Extension, Tulare Cty. pp. 897.

Shulman, Y., Nir, G., Fanberstein, L. & Lavee, S., 1983. The effect of cyanamide on the release from dormancy of grapevine buds. Sci. Hortic. 19, 97-104.

Swanepoel, J.J. & Baard, F., 1988. The correlation between potential and actual fertility in the grapevine. Dec. Fruit Grow. 38, 231-235.

Weyand, K.M. & Schultz, H.R., 2005. Physiological responses of minimal pruning systems to Gibberellic acid. Acta Hortic. 689, 117-124.

Williams, L.E., 2000. Bud development and fruitfulness of grapevines. In: Christensen, L.P. (ed). Raisin production manual, vol. 3393. UCANR Publications, California. pp. 24-29.

Chapter 8

General conclusions and recommendations

CHAPTER 8:
General conclusions and recommendations

8.1 BRIEF OVERVIEW

The study aimed to determine whether GA_{4+7} could be used as an alternative chemical thinning agent for cultivars, such as Sunred Seedless, that respond poorly to a GA_3 treatment in order to improve quality and reduce production costs without negatively affecting return fertility. In this study, the thinning effect of a standard concentration of 5 ppm GA_3 was compared to concentrations of GA_{4+7} ranging from 7.5 ppm to 120 ppm, over two seasons. The treatments of GA_3 and GA_{4+7} were applied at various phenological stages of the grapevine, ranging from 50% flowering to four days after berry set, over the two seasons. Due to an inadequate response to the thinning treatments applied in the first season, GA_{4+7} concentrations were increased in the second season, along with an adjustment to include an earlier phenological stage at application. The effect of these treatments was investigated to determine their effect on berry development and ripening, bunch structure and compactness, berry size distribution, rudimentary seed size and return fertility of Sunred Seedless. The study was performed in a commercial vineyard located on the premises of the ARC Infruitec-Nietvoorbij experimental farm in De Doorns. Results of this study will contribute to scientific published results regarding the thinning effect of GA_{4+7}, as well as the effect of GA_3 and GA_{4+7} on bunch structure, rudimentary seed size and return fertility of table grapes.

8.2 GENERAL DISCUSSION OF FINDINGS TO ORIGINAL OBJECTIVES

8.2.1 Objective 1: Identify GA_{4+7} treatments for the effective thinning of table grapes (Sunred Seedless) by establishing the most effective phenological stage for application as well as the minimum GA_{4+7} concentration required compared to the standard GA_3 treatment.

Bunch structure, compactness and berry size distribution results have to be assessed in order to determine the most effective phenological stage for applying a GA_{4+7} thinning treatment on Sunred Seedless, as well as the minimum concentration of GA_{4+7} required. Treatments of GA_{4+7} applied at different phenological stages and concentrations, ranging from 7.5 ppm to 120 ppm (over two seasons), did not prove to be effective for the thinning of Sunred Seedless. An increased sensitivity expressed as a significant increase in rachis diameter was observed for high rates as well as double applications of GA_{4+7} applied during 50% flowering when compared to a low rate and single application of 5 ppm GA_3. The highest GA_{4+7} concentration of 120 ppm, significantly increased the

number of shot berries reported per cm of lateral for treatments applied at 50% flowering and 80-100% flowering compared to the untreated control.

The earlier 5 ppm GA_3 (10%BS) treatment (2015/2016 season), applied at berry set, produced bunches with the most desirable berry size classification, with the highest percentage of normal berries and the lowest percentage of shot berries. Sunred Seedless is known to respond poorly to GA_3 treatments, compared to other commercial cultivars. Considering this inadequate response, the 5 ppm GA_3 thinning treatment applied at 80-100% flowering (2016/2017 season) delivered the most effective thinning treatment, taking the above mentioned results into consideration. The 5 ppm GA_3 treatment, applied at 80-100% flowering, significantly reduced the number total of berries per cm of lateral compared to untreated control.

The 5 ppm GA_3 treatment applied at 80-100% flowering and berry set should be evaluated further as thinning agent for Sunred Seedless and other cultivars or selections that respond poorly to standard GA_3 thinning treatments, normally applied at 1-2 ppm. The use of GA_{4+7} applied at different phenological stages and at a range of concentrations were not effective for the thinning of Sunred Seedless.

8.2.2 Objective 2: Compare the effect of different GA_3 and GA_{4+7} treatments applied at different phenological stages of Sunred Seedless on bunch structure, rudimentary seed size and return fertility.

8.2.2.1 Bunch structure

Based on the bunch and berry mass measurements, applications during flowering resulted in a better thinning effect of Sunred Seedless. Bunch and berry mass measurements at harvest didn't result in a specific trend with regard to a specific GA concentration and application timing combination that could be recommended for effective thinning in Sunred Seedless.

The mean rachis diameter increased significantly for GA treatments applied after flowering. As mentioned in Section 8.2.1, Sunred Seedless experienced an increased sensitivity towards high rates and double applications of GA_{4+7} applied at 50% flowering, with significantly increased rachis diameters compared to the standard 5 ppm GA_3 treatment.

Based on the subjective visual assessment of bunch compactness, applying a GA thinning treatment at 50% flowering is too early for Sunred Seedless, as it resulted in bunches with straggly appearance. From the earliest to the latest phenological stage, the mean bunch compactness score decreased significantly, *i.e.* bunches became more compact. This indicates that the longer a GA thinning treatment is delayed from flowering to berry set, the less effective the thinning results will be, resulting

in more compact bunches if applied around berry set. These findings correspond with the results obtained with the quantitative bunch compactness measurements. The mean total and normal number of berries per cm of lateral length, as well as the number of berries per cm of lateral length, were reduced significantly by GA treatments applied during flowering. The application of GA treatments during flowering reduced fruit set in Sunred Seedless, however, applying a GA thinning application too early during flowering could result in overthinning and thus yield bunches with a straggly appearance. As mentioned in Section 8.2.1, the 5 ppm GA_3 treatment applied at 80-100% flowering resulted in the most effective thinning, with a significantly reduced number of total berries per cm of lateral compared to the untreated control.

The formation of shot berries was increased when GA was applied during flowering, indicating that Sunred Seedless has a higher sensitivity for the formation of shot berries when GA is applied during flowering. Based on objective bunch compactness measurements, the most effective phenological stage to apply a GA thinning treatment for Sunred Seedless was at the 80-100% flowering stage. There was, however, a negative side effect of increased shot berry occurrence. An increase in shot berry occurrence was also observed with the use of higher GA_{4+7} concentrations and double applications at the 50% flowering stage.

The sensitivity of Sunred Seedless towards GA applications applied during early flowering, along with poor response for GA applications applied after flowering observed in this study, confirms why GA thinning treatments for this particular cultivar do not give economically acceptable results. More reoccurring trends regarding the bunch phenological stage at the time of application were observed in this study, rather than trends regarding a specific GA treatment and treatment rates. These results confirm that the timing of GA applications play a fundamental role in the treatment outcome for a specific cultivar.

8.2.2.2 Rudimentary seed size

The 5 ppm GA_3 (10%BS) treatment, applied at 80-100% flowering and berry set in the 2015/2016 season, significantly reduced the average rudimentary seed mass compared to the untreated control. A treatment effect was visible at berry set with a reduction in the total seed mass per berry for all GA treatments compared to the untreated control. Similar trends were not obtained during the 2016/2017 season. Seasonal differences were observed, with a reduction in the overall rudimentary seed mass reported for the 2016/2017 season compared to the 2015/2016 season. A higher average temperature for the period between flowering and the early stages of fruit development could have contributed to delayed embryo abortion, resulting in the higher seed mass recorded for the 2015/2016 season.

The application of GA treatments during flowering resulted in an improved rudimentary seed size distribution, with an increased percentage in small rudimentary seeds. A possible response is visible for GA applied during flowering with a reduction in the size distribution of the rudimentary seeds compared to the treatments applied post-flowering.

A trend was observed that applying GA treatments during flowering resulted in a decreased average seed mass per berry, as well as an improved rudimentary seed size distribution, with an increased percentage in small rudimentary seeds compared to GA applied during the early stages of berry development. The 5 ppm GA_3 treatments applied at 80-100% flowering and berry set were the most promising treatments based on bunch structure results, as well as rudimentary seed results.

8.2.2.3 Return fertility

All treatments reached commercially acceptable bud break percentages of ≥ 80% in potential fertility, as well as actual fertility assessments. The GA_3 and GA_{4+7} treatments applied did not affect the bud break of the experimental vineyard.

A reduction in the mean number of bunches per sprouted bud was reported from June 2016 to June 2017, for the potential fertility assessed through forced budding for similar treatments. The decrease in potential fertility recorded could be ascribed to a seasonal effect, due to no significant differences recorded between the untreated control and treatments within a season. Potential fertility assessed through bud dissections did not follow the same trend from June 2016 to June 2017, as mentioned above for forced budding. Potential fertility assessed through bud dissection in June 2016 indicate a significantly higher number of inflorescence primordia per bud for the untreated control compared to the 5 ppm GA_3 (10%BS), 7.5 ppm GA_{4+7} (10%BS) and the 15 ppm GA_{4+7} (10%BS) treatments.

The actual fertility determined in the vineyard in October 2016 indicated a significantly higher number of bunches per half-long bearer and number of bunches per vine for the untreated control, compared to the 5 ppm GA_3 (10%BS) treatment. The use of 5 ppm GA_3 reduced the actual fertility of Sunred Seedless in this study, after one season of treatment application, compared to the untreated control. The same results were not found for the GA_{4+7} treatments. This negative impact on the actual fertility must be considered when producers and/or researchers are considering higher dosages or multiple applications of GA_3, in further evaluations of a 5 ppm GA_3 thinning treatment of Sunred Seedless and other cultivars or selections.

A poor correlation between the potential fertility determined through bud dissection and forced budding was reported, compared to the actual fertility determined in the vineyard. Potential fertility

assessments should therefore not be used for crop estimations, but rather used to verify the pruning system recommended for a specific cultivar.

8.3 RECOMMENDATIONS FOR FUTURE STUDIES

In general, it is recommended to repeat the study for at least one more season, in order to verify results obtained, with specific reference to significant differences that were found for one of the two seasons. This could help determine if these differences could be repeated for another season, as well as to determine whether trends observed could develop into significant differences. In order to address research gaps, the following are recommended for repeating the existing trial and/ or similar new trials:

- In future, the phenological stages at application included in thinning trials performed on Sunred Seedless, can include 80-100% flowering and 10% berry set.
- In future, the viability of the primary bud, expressed as primary bud necrosis (PBN), should be noted for every bud position evaluated for potential fertility through bud dissections in order to determine whether a change in the fertility of the vine are due to treatments applied or PBN.
- To accurately determine the effect of treatments on return fertility, the actual fertility for the experimental vineyard should be determined before treatment application, as well as the season following the last treatment application.
- It is recommended that other PGRs such as ABA and ethylene, are included on their own and in combination with GA, to be evaluated as thinning agents for Sunred Seedless, as well as for other cultivars selected for similar trials.
- In future, a multidisciplinary approach is recommended, where parallel to the field trial evaluations of PGRs, genomic studies are also included, to identify and quantify GA signalling components and availability of bioactive GAs, in order to contribute to understanding differences in the response obtained with treatments applied in the field trial.
- If a cultivar's or selection's response to a specific PGR has not yet been established, or if standard practices do not deliver a commercially acceptable reaction, molecular studies should be performed first, in order to determine if and how the cultivar responds towards a specific PGR applied at different concentrations and phenological stages, under laboratory conditions. Promising PGRs and concentrations should then be advanced to a field study.

Addendum A

Table A.1: Berry diameter and length of marked Sunred Seedless bunches at harvest (2015/2016 season).

Treatment		Berry diameter (mm)				Berry length (mm)			
		Bunch phenological stage at application							
		80-100% Flowering	10% Berry Set	Berry Set	Berry Set + 4 Days	80-100% Flowering	10% Berry Set	Berry Set	Berry Set + 4 Days
T1	Untreated control	18.66 a	18.84 a	19.41 ab	.	21.87 b	22.10 ab	22.11 ab	.
T2	5 ppm GA3 (10%BS)	19.29 a	18.95 a	**19.82 a**	.	**23.17 a**	21.99 ab	**23.29 a**	.
T3	7.5 ppm GA4+7 (10%BS)	19.01 a	19.25 a	18.63 ab	.	22.12 b	22.44 ab	21.12 b	.
T4	15 ppm GA4+7 (10%BS)	18.89 a	19.08 a	18.42 b	.	22.29 b	22.30 ab	21.35 b	.
T5	30 ppm GA4+7 (10%BS)	18.98 a	18.59 a	18.43 b	.	21.87 b	21.23 b	21.10 b	.
T6	5 ppm GA3 (BS)	.	19.19 a	19.27 ab	18.53 bc	.	22.50 ab	21.90 ab	21.24 b
T7	7.5 ppm GA4+7 (BS)	.	18.84 a	18.81 ab	**18.18 c**	.	22.08 ab	21.86 ab	20.89 b
T8	15 ppm GA4+7 (BS)	.	18.87 a	19.31 ab	**20.03 a**	.	**22.84 a**	22.51 ab	**22.97 a**
T9	30 ppm GA4+7 (BS)	.	19.09 a	18.84 ab	19.53 ab	.	22.47 ab	21.98 ab	**22.91 a**
Mean		18.97 x	18.97 x	18.99 x	19.07 x	22.26 x	22.22 x	21.91 x	22.00 x
LSD p=0.05		0.70	0.86	1.22	1.11	0.79	1.32	1.52	0.94

Values designated by the same letters within a column or row do not differ significantly ($p \leq 0.05$).

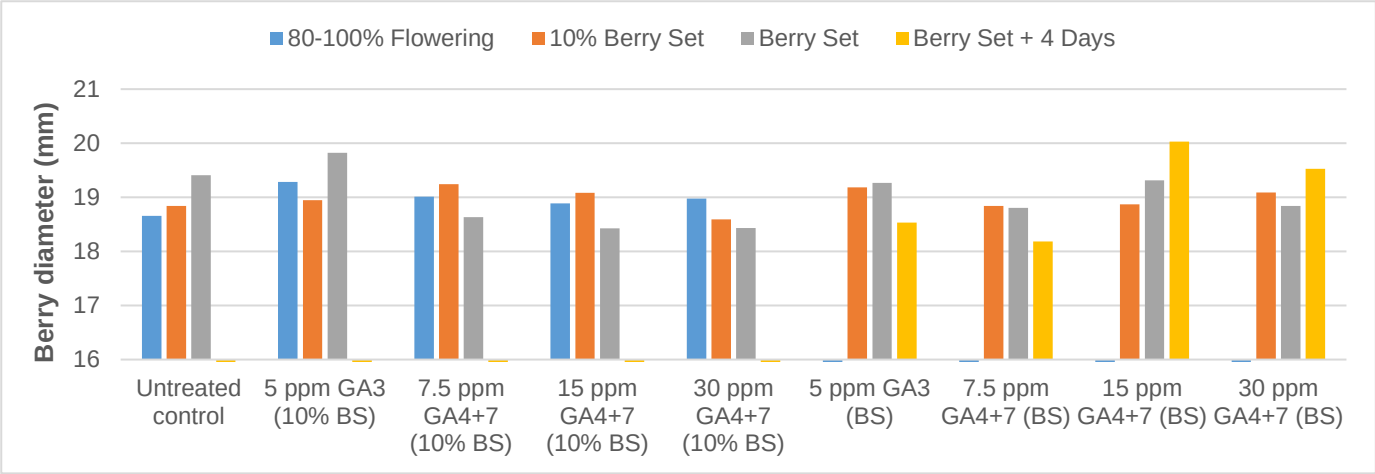

Figure A.1: Berry diameter of marked Sunred Seedless bunches at harvest (2015/2016 season).

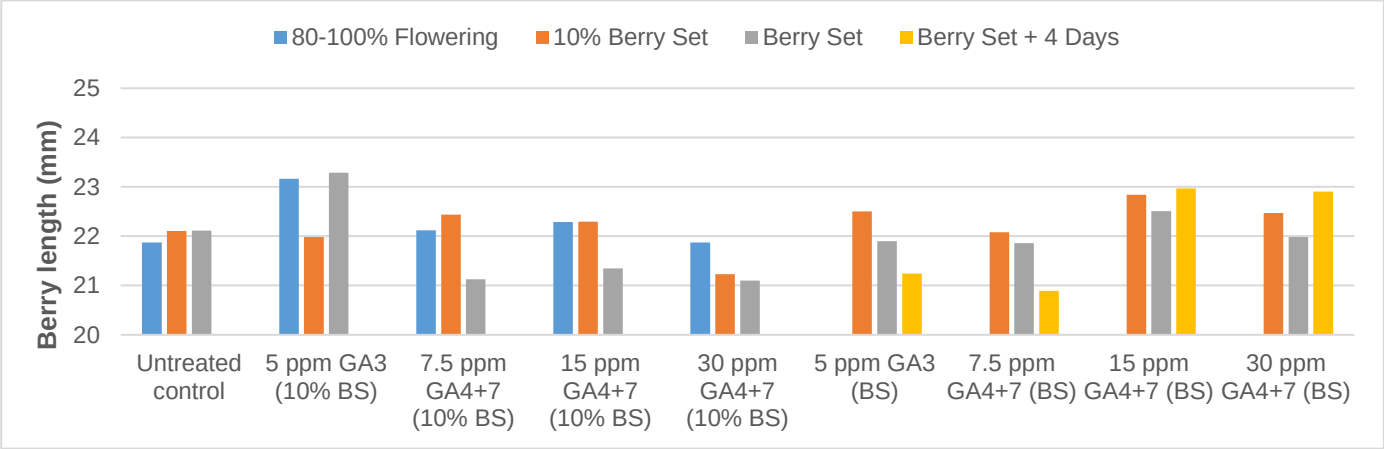

Figure A.2: Berry length of marked Sunred Seedless bunches at harvest (2015/2016 season).

Table A.2: Berry diameter and length of marked Sunred Seedless bunches at harvest (2016/2017 season).

Treatment		Berry diameter (mm)				Berry length (mm)			
		Bunch phenological stage at application							
		50% Flowering	80-100% Flowering	10% Berry Set	Berry Set	50% Flowering	80-100% Flowering	10% Berry Set	Berry Set
T1	Untreated control	18.32 a	19.05 a	19.19 ab	20.24 a	21.48 ab	22.46 a	22.62 bc	23.72 a
T2	5 ppm GA3 (T)	18.21 a	19.01 a	19.49 ab	20.12 a	22.49 ab	22.85 a	23.19 abc	23.70 a
T3	7.5 ppm GA4+7 (T)	19.45 a	19.06 a	**19.90 a**	19.65 a	22.92 ab	22.98 a	**24.09 a**	22.80 a
T4	15 ppm GA4+7 (T)	18.38 a	18.89 a	19.23 ab	19.58 a	22.28 ab	22.77 a	22.67 bc	22.94 a
T5	30 ppm GA4+7 (T)	18.16 a	18.81 a	**19.92 a**	19.76 a	22.01 ab	23.48 a	23.84 ab	23.16 a
T6	60 ppm GA4+7 (T)	18.90 a	18.97 a	19.66 ab	19.98 a	23.52 ab	23.21 a	23.96 ab	23.54 a
T7	120 ppm GA4+7 (T)	18.97 a	18.44 a	19.58 ab	20.20 a	**23.54 a**	22.92 a	23.26 abc	23.78 a
T8	60 ppm GA4+7 (T+S)	18.59 a	18.54 a	18.86 b	19.32 a	23.36 ab	22.80 a	**22.15 c**	22.87 a
T9	60 ppm GA4+7 (S)	18.01 a	18.97 a	19.50 ab	19.91 a	21.29 b	22.46 a	22.67 bc	23.26 a
Mean		**18.55 z**	**18.86 y**	**19.48 x**	**19.86 w**	**22.54 y**	22.88 xy	**23.16 x**	**23.31 x**
LSD p=0.05		1.57	1.08	0.82	1.19	2.23	1.63	1.36	1.66

Values designated by the same letters within a column or row do not differ significantly ($p \leq 0.05$).

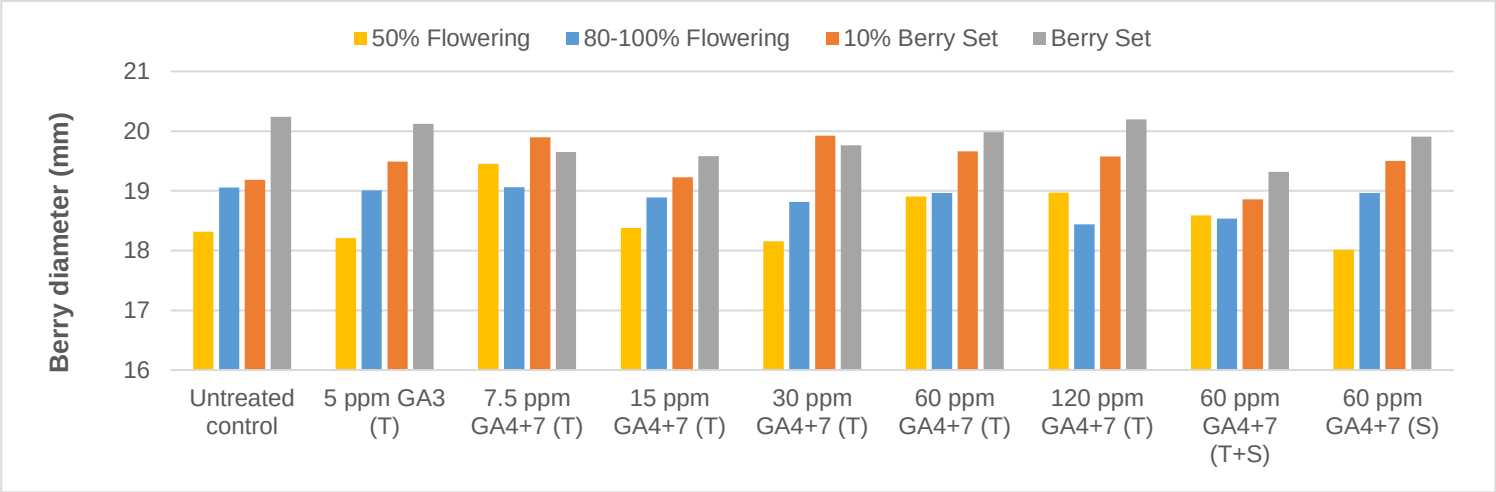

Figure A.3: Berry diameter of marked Sunred Seedless bunches at harvest (2016/2017 season).

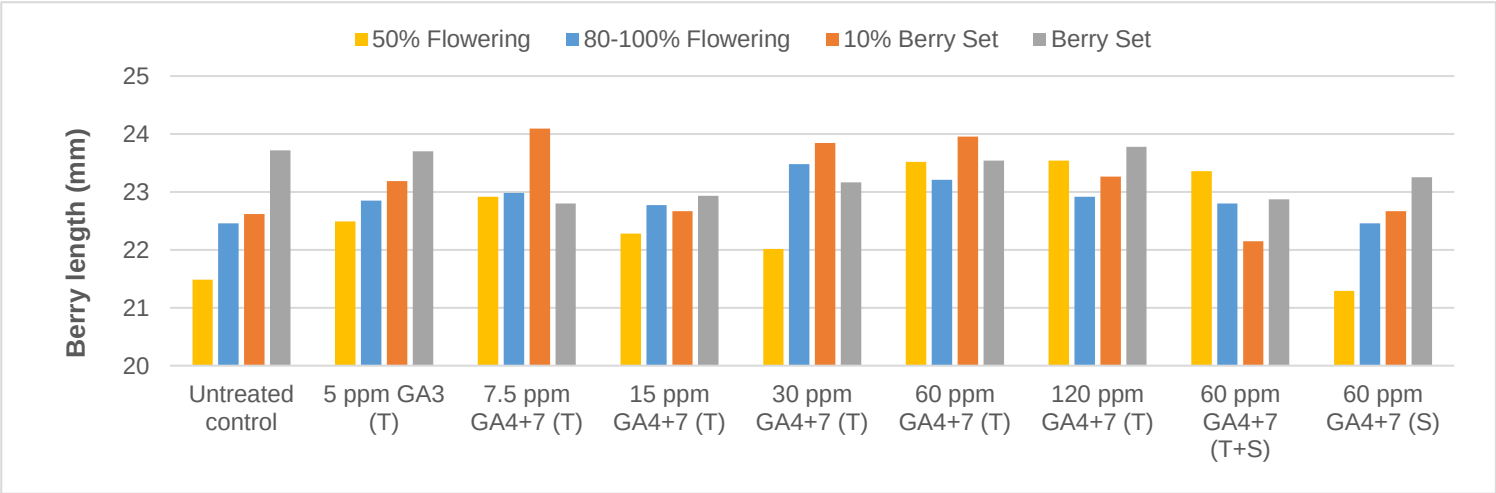

Figure A.4: Berry length of marked Sunred Seedless bunches at harvest (2016/2017 season).

Table A.3: Total soluble solids (TSS) and titratable acidity (TA) of marked Sunred Seedless bunches at harvest (2016/2017 season).

Treatment		Total soluble solids				Titratable acidity			
		Bunch phenological stage at application							
		50% Flowering	80-100% Flowering	10% Berry Set	Berry Set	50% Flowering	80-100% Flowering	10% Berry Set	Berry Set
T1	Untreated control	13.53 a	14.40 ab	15.08 a	15.65 a	7.49 ab	7.04 a	6.63 a	6.46 a
T2	5 ppm GA3 (T)	13.60 a	14.30 ab	14.83 a	16.58 a	7.74 ab	6.95 a	6.73 a	6.43 a
T3	7.5 ppm GA4+7 (T)	13.75 a	13.50 b	15.20 a	15.50 a	**8.36 a**	7.42 a	6.66 a	6.11 a
T4	15 ppm GA4+7 (T)	13.83 a	13.90 ab	14.43 a	15.30 a	7.44 b	7.26 a	6.86 a	6.44 a
T5	30 ppm GA4+7 (T)	13.93 a	14.48 ab	15.90 a	16.60 a	7.41 b	6.91 a	6.49 a	5.83 a
T6	60 ppm GA4+7 (T)	14.47 a	14.60 ab	15.80 a	16.30 a	6.94 b	6.89 a	6.35 a	6.08 a
T7	120 ppm GA4+7 (T)	13.90 a	14.43 ab	15.53 a	16.40 a	7.35 b	6.79 a	6.44 a	5.79 a
T8	60 ppm GA4+7 (T+S)	13.83 a	**15.08 a**	15.63 a	15.78 a	7.42 b	6.85 a	6.61 a	6.62 a
T9	60 ppm GA4+7 (S)	14.70 a	14.45 ab	15.65 a	16.65 a	7.18 b	7.47 a	6.46 a	6.27 a
Mean		**13.95 z**	**14.35 y**	**15.34 x**	**16.08 w**	**7.48 z**	**7.06 y**	**6.58 x**	**6.22 w**
LSD p=0.05		1.32	1.48	1.50	1.65	0.90	0.82	0.54	0.86

Values designated by the same letters within a column or row do not differ significantly ($p \leq 0.05$).

Table A.4: Average bunch mass (g) and berry mass (g) of marked Sunred Seedless bunches at harvest (2015/2016 season).

Treatment		Average bunch mass (g)				Average berry mass (g) (Normal berry size)			
		Bunch phenological stage at application							
		80-100% Flowering	10% Berry Set	Berry Set	Berry Set + 4 Days	80-100% Flowering	10% Berry Set	Berry Set	Berry Set + 4 Days
T1	Untreated control	861.0 a	980.8 a	**1174.8 a**	.	5.03 a	4.82 bc	5.48 ab	.
T2	5 ppm GA3 (10%BS)	708.3 a	864.5 ab	**1212.3 a**	.	5.13 a	4.73 bc	**5.62 a**	.
T3	7.5 ppm GA4+7 (10%BS)	703.3 a	929.8 a	865.0 cb	.	4.78 a	**5.43 a**	4.47 c	.
T4	15 ppm GA4+7 (10%BS)	706.8 a	879.8 ab	964.7 abc	.	4.71 a	4.91 abc	5.19 abc	.
T5	30 ppm GA4+7 (10%BS)	749.5 a	909.8 ab	924.7 abc	.	4.72 a	4.84 abc	4.70 bc	.
T6	5 ppm GA3 (BS)	.	727.0 ab	**833.0 c**	994.0 ab	.	4.66 bc	4.46 c	4.75 a
T7	7.5 ppm GA4+7 (BS)	.	664.0 b	847.5 c	741.3 b	.	**4.33 c**	**4.71 bc**	4.37 a
T8	15 ppm GA4+7 (BS)	.	**948.3 a**	972.5 abc	**1152.0 a**	.	5.07 ab	4.86 abc	5.11 a
T9	30 ppm GA4+7 (BS)	.	785.3 ab	886.0 cb	899.0 ab	.	4.86 abc	4.80 bc	5.17 a
Mean		**745.8 y**	854.4 xy	**964.5 x**	946.6 xy	4.9 x	4.9 x	4.9 x	4.9 x
LSD p=0.05		262.0	257.6	323.7	263.4	0.86	0.60	0.82	1.14

Values designated by the same letters within a column or row do not differ significantly ($p \leq 0.05$).

Table A.5: Average bunch mass (g) and berry mass (g) of marked Sunred Seedless bunches at harvest (2016/2017 season).

Treatment		Average bunch mass (g)				Average berry mass (g) (Normal berry size)			
		Bunch phenological stage at application							
		50% Flowering	80-100% Flowering	10% Berry Set	Berry Set	50% Flowering	80-100% Flowering	10% Berry Set	Berry Set
T1	Untreated control	373.5 ab	591.3 a	728.0 a	**709.0 b**	5.05 ab	4.65 a	5.19 ab	5.16 a
T2	5 ppm GA3 (T)	254.0 b	441.5 a	810.0 a	871.0 ab	4.80 ab	4.73 a	4.87 ab	5.80 a
T3	7.5 ppm GA4+7 (T)	346.0 ab	599.0 a	981.5 a	926.0 ab	5.36 a	5.30 a	5.40 a	5.09 a
T4	15 ppm GA4+7 (T)	403.3 ab	555.8 a	850.5 a	1019.0 a	4.70 ab	4.85 a	5.00 ab	5.32 a
T5	30 ppm GA4+7 (T)	362.3 ab	513.5 a	948.0 a	953.5 ab	4.66 ab	4.61 a	5.19 ab	5.52 a
T6	60 ppm GA4+7 (T)	354.0 ab	518.8 a	823.5 a	900.5 ab	4.79 ab	4.87 a	5.33 ab	5.64 a
T7	120 ppm GA4+7 (T)	412.8 ab	524.0 a	861.5 a	1099.5 a	4.47 ab	4.93 a	5.28 ab	5.45 a
T8	60 ppm GA4+7 (T+S)	274.8 ab	516.5 a	798.0 a	858.5 ab	4.73 ab	4.44 a	**4.82 b**	5.02 a
T9	60 ppm GA4+7 (S)	**442.7 a**	508.5 a	873.5 a	987.8 ab	**4.25 b**	4.71 a	4.95 ab	5.13 a
Mean		**358.1 z**	**529.9 y**	**852.7 x**	**925.0 w**	4.8 x	4.8 x	5.1 x	5.3 x
LSD p=0.05		174.2	196.2	276.3	284.8	1.07	0.95	0.56	0.83

Values designated by the same letters within a column or row do not differ significantly ($p \leq 0.05$).

Table A.6: Pre-harvest and at harvest bunch lengths of marked Sunred Seedless bunches (2015/2016 season).

Treatment		Bunch length (cm)							
		Bunch phenological stage at application							
		80-100% Flowering		10% Berry Set		Berry Set		Berry Set + 4 Days	
		Pre-Harvest	At Harvest	Pre-Harvest	At Harvest	Pre-Harvest	At Harvest	Pre-Harvest	At Harvest
T1	Untreated control	19.85 a	21.34 a	18.94 ab	20.85 abc	19.77 a	**16.73 b**	.	.
T2	5 ppm GA3 (10%BS)	20.02 a	20.10 a	20.05 a	20.61 abc	19.96 a	**22.80 a**	.	.
T3	7.5 ppm GA4+7 (10%BS)	20.13 a	20.91 a	19.40 ab	21.73 ab	18.38 a	20.72 ab	.	.
T4	15 ppm GA4+7 (10%BS)	19.50 a	20.53 a	20.29 a	20.99 abc	20.69 a	22.16 ab	.	.
T5	30 ppm GA4+7 (10%BS)	19.26 a	20.16 a	20.22 a	21.90 a	20.10 a	21.28 ab	.	.
T6	5 ppm GA3 (BS)	.	.	18.67 ab	**19.14 c**	19.32 a	20.56 ab	18.02 b	19.71 a
T7	7.5 ppm GA4+7 (BS)	.	.	19.24 ab	**18.99 c**	19.91 a	20.37 ab	19.76 ab	20.20 a
T8	15 ppm GA4+7 (BS)	.	.	19.34 ab	20.79 abc	20.10 a	21.32 ab	20.98 a	21.91 a
T9	30 ppm GA4+7 (BS)	.	.	**18.24 b**	19.63 bc	19.84 a	20.29 ab	18.82 b	19.54 a
Mean		19.75 x	20.61 x	19.38 x	20.51 x	19.79 x	20.69 x	19.40 x	20.34 x
LSD p=0.05		1.67	1.58	1.81	2.19	3.12	5.56	2.43	3.73

Values designated by the same letters within a column or row do not differ significantly ($p \leq 0.05$).

Table A.7: Pre-harvest and at harvest bunch lengths of marked Sunred Seedless bunches (2016/2017 season).

Treatment		Bunch length (cm)							
		Bunch phenological stage at application							
		50% Flowering		80-100% Flowering		10% Berry Set		Berry Set	
		Pre-Harvest	At Harvest	Pre-Harvest	At Harvest	Pre-Harvest	At Harvest	Pre-Harvest	At Harvest
T1	Untreated control	17.09 a	19.67 abc	18.99 ab	21.95 a	20.42 a	21.79 a	21.29 a	23.67 a
T2	5 ppm GA3 (T)	16.90 a	19.46 abc	18.48 ab	21.13 a	19.38 a	22.62 a	20.86 ab	22.38 a
T3	7.5 ppm GA4+7 (T)	14.66 ab	18.02 c	19.91 a	21.83 a	20.79 a	23.97 a	20.54 ab	22.23 a
T4	15 ppm GA4+7 (T)	16.68 ab	**21.21 ab**	18.55 ab	21.31 a	20.79 a	23.47 a	20.66 ab	23.09 a
T5	30 ppm GA4+7 (T)	16.47 ab	20.78 abc	18.89 ab	22.31 a	19.67 a	23.12 a	19.67 b	21.74 a
T6	60 ppm GA4+7 (T)	14.27 b	18.55 bc	17.12 b	20.82 a	21.50 a	23.59 a	20.34 ab	22.00 a
T7	120 ppm GA4+7 (T)	15.90 ab	19.54 abc	18.34 ab	20.18 a	20.38 a	23.82 a	20.56 ab	22.11 a
T8	60 ppm GA4+7 (T+S)	16.22 ab	19.46 abc	19.52 a	21.91 a	20.59 a	24.35 a	20.42 ab	22.35 a
T9	60 ppm GA4+7 (S)	17.06 a	**22.38 a**	18.52 ab	20.82 a	19.54 a	22.92 a	19.96 ab	22.04 a
Mean		16.14 z	19.90 z	18.70 y	21.36 y	20.34 x	23.29 x	20.48 x	22.40 w
LSD p=0.05		2.51	3.10	1.90	3.11	2.00	2.06	1.62	2.18

Values designated by the same letters within a column or row do not differ significantly ($p \leq 0.05$).