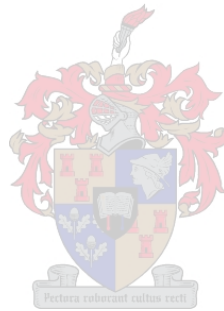


The influence of gibberellic acid (GA₃) for berry thinning and berry sizing on table grape production, quality and fertility of Prime

by

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DECLARATION

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SUMMARY

Table grapes are one of the major commercially grown non-climacteric fresh fruit crops worldwide. Over centuries the table grape industry became a niche market with increasing competition on the markets, putting pressure on table grape growers to produce quality grapes that meet market requirements nationally and internationally. To meet market requirements regarding bunch size and compactness, as well as berry size, colour, flavour, texture and firmness, viticultural practices for table grape production include the use of plant growth regulators (PGRs). Higher input costs are invested to meet these requirements. This lead to the critical focus on labour-intensive cultivation practices and whether alternative methods could be found to maintain high levels of fertility, production and quality.

The the aim of this study was to identify GA₃ application methods and volumes for thinning and sizing treatments of table grapes without negatively affecting fertility. The study was done in a commercial Prime vineyard, grafted onto Ramsey, in Paarl, Berg River Valley, South Africa. There are limited scientific publications reporting research results on this cultivar, specifically regarding the effect of different GA₃ application methods and volumes on production, quality and fertility.

Thinning and berry sizing treatments were applied according to commercial concentrations recommended for Prime. In this trial, different GA₃ application methods and volumes were evaluated. Two GA₃ treatments were applied during two phenological stages. The first application was the thinning treatment which was applied at 80-100% full bloom. The second application was the berry sizing treatment which was applied when the berries where at 7-8 mm diameter.

Six treatments were applied: Treatment 1 (NoThin + Dip (Control)), comprised of a no thinning application, followed by the berry sizing treatment applied by dipping. Treatment 2 (Thin + Dip) comprised a conventional thinning spray application, followed by a berry sizing treatment applied by dipping. Treatment 3 (Thin + 250 L/ha), Treatment 4 (Thin + 500 L/ha) and Treatment 5 (Thin + 1000 L/ha) comprised conventional thinning spray application, followed by berry sizing treatments applied by spraying with a mist blower with spray volumes of 250 L/ha, 500 L/ha and 1000 L/ha respectively. Treatment 6 (Thin + ESS) comprised a conventional thinning spray, followed by a berry sizing treatment, applied at 72 L/ha with an electrostatic spray pump (ESS).

In both seasons, before the thinning application was applied, 15 inflorescences per data experimental unit were marked according to a phenological stage. In the first season, ten inflorescences per data experimental unit were marked at 80-100% full bloom (FB) and five inflorescences were marked at 10% set (referred to in the table grape indusy as 110% full bloom). In the second season five inflorescences per data experimental unit were marked at 80-100% FB, five inflorescences were marked at 10% set and five inflorescences were marked at 100% set. No manual bunch preparation actions were applied to these marked

bunches and no berry sampling were done from them. These clusters were evaluated for bunch structure/compactness at harvest. This method was used to determine the optimum time for application of thinning treatments in terms of bunch structure at harvest.

The bud break percentage determined through forced budding in June 2015 and June 2016, as well as through assessment in the vineyard (November 2015) did not differ significantly between treatments and was above 80% for all treatments. Commercially acceptable levels of bud break were obtained in both seasons.

The potential and actual fertility decreased over the two seasons. In June 2015 Treatment 1 had a significantly higher potential fertility and Treatment 6 had a significantly lower potential fertility compared to the other treatments. In June 2016, no significant differences were found between treatments, although Treatment 6 again had the lowest potential fertility. It seems that Treatments 3 and 6 with lower application volumes and smaller droplet size are associated with lower fertility, possibly due to more effective coverage obtained on bunches (the target organs for berry sizing treatments), but also on the shoots and buds and that GA₃ applied to the buds had a negative effect on potential fertility. This was also reflected by the actual fertility and yield obtained in the November 2015, where Treatment 2 had the lowest yield as compared to Treatments 3 and 6 (only significant for Treatment 3).

Regarding manual thinning in both seasons, Treatment 1 required the longest time spent per ha and Treatments 2 and 5 required significantly less time, which can be ascribed to the larger berry size and % normal berries obtained with Treatment 2. No significant difference was found between the different spray applications (volumes). Therefore, the “best” method for application will depend on the effect on return fertility.

In both seasons, Treatment 1 required the most hours for manual thinning and consequently had the highest cost, verifying the need for chemical thinning of Prime, to save labour cost. Time and cost of manual thinning of Prime using Treatment 2, can be up to 40% lower than with Treatment 1. Time required and cost for Treatments 2 and 5 ranged from 942 to 2578 hours and R12 595 and R31 992, which were in line with the time and cost required for commercial Prime blocks.

Berry juice composition was not negatively affected by any of the treatments. The expected berry development and ripening patterns were found. Although a few significant differences were found regarding post-harvest quality, it did not practically impact the marketability of the grapes.

Regarding the bunch structure in the 2014/2015 season, there were few significant differences between treatments. With the thinning application applied at 80-100% FB the number of berries per cm lateral length, as well as the number of normal berries per cm lateral length of Treatment 1 was significantly higher compared to Treatment 2, indicating that the bunches of Treatment 1 were more compact than the bunches of Treatment 2. The 80-100% FB Treatment 1 had a significantly higher number of small berries per cm lateral

length compared to the other two treatments which can be linked to the longer time required for manual thinning of this treatment. In both seasons a trend was observed that a lower number of berries per cm lateral length (less compact bunches) was obtained with the thinning application applied at 80-100% FB compared to the later applications.

The results of this study contribute to the available published scientific results regarding the effect of GA₃ application methods (volumes) on fertility of table grapes. Based on the results after the first two seasons of the trial, the following are recommended regarding identifying GA₃ application methods and volumes for effective thinning and sizing treatments of table grapes without negatively affecting fertility:

- Treatment 2 (Chemical thinning with a standard GA₃ spray application, followed by a GA₃ dipping treatment for berry sizing) had the largest berries, less compact bunches and the highest percentage normal berries. This treatment also required the least time for manual thinning.
- Practical implementation of Treatment 2 (Chemical thinning with a standard GA₃ spray application, followed by a GA₃ dipping treatment for berry sizing) in commercial table grape production requires availability of sufficient labour. This is already practically applied by several producers in the industry in situations where they have practical experience of a decline in fertility after GA₃ applications.
- Current available results indicate that the lower spray application volumes Treatment 3 (250 L/ha) and Treatment 6 (ESS 72 L/ha) were associated with a decrease in fertility, while with Treatment 4 (500 L/ha) no indication of a negative effect on fertility was found. Therefore, repetition of the trial is needed to verify these results and to investigate whether the 500 L/ha spray application volume could be used instead of the current standard industry practice of using 1000 L/ha for the majority PGRs and other spray applications. Using an application volume of 500 L/ha instead of 1000 L/ha will have several practical and economic benefits, in terms of more hectares being sprayed with a one tank mix, decreasing the water foot print as well as the carbon foot print.
- It is recommended to repeat the trial for at least one more season to verify results obtained and test repeatability.

OPSOMMING

Tafeldruiwe is een van die grootste kommersieel geproduseerde nie-klimakteriese vars vrugtesoorte wêreldwyd. Oor die jare het die tafeldruiwemark 'n 'niche' mark geword, met groeiende kompetisie en druk op tafeldruiweprodusente om gehalte druiwe te produseer wat aan markvereistes voldoen, nasionaal en internasionaal. Om aan markvereistes ten opsigte van trosgrootte en -kompaktheid, asook korrelgrootte, -kleur, -geur, -tekstuur en -fermheid te voldoen, word die gebruik van plantgroeireguleerders (PGRs) ingesluit by wingerboupraktyke vir tafeldruiweproduksie. Hoër insetkoste word aangegaan om aan hierdie vereistes te voldoen. Dit het gelei tot die kritiese fokus om alternatiewe metodes te vind vir arbeidintensiewe wingerdpraktyke en om nog steeds hoë vlakke van vrugbaarheid, produksie en gehalte te handhaaf.

Die doel van die studie was identifisering van GA₃-toedieningsmetodes en -volumes vir uitdunning- en korrelvergrotingbehandlings van tafeldruiwe, sonder om vrugbaarheid te benadeel. Hierdie studie is uitgevoer in 'n kommersiële Prime wingerd, geënt op Ramsey, in die Paarl, Bergriviergebied, Suid-Afrika. Daar is beperkte wetenskaplike publikasies beskikbaar met navorsingresultate oor hierdie kultivar en spesifiek ten opsigte van die effek van verskillende GA₃-toedieningsmetodes en -volumes op produksie, gehalte en vrugbaarheid.

Uitdunning- en korrelvergrotingbehandelings is toegedien volgens kommersiële konsentrasies aanbeveel vir Prime. In hierdie proef is verskillende GA₃-toedieningsmetodes en -volumes geëvalueer. GA₃-behandelings is toegedien gedurende twee fenologiese stadiums. Die uitdunbehandeling is toegedien by 80-100% volblom. Die korrelvergrotingbehandeling is toegedien by 7-8 mm korreldeursnee.

Ses behandelings is toegedien: Behandeling 1 (geen uitdunning + doop (kontrole)), bestaande uit 'n geen uitduntoediening, gevolg deur 'n korrelvergrotingtoediening deur middel van 'n doop-aksie; Behandeling 2 (uitdun + doop) bestaande uit 'n konvensionele uitduntoediening, gevolg deur 'n korrelvergrotingtoediening deur middel van 'n doop-aksie; Behandeling 3 (uitdun + 250 L/ha), Behandeling 4 (uitdun + 500 L/ha) en Behandeling 5 (uitdun + 1000 L/ha) bestaande uit 'n konvensionele uitduntoediening, gevolg deur 'n korrelvergrotingtoediening met 'n newelblaser met toedieningsvolumes van onderskeidelik 250 L/ha, 500 L/ha en 'n 1000 L/ha en Behandeling 6 (uitdun + ESS) bestaande uit 'n konvensionele uitduntoediening, gevolg deur 'n korrelvergrotingtoediening teen 72 L/ha met 'n elektrostatiese spuitpomp (ESS).

In albei seisoene, voordat die korreluitdunbehandelings toegedien is, is 15 blomtrosse per data eksperimentele eenheid gemerk volgens 'n spesifieke fenologiese stadium. In die eerste seisoen is tien blomtrosse by 80-100% volblom (VB) gemerk en 5 blomtrosse by 10% set. In die tweede seisoen is 5 blomtrosse by 80-100% VB gemerk, 5 blomtrosse by 10% set en 5 blomtrosse by 100% set, vir elke data eksperimentele eenheid. By hierdie gemerkte

trosse is geen trosvoorbereidingsaksies, asook geen korrelversameling gedoen nie. Hierdie trosse is geëvalueer vir trosstruktuur/kompaktheid by oes. Hierdie metode is gebruik om die mees optimale blomstadium vir toedien van uitdunningsbehandelings by Prime te bepaal, op grond van trosvoorkoms by oes.

Die bot% wat bepaal is in Junie 2015 en Junie 2016 deur middel van uitbotproewe, asook in die wingerd (November 2015), het nie betekenisvol verskil tussen behandelings nie en was hoër as 80% vir alle behandelings. In beide seisoene is kommersiële aanvaarbare vlakke van bot verkry.

Die potensiële en werklike vrugbaarheid het afgeneem oor die twee seisoene. In Junie 2015 het Behandeling 1 'n betekenisvol hoër potensiële vrugbaarheid gehad en Behandeling 6 het 'n betekenisvol laer potensiële vrugbaarheid gehad in vergelyking met die ander behandelings. In Junie 2016, was daar geen betekenisvolle verskille tussen behandelings nie, hoewel Behandeling 6 die laagste potensiële vrugbaarheid getoon het. Dit wil voorkom asof Behandelings 3 en 6 met 'n laer toedieningsvolume en kleiner druppelgrootte met 'n laer vrugbaarheid geassosieer word, moontlik as gevolg van meer effektiewe bedekking wat verkry word op trosse (die teikenorgaan vir korrelvergrotingbehandelings), maar ook op die lote en ogies en dat GA₃ wat toegedien is op die ogies 'n negatiewe uitwerking op potensiële vrugbaarheid het. Dit is ook weerspieël deur die werklike vrugbaarheid en opbrengs wat gekry is in November 2015, waar met Behandeling 3 en 6 in vergelyking met Behandeling 2 die laagste opbrengs verkry het (slegs betekenisvol vir Behandeling 3).

Met betrekking tot hand-uitdunning in beide seisoene, het Behandeling 1 die langste tyd per ha vereis en Behandelings 2 en 5 betekenisvol die minste tyd, wat toegeskryf kan word aan die groter korrelgrootte en hoër % normale korrels wat verkry is met Behandeling 2. Daar was geen betekenisvolle verskille tussen die verskillende spuittoedieningsmetodes (volumes) nie. Daarom sal die "beste" metode vir toediening bepaal word op grond van die invloed op vrugbaarheid.

In beide seisoene, het Behandeling 1 die meeste ure vir handuitdunning vereis en gevolglik ook die hoogste koste gehad. Dit bevestig die behoefte aan chemiese uitdunning van Prime, om arbeidskoste te bespaar. Tyd en koste van hand-uitdunning van Prime met toepassing van Behandeling 2, kan tot 40% laer wees as met Behandeling 1. Tyd benodig en koste vir toepassing van Behandeling 2 en 5 het gevarieer tussen 942 en 2578 uur en R12 595 en R31 992, wat in lyn is met die tyd en koste wat benodig word vir kommersiële Prime blokke.

Die korrelsapsamestelling is nie negatief beïnvloed deur enige van die behandelings nie. Die verwagte korrelontwikkeling- en rypwordingspatrone is gevind. Alhoewel enkele betekenisvolle verskille voorgekom het met betrekking tot na-oes gehalte, het dit nie 'n praktiese impak op bemarkbaarheid van die druiwe gehad nie.

Met betrekking tot trosstruktuur in die 2014/2015 seisoen, was daar min betekenisvolle verskille tussen behandelings. Vir die uitdunningbehandeling toegedien by 80-100% VB, was

die aantal korrels per cm laterale lengte, asook die aantal normale korrels per cm laterale lengte van Behandeling 1 betekenisvol hoër in vergelyking met Behandeling 2, wat aandui dat die trosse van Behandeling 1 meer kompak was as trosse van Behandeling 2. Die 80-100% VB Behandeling 1 het 'n betekenisvol hoër aantal klein korrels per cm laterale lengte tot gevolg gehad in vergelyking met die ander twee behandelings, wat gekoppel kan word aan die langer tyd wat benodig was vir hand-uitdunning van hierdie behandeling. In beide seisoene is waargeneem dat minder korrels per cm laterale lengte (minder kompakte trosse) verkry is met die uitdunbehandeling toegedien by 80-100% VB, in vergelyking met die later toedienings.

Die resultate van hierdie studie dra by tot beskikbare wetenskaplike gepubliseerde resultate oor die effek van GA₃-toedieningsmetodes (volumes) op vrugbaarheid van tafeldruiwe. Op grond van die resultate na afloop van die eerste twee seisoene se behandelings, kan die volgende aanbevelings gemaak word met betrekking tot identifisering van GA₃-toedieningsmetodes en volumes vir effektiewe uitdunningbehandelings en vergrotings- van tafeldruiwe sonder 'n negatiewe invloed op vrugbaarheid:

- Met Behandeling 2 (Chemiese uitdunning met 'n standaard GA₃-toediening, gevolg met 'n GA₃-doopbehandeling vir korrelvergroting) is die grootste korrels, minder kompakte trosse en hoogste persentasie normale korrels verkry. Hierdie behandeling vereis ook die minste tyd vir hand-uitdunning.
- Praktiese toepassing van Behandeling 2 (Chemiese uitdunning met 'n standaard GA₃-toediening, gevolg deur 'n GA₃-doopbehandeling vir korrelvergroting) in kommersiële tafeldruiweproduksie vereis beskikbaarheid van voldoende arbeid. Dit word reeds toegepas deur verskeie produsente in die bedryf, in situasies waar daar reeds praktiese ervaring van afname in vrugbaarheid na GA₃-toedienings bestaan.
- Huidige beskikbare resultate dui daarop dat die laer toedieningsvolumes, naamlik Behandeling 3 (250 L/ha) en Behandeling 6 (ESS 72 L/ha) verband hou met 'n afname in vrugbaarheid, terwyl by Behandeling 4 (500 L/ha) geen aanduiding van 'n negatiewe uitwerking op vrugbaarheid gevind is nie. Daarom is herhaling van die studie nodig om hierdie resultate te verifieer en vas te stel of die 500 L/ha spuittoedieningsvolume gebruik kan word in plaas van die huidige standaardbedryfspraktyk van 1000 L/ha vir die meerderheid van PGRs en ander toedienings. Die toedieningsvolume van 500 L/ha in plaas van 1000 L/ha het verskeie praktiese en ekonomiese voordele, naamlik meer hektaar kan gespuit word met net een tenkmengsel, verlaagde watervoetspoor, asook verlaagde koolstofvoetspoor.
- Dit word aanbeveel dat die proef vir minstens nog een seisoen herhaal moet word, om resultate te bevestig en herhaalbaarheid te toets.

This thesis is dedicated to my family, especially to my mother, Riana, my father, Rowan and my sister, Anesmé for their support and encouragement. For never letting me lose focus and keeping me moving forward.

BIOGRAPHICAL SKETCH

Larissa van der Vyver was born in Volksrust on 30 November 1991. She matriculated at Strand High School in 2009. Larissa enrolled at Stellenbosch University in 2010 and obtained the BScAgric-degree in Viticulture and Oenology in December 2013. She then enrolled for the MScAgric-degree in Viticulture degree in 2014 at Stellenbosch University.

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PREFACE

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

- Chapter 1 General introduction and project aims**
- Chapter 2 Literature review**
- Chapter 3 Methods and materials**
- Chapter 4 Results and discussion**
- Chapter 5 General conclusion and recommendations**

TABLE OF CONTENTS

Chapter 1. General introduction and project objectives	1
1.1 Introduction	2
1.2 Project aims and objectives	5
1.2.1 Project aims	5
1.2.2 Objectives	5
1.3 Literature cited	5
Chapter 2. Literature review	7
2.1 Introduction	8
2.2 Overview of grapevine phenology	9
2.2.1 Bud morphology	9
2.2.2 Vegetative cycle	10
2.2.2.1 Shoot growth	10
2.2.2.2 Root growth	11
2.2.3 Reproductive cycle	11
2.2.3.1 Flower development	13
2.2.3.2 Flowering	15
2.2.3.3 Set	16
2.2.3.4 Berry development	17
2.2.3.5 Seed development	20
2.2.4 Grapevine fertility	20
2.2.4.1 Factors affecting grapevine fertility	21
2.2.4.2 Methods for assessing grapevine fertility	27
2.3 Overview of plant growth regulator use in table grape production	28
2.3.1 Gibberellin	30
2.3.1.1 GA ₃ mechanism / Role in plant	32
2.3.1.2 Cluster elongation, stem elongation and compactness	33
2.3.1.3 Berry thinning	33
2.3.1.4 Berry sizing	34
2.3.1.5 Fruit quality and post-harvest quality after storage	36
2.3.1.6 Leaf area development	36
2.3.1.7 GA ₃ and CPPU	37
2.4 Comparing different plant growth regulator application methods for thinning and/or berry sizing in table grape production	37
2.4.1 Spray application	37
2.4.1.1 Coverage, water volumes	37
2.4.1.2 Conventional	38
2.4.1.3 Ultra-low volume or Electrostatic spray	38
2.4.2 Dipping of bunches	40
2.4.3 Recommendations for best practices to follow based on current knowledge	40
2.5 The effect of plant growth regulator treatments for thinning and berry sizing on table grape return fertility	41
2.5.1 Gibberellic acid (GA ₃)	41
2.5.2 CPPU (Forchlorfenuron)	42
2.5.3 Ethephon	43
2.6 Conclusions	43
2.7 Literature cited	44

Chapter 3. Materials and Methods	52
3.1 Experimental vineyard	53
3.1.1 Weather data	53
3.1.2 Phenology	55
3.2 Experimental layout	56
3.3 Statistical procedures	57
3.4 Treatments	57
3.4.1 Marking of bunches according to phenological stage	60
3.4.2 Application of fluorescent product to assess spray coverage	61
3.5 Manual thinning: timing experiment	61
3.6 Pre-harvest evaluation	62
3.6.1 Field sampling	62
3.6.2 Berry development and ripening	62
3.6.3 Organic acid	62
3.6.4 Bunch structure	63
3.7 Harvest evaluation	63
3.7.1 Yield and Pack out	63
3.7.2 Bunch structure	64
3.8 Pruning measurements	66
3.9 Post-harvest evaluation	66
3.10 Bud fertility of the experimental block	67
3.10.1 Potential fertility	67
3.10.1.1 Plant material	67
3.10.1.2 Forced budding in glasshouse	67
3.10.1.3 Bud dissection	68
3.10.2 Actual Fertility	69
3.11 Literature cited	69
Chapter 4. Results and discussion	71
4.1 Introduction	72
4.2 Fertility	72
4.2.1 Potential and actual fertility before the trial commenced	72
4.2.1.1 Potential fertility (Bud dissection) and actual fertility	72
4.2.2 Potential and actual fertility after treatments were applied	73
4.2.2.1 Potential fertility (Forced budding)	74
4.2.2.2 Potential fertility (Bud dissection)	76
4.2.2.3 Actual fertility	76
4.3 Manual thinning (timing)	77
4.4 Berry development and ripening	79
4.4.1 Berry diameter and length	79
4.4.2 Mass	81
4.4.3 Total Soluble Solids	81
4.4.4 Titratable acids	82
4.4.5 Malic acid	83
4.5 Yield	84
4.6 Berry size classification of marked bunches	86
4.6.1 2014/2015 season	86
4.6.2 2015/2016 season	87
4.7 Bunch structure	89
4.7.1 2014/2015 season	89
4.7.2 2015/2016 season	89
4.8 Bunch compactness	90

4.8.1	2014/2015 season	90
4.8.2	2015/2016 season	90
4.9	Post-harvest quality	93
4.10	Summary	96
4.11	Literature cited	97

Chapter 5. General conclusion and recommendations	101
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ADDENDUM A	104
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ADDENDUM B	105
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ADDENDUM C	106
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Chapter 1

GENERAL INTRODUCTION AND PROJECT OBJECTIVES

CHAPTER 1: GENERAL INTRODUCTION AND PROJECT OBJECTIVES

1.1 Introduction

Table grapes are one of the major fruit industries in South Africa, with 18 212 hectares under production (SATI, 2015). During the 2014/2015 South African table grape season the largest harvest ever was recorded with 58,5 million 4.5 kg cartons exported (SATI, 2015). There is a continued increase in the demand for seedless cultivars (SATI, 2015) due to the ease of eating, contributing to higher prices and a greater return on investment for the producer (Casanova *et al.*, 2009; Özer *et al.*, 2012).

In South Africa, Prime is the second largest exported cultivar, comprising a total of 1560 hectares, with 94% of these vineyards between 0-15 years old. Prime is a white seedless cultivar that was developed at the Volcani Institute in Israel, who holds the patent right. In South Africa, Hoekstra Farms holds the patent right for Prime.

This study was conducted on Prime, because it is one of South Africa's major table grape cultivars and there are limited scientific publications reporting research results on this cultivar. Prime is a very early ripening cultivar, has an amber colour, a Muscat flavour, crisp taste and excellent shelf life (Perl *et al.*, 2000, 2003).

In its natural state, Prime produces small round berries (Van Der Merwe, 2014; SATI, 2015) and well filled bunches without being too compact (Raath, 2012). When prepared for export, large size berries for an early seedless are obtained (minimum berry diameter of 18 mm, average mass of 7-8 g) (Perl *et al.*, 2003). Prime is prone to millerandage and consequently shot berries which need to be removed manually or by chemical thinning treatments (Van Der Merwe, 2014; SATI, 2015).

For production of export grapes it is recommended to treat Prime with 1-2 ppm GA₃ at bloom, to decrease the number of berries and after 100% set with 20 ppm GA₃ to increase berry size (Van Der Merwe, 2014). It has been reported that if these dosages are exceeded, a decline of fertility is observed (Van Der Merwe, 2014; SATI, 2015).

Prime has a poor to average vigour (Van Der Merwe, 2014). Prime is very fertile and can be spur pruned or with half long bearers, depending on the growing area (Van Der Merwe, 2014). In the Berg River Valley, Prime is predominantly pruned with half long bearers due to the fertility which is observed to be positioned between bud position no. 3 to no. 9.

The trial was conducted in the Berg River Valley, which is South Africa's third largest table grape production region, comprising a total of 4053 hectares (22% of the total planted hectares under table grapes) (SATI, 2015). Prime is harvested between week 48 and week 2 in the Berg River Valley.

The South African table grape industry is a very labour intensive industry, but also realises a high net income for producers (Elgendy *et al.*, 2012). With increased costs in terms of establishment, production and labour, the industry needs to strive for sustainable and economically viable table grape production. With the increasing pressure to meet market requirements nationally and

internationally, higher input costs are invested to meet these requirements. This leads to the critical focus on labour-intensive cultivation practices and efforts to identify alternative less labour-intensive practices to decrease input costs, while maintaining high levels of fertility, production and quality.

To meet market requirements regarding table grape bunch size and compactness, as well as berry size, colour, flavour, texture and firmness (Gil *et al.*, 1994; Özer *et al.*, 2012; Raban *et al.*, 2013), viticultural practices include the use of plant growth regulators (PGRs) (Roller, 2003). PGRs are a population of endogenous molecules and synthetic compounds with similar structures to the natural occurring plant hormones which play an important role in regulating growth and development of plants (Roberts & Hooley, 1988; Korkutal *et al.*, 2008).

The five main PGR groups are gibberellins (GA), cytokinins (CK), auxins, abscisic acid (ABA) and ethylene (Roberts & Hooley, 1988; Korkutal *et al.*, 2008; Durner, 2013). An overview of the major PGRs used in table grape production is given in section 2.3 in Chapter 2.

Gibberellin is a group of naturally occurring plant hormones involved in various aspects and functions of growth and development in the plant/grape (Roller, 2003; Durner, 2013). Functions of GA₃ in the plant/grape are to stimulate stem elongation, affect floral sex expression, stimulate seed germination, inhibit leaf senescence and inhibit root growth (Durner, 2013).

In the table grape industry GA₃ is one of the most used PGR's to thin out clusters, decrease berry set (Singh *et al.*, 1978; Özer *et al.*, 2012) and increase berry size, as a result of the stimulation of cell division and cell elongation (Dokoozlian, 2000), especially in seedless table grape cultivars that naturally set compact bunches with small berries. GA₃ also has a "stretching" effect on bunches, which contributes to decreasing compactness of tight clusters (Roller, 2003) and reduce bunch rot (Hed *et al.*, 2011).

The use of GA₃ on seeded cultivars is limited due to the seeds being a natural or endogenous source of GA₃ (Dokoozlian, 2000). To obtain the berry size required by markets, seedless cultivars are treated with GA₃, due to the lack of seeds as natural sources of GA₃ (Dokoozlian, 2000).

In table grape production, there are three main objectives with GA₃ treatments, each requiring application at a specific phenological stage for the desired outcome, namely in the case of Prime: treatment for a stretching effect will be applied when inflorescence length is at 8 cm, treatment for a thinning effect will be applied at 10% set and treatments for a berry sizing effect will be applied at 7-8 mm berry diameter (Van Der Merwe, 2014).

Berry thinning can be achieved by spraying vineyards with PGRs and/or applying manually thinning of shot berries after set, which makes it a time-consuming practice (Orth, 1990a; Gil *et al.*, 1994). Table 1 indicates time spent and cost of manual thinning for three commercial scenarios, where GA₃ thinning and sizing applications were applied according to standard practices and costs were calculated based on the minimum labour cost per hour according to the Department of Labour Agriculture South Africa.

With regard to manual thinning, Prime is a more labour intensive cultivar than Crimson Seedless, which is reflected by the time required and cost of manual thinning (up to 178% higher for Prime

compared to Crimson Seedless). Differences between Prime blocks regarding time and cost of manual thinning can be linked to the occurrence of millerandage – if more shot berries are set, more time is spent to remove them.

Table 1 Time spent and cost of manual thinning for three commercial table grape block (Mouton, B., 2016, personal communication & Myburgh, B., 2016, personal communication)

Scenario	Bunch mass (g)	Hours/ ha	Hourly rate	R/ha	R/bunch
Scenario 1, commercial Prime, Berg River Valley	500-700	1755	R11.65	R20 426	R0.38
Scenario 2, commercial Prime, Orange River	600-625	945	R14.19	R13 411	R0.30
Scenario 3, commercial Crimson Seedless, Berg River Valley	500-800	630	R11.65	R7346	R0.12

GA₃ is applied for berry sizing of cultivars where the natural berry size does not meet the requirements for commercial table grapes (Abu-Zahra & Salameh, 2012) and to improve the quality (Wolf & Loubser, 1994). The three traditional ways to enlarge berries are through crop control, girdling and use of GA₃ applications (Orth, 1990b). GA₃ applications are usually done either by spraying the whole vine or by dipping young bunches in a GA₃ solution (Orth, 1990b; Abu-Zahra & Salameh, 2012), with the latter technique being very labour intensive. The manual dipping technique is specifically used because it is believed the decreased fertility associated with GA₃ spray applications can be prevented/limited. There are several reports from the industry that decreased bud fertility is linked to GA₃ treatment, but very few published research results are available to support these practical observations.

Some negative consequences of GA₃ treatments that have been mentioned by producers and reported by researchers are that GA₃ application delays maturity, increases cluster rigidity and berry shatter (Guelfat-Reich & Safran, 1973; Retamales & Cooper, 1993; Han & Lee, 2004; Raban *et al.*, 2013) and also reduces bud fertility in the following season (Orth, 1990b; Dokoozlian, 2000). It has been reported for Prime that if the recommended dosages are exceeded (1-2 ppm GA₃ for thinning and 20 ppm GA₃ for berry sizing), a decline in fertility was observed (Van Der Merwe, 2014; SATI, 2015).

The rationale behind this study was to determine whether the producer can apply the same active ingredient (GA₃) dosage per hectare using lower application volumes than the current standard industry practice, without negatively affecting fertility, production and quality.

1.2 Project aims and objectives

1.2.1 Project aims

The purpose of the study was to determine the influence of gibberellic acid (GA₃) for berry thinning and berry sizing on table grape production, quality and return fertility of Prime.

Main aim: Establish the effect of different GA₃ application methods and volumes for berry thinning and berry sizing treatments of table grapes without negatively affecting fertility.

Sub aim: Reduce labour inputs and production costs

1.2.2 Objectives

- Objective 1 - Identify GA₃ application methods and volumes for effective thinning and sizing treatments of table grapes without negatively affecting fertility
- Objective 2 - Limit manual bunch preparation to a minimum, to reduce labour inputs and production costs

The significance of this study for the table grape industry was to:

- Obtain scientific results regarding the effect of GA₃ application methods (volumes) on fertility of table grapes
- Establish and identify GA₃ application methods and volumes for effective thinning and sizing treatments of table grapes without negatively affecting fertility
- Contribute to reducing labour inputs and production costs

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Chapter 2

LITERATURE REVIEW

**Return fertility of table grapes as affected by
GA₃ application method (volume) for thinning
and sizing treatments**

CHAPTER 2: LITERATURE REVIEW: Return fertility of table grapes as affected by GA₃ application method (volume) for thinning and sizing treatments

2.1 Introduction

Table grapes are one of the major commercially grown non-climacteric fresh fruit worldwide with production still increasing (Coombe & Hale, 1973). Increasing competition on markets is putting pressure on table grape growers to produce quality grapes that meet market requirements.

Consumer satisfaction requirements regarding grape appearance and eating quality include berry and cluster size, shape, colour, compactness, packaging, sugar and organic acid balance, as well as lack of defects like decay, stem browning and berry softness (Mullins *et al.*, 1992; Roller, 2003; Muñoz-Robredo & Robledo, 2011; Rizzuti *et al.*, 2015; Sonnekus, 2015).

The consumer's decision to purchase table grapes is influenced by the factors mentioned above, but also by whether it is seeded or seedless (Orth, 1990b). Over the years a preference shift for fresh consumption was made from seeded berries to seedless berries (Perl *et al.*, 2000), with more than 80% of table grapes being sold on fresh markets at present being seedless (SATI, 2015).

Table grapes must also have a good shelf life, without developing any post-harvest defects. Post-harvest quality of table grapes can be negatively influenced by physical, physiological or pathological factors that may have occurred in the vineyard (pre-harvest) or after harvest (in the pack house or during cold storage) (Zoffoli *et al.*, 2009; Özer *et al.*, 2012).

Berry size is a very important quality factor influencing sales of table grapes (Abu-Zahra & Salameh, 2012). Therefore in the international market the best prices for table grapes are obtained with large berries (Zoffoli *et al.*, 2009).

Berry size and all the other size/compactness requirements can be obtained through application of viticultural practices such as adjusting the crop load (Dokoozlian & Hirschfeld, 1985), by applying manual bunch thinning, trunk girdling and using plant growth regulators (PGRs) (Orth, 1990b; Zoffoli *et al.*, 2009; Abu-Zahra & Salameh, 2012).

A 'plant hormone' is defined as (Durner, 2013): A naturally occurring organic substance produced by the plant, which at very low concentrations controls plant growth and development through effects on cell division, elongation and differentiation in the tissue of synthesis or elsewhere in the organism.

The five main PGR groups are gibberellins (GA), cytokinins (CK), auxins, abscisic acid (ABA) and ethylene. In section 2.3 in Chapter 2 an overview of PGRs used in table grape production is given.

Gibberellic acid (GA₃) is applied to certain seedless cultivars to improve quality and yield, but the application method is cultivar dependant (as explained in Chapter 1) and incorrect applications may lead to serious damage to the vine and bunches (Dass & Ranhawa, 1967; Orth, 1990b). It has been used since the 1960's to increase the size in seedless grapes (Mullins *et al.*, 1992).

To produce quality grapes, cost-effective management practices need to be identified, without causing negative effects. Therefore, in the following sections factors that influence fertility, as well as viticultural practices that are needed to achieve the market requirements, are discussed.

2.2 Overview of grapevine phenology

Guidelines regarding the timing of viticultural practices or chemical treatments are often linked to grapevine phenological stages. Therefore, the phenology (study of events or growth stages of plants) of the grapevine needs to be understood. By understanding the implications of each action, according to a phenological stage, improved decision making can take place and an optimal outcome could be reached in terms of fertility, yield and quality (Mullins *et al.*, 1992).

In this section an overview of grapevine bud morphology and phenological stages will be presented. A detailed description of each phenological stage was developed by Lorenz and modified by Eichhorn and Lorenz (cited by May, 2000; Bennett, 2002). In Fig. 2 the phenological cycle of Prime in the Berg River Valley is presented.

2.2.1 Bud morphology

The grapevine has the capacity to form buds that arise in the leaf axils all along the length of the current season's shoot. These buds are called the "prompt buds" which may burst in the current season and form lateral shoots (Winkler *et al.*, 1962; Khanduja & Balasubrahmanyam, 1972; Pongracz, 1978; Srinivasan & Mullins, 1981b; Mullins, 1986; May, 2000; Williams, 2000; Iland *et al.*, 2011).

The first leaf of the lateral shoot is reduced to a bract and the bud which develops in the axil of the bract may remain dormant or undeveloped for a season or longer and is called the latent bud (Winkler *et al.*, 1962; Srinivasan & Mullins, 1981b; Mullins, 1986), which can also be referred to as the compound bud ("eye") (Winkler & Shemsettin, 1937; Lavee *et al.*, 1967; Khanduja & Balasubrahmanyam, 1972; Srinivasan & Mullins, 1981b; Mullins, 1986; Morrison, 1991; Williams, 2000; Bennett, 2002) (Figure 1). The compound bud will remain in a dormant state through winter and will resume its growth in the following spring (Khanduja & Balasubrahmanyam, 1972; Morrison, 1991).

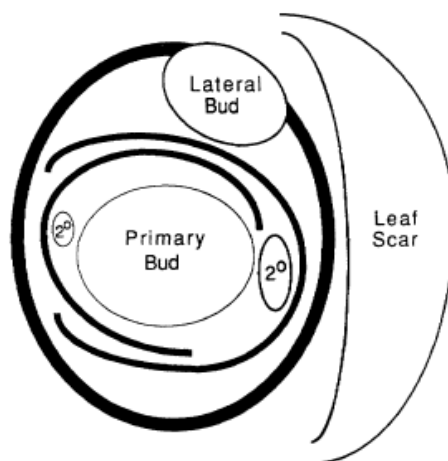


Figure 1 Schematic diagram of the compound bud of *Vitis vinifera* with the primary bud and two secondary buds (Morrison, 1991)

The compound bud contains three buds of unequal development stages (Vasconcelos *et al.*, 2009). One bud, namely the primary bud, is larger and more advanced than the other two buds according to their development, namely the secondary and tertiary buds (Snyder, 1933; Khanduja & Balasubrahmanyam, 1972; Morrison, 1991; Bennett, 2002; Vasconcelos *et al.*, 2009; Iland *et al.*, 2011). Primary, secondary and tertiary buds, may contain leaf and inflorescence primordia (primordia are "precursors"). A fertile bud contains inflorescence primordia (Winkler & Shemsettin, 1937; Lavee *et al.*, 1967; Khanduja & Balasubrahmanyam, 1972; Srinivasan & Mullins, 1981b; Mullins, 1986; Morrison, 1991; Williams, 2000; Bennett, 2002). The position of fertile buds on the cane differs between cultivars and this determines the pruning method to be used.

2.2.2 Vegetative cycle

2.2.2.1 Shoot growth

The seasonal shoot growth cycle of the grapevine has been described by several authors, including Winkler *et al.* (1962), Pongracz (1978), Mullins *et al.* (1992), Iland *et al.* (2011) and Bennett (2002).

Bud break will only occur when the daily mean maximum temperatures during spring are above a base temperature of 10°C (Winkler *et al.*, 1962; Pongracz, 1978; Bennett, 2002). Bud break is marked when the first green tip leaf tissue are visible, E-L 4 to 7 according to the modified E-L system of grapevine growth stages (Bennett, 2002). According to May (2000) the timing of bud break has a major influence on the course of the subsequent vegetative growth and reproductive development.

The first signs of shoot growth is when the first leaf separates from the shoot tip and gives rise to the following leaves that needs to separate (Bennett, 2002). As shoot growth

continues, from a compound bud, the first inflorescence can be seen clearly when five leaves have separated and the shoot is about 10cm long (Bennett, 2002).

Between E-L 15 and 18 the shoot starts to elongate, inflorescences are more well developed and will appear usually from node four onwards opposite a leaf (Bennett, 2002). Shoot development is rapid during the first eighth to ten weeks after bud break, followed by a period where a constant growth rate is maintained, while after flowering a decrease in growth rate is experienced until growth ceases (Winkler *et al.*, 1962; Bennett, 2002). The shoots will start to change in colour during stage III of berry development, from green to yellow and then to brown as they become lignified (Pongracz, 1978; Bennett, 2002).

It is important that the leaves stay active after harvest to build up carbohydrate reserves in storage tissue for growth and fruit cluster development the following spring (Winkler *et al.*, 1962). Depending on the climate, the leaves remain green for several weeks after harvest before changing into their autumnal colours (Pongracz, 1978; Bennett, 2002). When the grapevines have completed leaf fall the vines will enter endodormancy (Pongracz, 1978; Bennett, 2002).

2.2.2.2 Root growth

The roots are the main storage organs of the vine where nutrient reserves are stored during the post-harvest period, to be used in early spring of the next season, when initial growth occurs (Archer, 1981). Shortly after shoot growth commences in spring, a flush of root growth is experienced, which will reach a peak at flowering, followed by a second flush of root growth after the fruit has been harvested (Mullins *et al.*, 1992). These flushes consist of new root production arising from the permanent root system and is important in relation to the uptake of water and mineral nutrient (Mullins *et al.*, 1992) and production of hormones such as cytokinins.

2.2.3 **Reproductive cycle**

During the reproductive cycle each compound bud has the potential to become a flower bud (containing inflorescence primordia, have rudimentary leaves and flower clusters) or a leaf bud (producing a sterile shoot, that bears only leaves and tendrils) (Winkler *et al.*, 1962; Khanduja & Balasubrahmanyam, 1972). A single compound bud may contain more than one inflorescence primordia if all the latent buds in the compound bud contain inflorescence primordia (Bennett, 2002; Iland *et al.*, 2011).

The potential yield of the next season is already determined during the current season (Dunn & Martin, 2000; Williams, 2000). Inflorescence primordia for the next season's crop are formed in the axils of leaf primordia of the primary latent buds during late spring and summer (Dunn & Martin, 2000) of the current season, at about the same time that inflorescences on the shoots are flowering (Snyder, 1933; Winkler & Shemsettin, 1937; Morrison, 1991; Iland *et al.*, 2011). It takes about 15 months from inflorescence primordia

initiation in the spring of season 1 (Figure 2a) until harvesting of the bunch in season 2 (Figure 2b) (Bennett, 2002; Iland *et al.*, 2011), but this duration may vary between cultivars and regions.

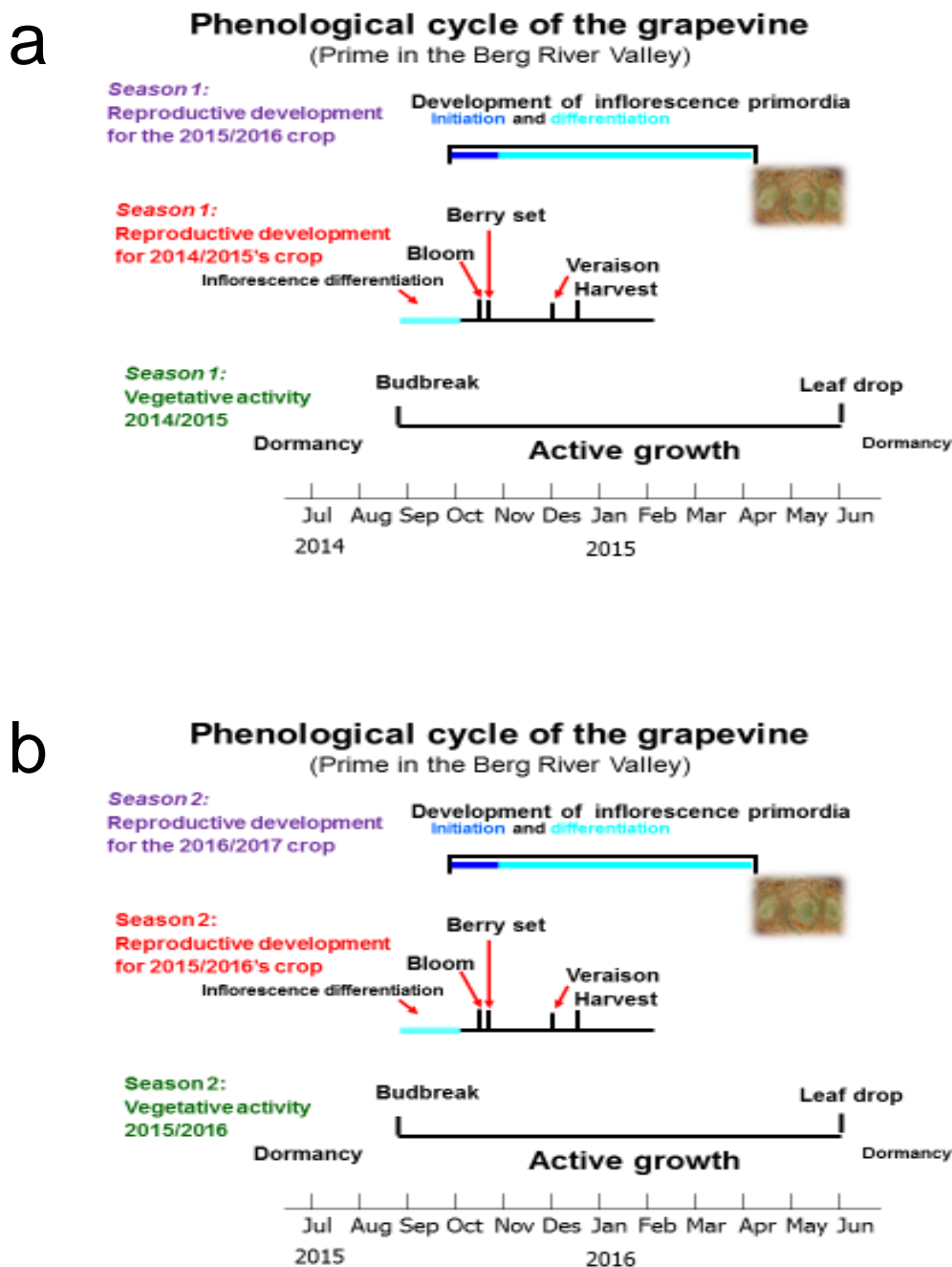


Figure 2 Phenological cycle of Prime in the Berg River Valley; a. Season 1, b. Season 2 (adapted for the cultivar and site from Vasconcelos *et al.* (2009))

2.2.3.1 Flower development

Induction

The process of inflorescence primordia development will commence with the induction phase where a physiological stimulus is received, resulting in the initiation phase, where morphological consequences of this stimulus will lead ultimately to flowering (Mullins *et al.*, 1992; May, 2000; Iland *et al.*, 2011).

Induction occurs sometime before initiation, up to for 18 days for Sultana and 20 days for Muscat of Alexandria (May, 2000; Iland *et al.*, 2011). It is suggested by Lavee *et al.* (1967) that the induction impulse originates from the leaves located at and above each bud.

The formation of the inflorescence primordia in the grapevine bud are divided into three phases. The first two phases are already completed during the current season, while the third and final phase starts during the current season, but is only completed shortly before and during bud break in the next season (Srinivasan & Mullins, 1981b; Swanepoel & Archer, 1988; Mullins *et al.*, 1992; Bennett, 2002; Watt *et al.*, 2008). These three phases can be summarised as follows:

The first phase is the *formation of the anlagen* or uncommitted primordia (*initiation*). The anlagen or uncommitted primordia are club-shaped meristematic protuberances by the apices of the primary buds on shoots of the current season (Srinivasan & Mullins, 1981b; Mullins *et al.*, 1992; Bennett, 2002; Iland *et al.*, 2011).

The second phase is the *formation of inflorescence primordia (differentiation)*. Differentiation is the process whereby unspecialised cells change into specialised cells, tissues and organs (Srinivasan & Mullins, 1981a). Anlagen can either develop into inflorescence primordia, tendril primordia or even shoot primordia (Srinivasan & Mullins, 1981b; Mullins *et al.*, 1992; Iland *et al.*, 2011). The directed anlagen will experience repeated branching to form a conical structure and shortly thereafter the latent buds will enter dormancy (Srinivasan & Mullins, 1981b; Mullins *et al.*, 1992; Bennett, 2002).

The third and final phase is the *formation of the flowers*, where inflorescence primordia undergo differentiation resulting in individual flowers, from around the time of bud break of the following season (Srinivasan & Mullins, 1981b; Bennett, 2002; Iland *et al.*, 2011).

Initiation

The initiation phase can be described as where a difference in time and development is found, between the induction and differentiation phases (Lavee *et al.*, 1967; Sommer *et al.*, 2000).

With the onset of induction, between 11 and 22 unfolded leaves, depending on the cultivar, are found on the main shoot (Vasconcelos *et al.*, 2009). According to Swanepoel & Archer (1988) initiation of the first anlage, in the cultivar Chenin blanc, commenced 12-15 days

before flowering and was completed after seven days, when 12 expanded leaves were on the main shoot.

Anlagen formation

The time of initiation and the rate formation of anlagen (uncommitted primordia) by the apices of latent buds (Mullins, 1986; Watt *et al.*, 2008), dependent on the position of the winter bud on the cane and the cultivar, and that will determine whether leaves or inflorescences are produced, depending on the vine's development stage and environmental conditions during primordia formation (Snyder, 1933; Pratt, 1971; Morrison, 1991; Williams, 2000; Vasconcelos *et al.*, 2009).

When the first anlagen appears to separate from the apex, the latent bud is no longer considered to be in the vegetative growth phase but in the reproductive growth phase and is used as indicator that the inflorescence axis has begun to form (Srinivasan & Mullins, 1981b; Swanepoel & Archer, 1988; Mullins *et al.*, 1992; Williams, 2000).

Conditions during separation will determine whether the anlagen may become inflorescence or tendril primordia, since they are homologous organs (Pratt, 1971; Srinivasan & Mullins, 1979; Mullins, 1986; Swanepoel & Archer, 1988; Morrison, 1991; Vasconcelos *et al.*, 2009). Where rapid shoot growth is found, the uncommitted primordia will develop into a tendril (Vasconcelos *et al.*, 2009).

During anlagen formation, a clear difference is visible between anlagen, which are broad, blunt, obovate structures and lacking stipular scales and the leaf primordia, which are narrow pointed structures with stipular scales (Mullins *et al.*, 1992). The continued development of each anlage starts with its division into two unequal parts, the larger *inner arm* and the smaller *outer arm*. The inner arm will give rise to the main body (*rachis*) of the cluster, while the outer arm will give rise to either a wing or a large branch at the top of the cluster (Pratt, 1971; Mullins, 1986; Mullins *et al.*, 1992; Vasconcelos *et al.*, 2009; Iland *et al.*, 2011).

Formation of inflorescence primordia

Differentiation is a critical phase where the formation of inflorescence and leaf primordia for the following season are concurrent to each other, determining the fertility of the compound bud (Williams, 2000). Differentiation will commence in the basal buds of the shoot and will continue in an apical direction (Sommer *et al.*, 2000). The differentiation phase is influenced by various environmental factors which influence growth and development in the grapevine (Khanduja & Balasubrahmanyam, 1972).

During the differentiation phase the anlagen will undergo extensive branching (Srinivasan & Mullins, 1981b; Swanepoel & Archer, 1988; Mullins *et al.*, 1992; Williams, 2000; Iland *et al.*, 2011). Twenty-one days after formation of the first anlagen, the inflorescence primordia are formed. The inflorescence primordium has a conical shape, appears like a small bunch of grapes in which each berry-like branch primordium consists of undifferentiated meristematic

tissue. This phase will be completed four days after the appearance of the fully developed inflorescence primordia (Srinivasan & Mullins, 1981b; Swanepoel & Archer, 1988; Mullins *et al.*, 1992; Williams, 2000; Iland *et al.*, 2011). During the last few days of the differentiation of the first anlagen the initiation of the second anlagen will commence (Swanepoel & Archer, 1988).

There is no morphological signs to distinguish between a differentiated and a non-differentiated bud, distinct morphological signs will only be visible during differentiation in the following season (Winkler & Shemsettin, 1937; Lavee *et al.*, 1967; Swanepoel & Archer, 1988; Iland *et al.*, 2011).

Inflorescence primordia will increase in size during the early season, where after it will slow down and no further differentiation of the anlagen will occur from about eight to ten weeks after flowering in the current season. The latent bud will then enter dormancy (Winkler & Shemsettin, 1937; Srinivasan & Mullins, 1981b; Mullins *et al.*, 1992; Sommer *et al.*, 2000; review by Vasconcelos *et al.*, 2009; Iland *et al.*, 2011). A clear distinction can be made from the fully matured latent buds whether the buds are fruitful (containing inflorescence primordia) or not (containing tendril primordia) (Iland *et al.*, 2011). Individual flower parts will only differentiate after recommencement of growth in the next spring (review by Vasconcelos *et al.*, 2009).

Formation of flowers

The final phase of flower differentiation continues from before the buds open in spring and for a short time thereafter in the dormant latent buds or newly formed buds when activated in spring on the developing shoot (Winkler & Shemsettin, 1937; Lavee *et al.*, 1967; Agaoglu, 1971; 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). The essential organs of the flower are already formed within 10-15 days of the appearance of the inflorescence after bud break (Agaoglu, 1971; Swanepoel & Archer, 1988; Bennett, 2002).

2.2.3.2 Flowering

As the final phase of individual flower development comes to an end, flowering will start (Iland *et al.*, 2011). The onset of flowering, E-L 19 to 25, is where the calyptra will separate or fall from the flower base exposing the stamens and pistils (Winkler *et al.*, 1962; Pongracz, 1978; Dokoozlian, 2000a; Bennett, 2002; Iland *et al.*, 2011). The petals will separate themselves at their base and be lift off as a cap (Iland *et al.*, 2011), hence the term “capfall”. When 50% of all the caps are off, E-L 23, it is known as full flowering and cap-fall is completed in E-L 26 (Bennett, 2002).

Cultivar and climate conditions will determine when flowering will occur, the acceptable period is between six to eight weeks after shoot growth commenced and flowering will be enhanced when temperatures range between 29°C and 35°C, making this period a very

critical period in the annual growth of vine (Winkler *et al.*, 1962; Pongracz, 1978; Dokoozlian, 2000a; Iland *et al.*, 2011).

Flowering marks the end point of a long and slow development of inflorescences of the current season and the beginning of initiation of the inflorescence primordia of the following season (Coombe & Dry, 1988; Bennett, 2002).

2.2.3.3 Set

After full flowering is achieved, pollination and fertilization will take place leading up to berry set. Coombe (1960) defined berry set as the changeover from a static condition of a flower ovary to the rapid growth conditions of a young fruit. For grapevines, when fruit set is successful, a single grape flower develops into a single berry (Iland *et al.*, 2011). Berry set marks the beginning of the fruit development period (Winkler *et al.*, 1962; Pongracz, 1978; Iland *et al.*, 2011). Successful fruit set is one of the major yield-determining events (Iland *et al.*, 2011).

Active cell division will contribute to rapid enlargement to transform ovaries into berries, even though little cell expansion is experienced (Mullins *et al.*, 1992; Dokoozlian, 2000a; Bennett, 2002; Bangerth, 2004; Iland *et al.*, 2011; Goussard, 2012). Berry set is strongly influenced by temperatures, therefore the duration may differ between grape cultivars and regions, but it is usually completed within two to three weeks after flowering (Pongracz, 1978; Bennett, 2002).

During set, three known plant growth regulators namely auxins, gibberellins and cytokinins are involved at the same time, promoting set and growth of the berry (Weaver *et al.*, 1962; Srinivasan & Mullins, 1981b; Mullins *et al.*, 1992; Bennett, 2002). The role of plant growth regulators is discussed in section 2.3. The completion of berry set is marked when all berries that did not set, drop off. This phenomenon is called berry shatter (Pongracz, 1978).

In grapevines, three types of set occur (May, 2004; Iland *et al.*, 2011), which are diagrammatically presented by May (2004):

- (i) *Normal set*, is where the normal sequence of pollination, fertilization and seed development takes place (Winkler *et al.*, 1962; Iland *et al.*, 2011). A direct correlation is reported by researchers (cited by Winkler *et al.*, 1962) between berry size and the number of seeds per berry due to the stimulus of pollination, fertilization and seed development (Winkler *et al.*, 1962; Iland *et al.*, 2011). This also has a direct effect on the level of auxin and gibberellin, which are important factors determining berry size (Bangerth, 2004). It has been found that the number of seed that is found in berries can vary up to four seeds per berry and also that not all the berries on a bunch of seeded cultivars have the maximum seed number (Dokoozlian, 2000a; Iland *et al.*, 2011).
- (ii) *Stimulative parthenocarpy*, is where only the stimulus of pollination, no fertilization, is needed for berry set, with the result of no normal ovule development after flowering,

only a defective embryo sac is formed (Winkler *et al.*, 1962; Mullins *et al.*, 1992; Dokoozlian, 2000a; Perl *et al.*, 2000; Iland *et al.*, 2011). True parthenocarpy is the only true method by which no seeds develop after flowering (Dokoozlian, 2000a). According to May (2004) true parthenocarpy does not occur in grapevines (Iland *et al.*, 2011).

Stimulative parthenocarpic percentage fruit set is relatively low with small final berry size, because of limited auxin or gibberellin supply due to the absence of seed development (Winkler *et al.*, 1962). However, with viticultural practices and the use of hormone sprays the size can be improved. Therefore when parthenocarpic berries are treated with GA₃ at a very early stage such as 80-100% full flowering, the small berries will enlarge (Coombe & Dry, 1992). Cultural practices like girdling and the use of PGRs could also contribute to increase berry size and improving the fruit set (Iland *et al.*, 2011).

- (iii) *Stenospermocarpy*, is where the berries appear to be seedless but contain one or more aborted seeds (Winkler *et al.*, 1962; Mullins *et al.*, 1992; Dokoozlian, 2000a). Seedless cultivars do not set fruit without fertilization and the flowers have normal appearance and produce good pollen (Winkler *et al.*, 1962; Iland *et al.*, 2011). The majority of current commercially important table grape cultivars set through stenospermocarpy, for example Prime, Crimson Seedless, Sultanina, Sugraone, Regal Seedless, Flame Seedless, Midnight Seedless and Sable Seedless.

During fruit development, seeds normally begin to develop after fertilization, but abort the embryo two weeks after fertilization (Coombe, 1960; Nitsch *et al.*, 1960; Winkler *et al.*, 1962; Mullins *et al.*, 1992; Dokoozlian, 2000a; Perl *et al.*, 2000; Roller, 2003; Iland *et al.*, 2011).

Seedless table grape cultivars usually have a naturally small berry size. According to Iland *et al.* (2011) the natural hormone levels are sufficient for fertilization, but not to carry the seed development through to a mature seed. Even though the seeds abort, the embryo growth, partially developed seeds or seed traces can still be found inside the berry (Winkler *et al.*, 1962; Dokoozlian, 2000a; Perl *et al.*, 2000; Iland *et al.*, 2011). The size of the seed traces are also linked to when embryo abortion occurred during fruit growth (Dokoozlian, 2000a).

There are two groups of factors that regulate fruit set in the grapevine: (i) Growth regulators that originate at sites other than the cluster itself; and (ii) The supply of organic nutrients that originate from organs external to the developing cluster (Winkler *et al.*, 1962). Strong evidence has been provided by Mullins (cited by Winkler *et al.*, 1962) that the second factor is solely the regulator of fruit set.

2.2.3.4 Berry development

Berry development is a process with two distinct processes, firstly the growth of the berries and secondly the ripening of the berries. Ripening involves various physiological, biochemical and development changes that occur in a coordinated and genetically regulated manner (Paul *et al.*, 2012).

Each cultivar has its own period required for complete berry development, but an estimate can be made at approximately 100 days from flowering/full flowering until full maturity (Harris *et al.*, 1968). The general *Vitis vinifera* berry development will be discussed in this chapter.

Berry development follows a double sigmoid curve, which is characterised by three stages (Figure 3) (Harris *et al.*, 1968; Coombe & McCarthy, 2000; Bennett, 2002; Sonnekus, 2015), depending on the environmental conditions, cultivar and cultivation practices (Coombe, 1973). The stages are: *Stage I*, the period of berry growth, where berries are small, hard and green; *Stage II* (lag stage), where berry growth slows down; and *Stage III*, the period from véraison up to maturity (Harris *et al.*, 1968; Coombe & Hale, 1973; Davies *et al.*, 1997; Bennett, 2002).

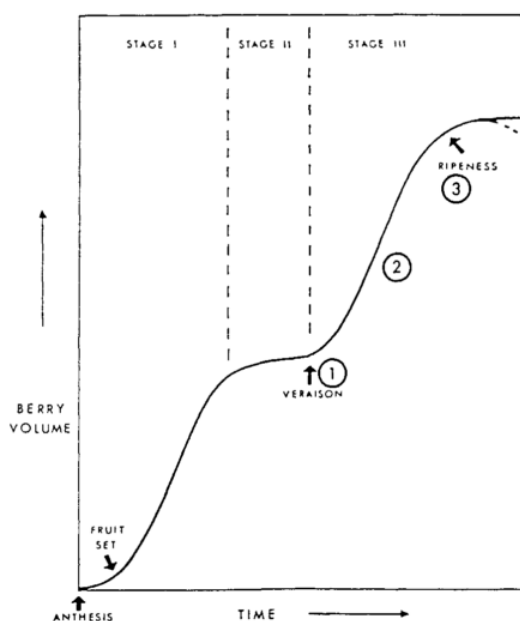


Figure 3 The development stages of grape berry (Coombe, 1992)

Stage I, also referred to as the first growth period and berry formation, happens immediately after fruit set where the pericarp growth is a result of partly cell division and mainly cell enlargement (Winkler *et al.*, 1962; Harris *et al.*, 1968; Mullins *et al.*, 1992; Coombe & McCarthy, 2000; Dokoozlian, 2000a; Bennett, 2002; Conde *et al.*, 2007). Most of the cell division in the pericarp takes place between five to ten days before and after flowering, but will only end three weeks after flowering (Winkler *et al.*, 1962; Harris *et al.*, 1968; Mullins *et al.*, 1992; Iland *et al.*, 2011). PGR treatments aimed at improving berry size, are usually applied during Stage I (see section 2.3.1.4).

During the first growth period of seedless (stenospermocarpic) cultivars such as Prime, the berry is formed and the seed embryos are produced, but will abort their growth two weeks after fertilization (Coombe, 1960; Nitsch *et al.*, 1960; Winkler *et al.*, 1962; Mullins *et al.*, 1992; Dokoozlian, 2000a; Perl *et al.*, 2000; Roller, 2003; Conde *et al.*, 2007; Iland *et al.*, 2011). Even though the seeds abort the embryo growth, partially developed seeds or seed

traces can still be found inside the berry (Winkler *et al.*, 1962; Dokoozlian, 2000a; Perl *et al.*, 2000; Iland *et al.*, 2011).

During Stage I (45 days after flowering), sugar content is still low, while organic acids start to accumulate and is measured as titratable acidity (TA) (Coombe, 1960; Winkler *et al.*, 1962; Mullins *et al.*, 1992; Coombe & McCarthy, 2000; Dokoozlian, 2000a; Kennedy, 2002). The three main organic acids are: tartaric acid which is the most important acid, followed by malic and citric acid (Soyer *et al.*, 2003; Conde *et al.*, 2007; Muñoz-Robredo & Robledo, 2011; Sonnekus, 2015).

During *Stage II*, the lag phase, which can last between 7-40 days (Harris *et al.*, 1968; Mullins *et al.*, 1992; Dokoozlian, 2000a; Conde *et al.*, 2007; Iland *et al.*, 2011), organic acid will reach a maximum with malic acid the highest (Conde *et al.*, 2007; Muñoz-Robredo & Robledo, 2011; Sonnekus, 2015). Berries are still green and hard and in seeded cultivars, embryo development is still rapid (Winkler *et al.*, 1962; Mullins *et al.*, 1992; Coombe & McCarthy, 2000; Sonnekus, 2015).

According to Pratt (1971), Coombe & Hale (1973), Raath (2012) and Sonnekus (2015) seedless table grape cultivars usually do not show a clear lag phase, which results in a less defined stages in the growth curve. Therefore cultivars which tend to ripen early in the season have no or a short lag stage compared to cultivars that ripen later in the season, which have a clear extended lag phase (Coombe, 1976; Mullins *et al.*, 1992).

Before the onset of *Stage III*, véraison is reached where the berries will become less green in colour (lose chlorophyll) and increase in volume while flavonoids accumulate in the skin and malate and tartrate in the pericarp decrease (Harris *et al.*, 1968; Coombe & Hale, 1973; Mullins *et al.*, 1992; Davies *et al.*, 1997; Coombe & McCarthy, 2000; Bennett, 2002; Iland *et al.*, 2011). Due to the increase in berry volume, the concentrations of tartaric and malic acid will decrease significantly (Kennedy, 2002; Sonnekus, 2015). After véraison (45 – 110 days after flowering) the pericarp width will increase two to three times because of cell enlargement in the pericarp and septum (Coombe, 1960). At this stage no cell division is taking place (Coombe, 1960).

In *Stage III* the pericarp growth is only dependent on cell enlargement (Winkler *et al.*, 1962; Harris *et al.*, 1968; Robinson & Davies, 2000). The berry will start to take the shape of a round, oval or long berry (Winkler *et al.*, 1962; Harris *et al.*, 1968). The growth of the pericarp tissue accounts for over half of the volume of mature berries and will determine the ultimate shape of the berry, which is the product of both cell division and cell enlargement (Harris *et al.*, 1968). Berry size is genetically encoded in the cultivar, but environmental factors can influence the size by affecting either the cell size or cell number quantities (Harris *et al.*, 1968; Mullins *et al.*, 1992; Dokoozlian, 2000a).

After the lag phase an abrupt decline in total acid is found, with an increase in sugar accumulation that will commence six to seven weeks after flowering and will continue until

harvest (Harris *et al.*, 1968; Dokoozlian, 2000a; Robinson & Davies, 2000). To reach full maturity, organic acids that accumulated will start to decrease steadily and the sugar content will increase at the same time (Harris *et al.*, 1968).

According to Davies *et al.* (1997) the trigger for ripening is likely linked to the influence of a number of hormonal factors. Since no specific hormone is related to the ripening of non-climacteric fruit (Coombe & Hale, 1973), three hormones have been identified which may be associated with regulating grape berry maturation processes namely abscisic acid, ethylene and brassinosteroids (Conde *et al.*, 2007).

2.2.3.5 Seed development

In seeded cultivars, seed development is mostly completed during the berry formation phase (Iland *et al.*, 2011). The seeds develop from fertilised ovules and contain three components namely an embryo, endosperm and protective tissue (Iland *et al.*, 2011).

Researchers cited by Iland *et al.* (2011) concluded seed development affects berry growth, size and final berry weight. Endogenous growth promoters do have an influence on seed development (Coombe, 1960) and the number of seeds per berry is directly linked with endogenous gibberellins concentration (Casanova *et al.*, 2009). Coombe (1960) also found that there is gibberellin activity in the ovaries of seedless cultivars at flowering.

The concentration of gibberellins in seeded and seedless berries does vary, but follows a similar pattern. Seeded berries start off with a very high concentration at fruit set, decreasing to low values after three weeks, reaching a second peak two weeks later and then remains low during fruit ripening (Casanova *et al.*, 2009).

Due to the low levels of GA₃ in seedless berries (due to the lack of seeds), the application of GA₃ at or soon after flowering will compensate to increase the concentration in the berry, which will give the enlargement effect (Coombe, 1960), as well as increase the sink activity (Casanova *et al.*, 2009).

2.2.4 Grapevine fertility

Fertility (fruitfulness) is defined as the number of inflorescence primordia per bud (Swanepoel & Baard, 1988). The mean bunch number per shoot is also often used as index of bud fertility (Iland *et al.*, 2011). As inflorescence primordia of the vine are already initiated within the bud during the preceding growing season, making prediction of potential fertility possible during the current winter (Barnard, 1932; Swanepoel & Archer, 1988; Swanepoel & Baard, 1988; Iland *et al.*, 2011; Molitor *et al.*, 2012).

Fertility of the grapevine is of utmost importance, since it will determine the size of the crop (Swanepoel & Archer, 1988). According to Vasconcelos *et al.* (2009) seasonal variation in fertility contributes to variations that are found in yield, but yield fluctuations are less sensitive to the variations in the number of berries per cluster, as well as berry size.

Barnard (1932) reported that fertility can vary between seasons and also stated that a better understanding of factors that control fertility will benefit the producer in management of the vines.

2.2.4.1 Factors affecting grapevine fertility

Genetic factors

The genetic factors of each cultivar are fixed characteristics of the vine (Bennett, 2002). Flower formation occurs through a series of sequential steps under strict genetic control (Krizek & Fletcher, 2005).

In some cultivars the buds closer to the basal part of the cane may be more fruitful than buds along the length of the cane, whilst other cultivars an increase in fertility are found towards the central part of the cane (Khanduja & Balasubrahmanyam, 1972; Bennett, 2002), Prime falls under the latter. In Table 2 pruning systems recommended for table grape cultivars produced in South Africa are listed. Based on the position of the fertile buds, Prime can be pruned with spurs or half long bearers.

Each cultivar also has its own proportion of buds in which differentiation to flower primordia occur, for example in Sultana 30-40% of the buds differentiate and in Alphonse Lavallee 100% is reached (Lavee *et al.*, 1967). In Sultana the number of differentiating buds increased gradually from the base to a maximum in buds 5-11 and in Alphonse full differentiation was found even in the basal buds (Lavee *et al.*, 1967).

Table 2 Pruning system for table and raisin grapes (compiled from: Van Der Merwe (2014), Avenant, J.H., 2016, personal communication)

Spur (2 buds)	Half long bearer (4-8 buds)	Cane (12-16 buds)
Alphonse Lavallée Bien Donné Bonheur Ebony Star Erlihane Flame Seedless La Rochelle Midnight Beauty Muska, Pirobella Queen of the Vineyard Prime Regal Seedless Ronelle Sable Seedless Sundance Sunred Seedless Victoria	Barlinka (6-8) Bien Donné (4-6) Bonheur (4-6) Crimson Seedless (8-10) Dan-ben-Hannah (4-6) Dauphine (6-8) Flame Seedless (6-8) Majestic (4-6) New Cross (4-6) Prime (4-6 buds) Redglobe (6-8) Waltham Cross (8) White Gem (4-6)	Crimson Seedless (10) Sugraone Sultanina Ralli Seedless (10) Autumn Royal (10)

Morphological factors

Fertility is related to the bud and its potential to form a fruitful bud. It is also related to cane length, diameter and internode length, as well as origin of the cane (Khanduja & Balasubrahmanyam, 1972). The most fruitful buds occur on one-year old canes arising from second-year old wood (Khanduja & Balasubrahmanyam, 1972).

Studies done on Pinot gris, Riesling, Auxerrois and Sauvignon Blanc indicated a relationship between cane diameter and bud fertility namely that decreased inflorescence number per shoot was associated with a decrease in cane diameter, while excess vigour has been associated with poor bud fertility (review by Vasconcelos *et al.*, 2009). Avenant (1998) recommended for Festival Seedless that canes should have a diameter of at least 10mm.

Hulmani *et al.* 1967 (cited by Khanduja and Balasubrahmanyam, 1972) reported that with increasing vigour of the cane, the region of maximum fertility was located higher up on the cane.

Physiological factors

Due to the differentiation phase that will take place before the bud enters dormancy, carbohydrate accumulation is of great importance to determine the percentage starch in the annual wood which is also associated with fertility. Inadequate supply of nutrients at the time of differentiation will lead to poor differentiation of the buds towards the base and distal end of the cane (Khanduja & Balasubrahmanyam, 1972).

Theories have been proposed regarding the biochemical mechanism of flower bud differentiation involving leaves and Srinivasan & Mullins (1981b) confirmed that leaves are

necessary in the process of differentiation, since it is a strong sink for root-produced cytokinin. With the transfer of the impulse for differentiation from leaves to the buds, the time of induction could be determined by means of defoliation (Lavee *et al.*, 1967). Defoliation can prevent or reduce differentiation (Lavee *et al.*, 1967).

The relationship between the number of leaves and time of induction of inflorescences has been described as an accumulation of metabolites (carbohydrates synthesized in the leaves) that are needed for differentiation (Lavee *et al.*, 1967; Khanduja & Balasubrahmanyam, 1972). In a field study conducted by Lavee *et al.* (1967), 18-21 leaves were necessary in both Alphonse Lavallée and Sultanina to complete induction (Swanepoel & Archer, 1988).

Lavee *et al.* (1967) suggested a possible correlation between the general vigour of a cultivar and its fertility. Excessive vigour is associated with decreased fertility, and normal vigour favours fertility (cited by Winkler, A.J., Cook, J.A., Kliewer, W.M. & Lider, 1962).

Plant hormones

Plant hormones play a major role in fertility of the grapevine, with the two main regulators of flowering being gibberellin and cytokinin (Vasconcelos *et al.*, 2009).

Anlagen formation is influenced by hormone levels found in the vine, as well as exogenous applications, to determine the final outcome, for example: a tendril primordium could develop into an inflorescence when it is treated with cytokinin and an inflorescence which is treated with gibberellins can convert to tendrils (Srinivasan & Mullins, 1981b). Inflorescence primordia development during bud break is a cytokinin-requiring process, where cytokinin is translocated to the developing inflorescence via the ascending sap that is synthesized in the roots (Mullins & Rajasekaran, 1981; Srinivasan & Mullins, 1981b; Lavee, 1987).

According to Chailakhyan (1977) (cited by Srinivasan & Mullins, 1981b) and Williams (2000), GA influences anlage formation and the direction of the anlagen development. At an early stage GA will promote fertility since the anlagen formation is a GA-requiring process, but later GA has an inhibiting effect during formation of flowers, directing the 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 are detectable in the xylem sap of the grapevines (Srinivasan & Mullins, 1981b; Bennett, 2002). Grapevines are sensitive to exogenous gibberellins and if exogenous gibberellins are applied to the grapevine when it is at its most sensitive, gibberellins at concentrations as low as 3µmol/L can have an induced bursting effect on the latent buds in the current season and inhibit formation of inflorescences from the anlagen (Srinivasan & Mullins, 1981b; Mullins, 1986).

It was reported that when GA is applied before the initiation process, it will have an inhibiting effect on flower bud formation, as it directs the anlagen formation to tendrils, but when

applied after flower bud formation little effect was found (Khanduja & Balasubrahmanyam, 1972; Mullins *et al.*, 1992; Williams, 2000).

Due to inflorescences and tendrils being homologous organs, the formation are controlled by the action of gibberellins, therefore when gibberellin activity is suppressed inflorescences may form instead of tendrils (Mullins *et al.*, 1992; Bennett, 2002; May, 2004; Iland *et al.*, 2011).

Several researchers reported that CK have an influence on fertility during formation of inflorescence primordia and formation of flowers (Khanduja & Balasubrahmanyam, 1972; Srinivasan & Mullins, 1979; Mullins, 1986; Williams, 2000; Bennett, 2002; Iland *et al.*, 2011). It has been suggested that a continuous influx of endogenous CK into the anlagen is needed for flower formation in the grapevine (Srinivasan & Mullins, 1980, 1981b).

Flower formation is a cytokinin-controlled process and high levels of cytokinin activity are found in the xylem sap of the grapevine during bud break and flowering (Mullins *et al.*, 1992; Williams, 2000; Bennett, 2002). Cytokinins are mainly found in the root apices and is involved in the regulation of flower differentiation (Srinivasan & Mullins, 1981b; Mullins *et al.*, 1992; Bennett, 2002). It was also found that longer pruned canes had more CKs available for reproductive development than spur-pruned vines (Vasconcelos *et al.*, 2009).

Sachs and Hackett (1976) (cited by Srinivasan & Mullins, 1981b) found evidence that flower formation in *Bougainvillea*'s is related to gibberellin:cytokinin balance and that this balance controls flowering by affecting the distribution of metabolites. Mullins *et al.* (1992) also stated that it appears that the initiation and development of the inflorescence primordia are regulated by the interaction between gibberellins and cytokinins.

Carbohydrate reserves

Carbohydrate reserves and grapevine bud fertility have a close relationship and adequate levels of carbohydrate reserves affect continued development of inflorescences during the bud burst stage (Bennett, 2002). The mean primordia size is positively influenced by the carbohydrate supply during inflorescence initiation, therefore the amount of carbohydrate which is stored in the winter buds may influence the early bunch development in spring (Antcliff & Webster, 1955). Reductions in carbohydrate reserves were reported to be associated with decreases in inflorescence number per shoot and flower number per inflorescence (Bennett *et al.*, 2005).

A positive correlation was found by Thomas and Barnard (1937) (cited by Sommer *et al.*, 2000) between the starch concentration in the respective node positions of the annual wood in the winter and fertility the following spring. These results were confirmed by the study of Sommer *et al.* (2000). Decreased photosynthesis during or shortly after flowering negatively affect bud fertility (Vasconcelos & Koblet, 1990; Bennett, 2002). According to Bennett (2002) carbohydrates play just as an important role as hormones in determining fertility.

Nutrient reserves

Nutrition places a major role in ensuring the formation of inflorescence primordia (Khanduja & Balasubrahmanyam, 1972; Mullins *et al.*, 1992; Williams, 2000; Vasconcelos *et al.*, 2009; Iland *et al.*, 2011).

The initiation phase of flower primordia in the buds has shown to be severely influenced by nitrogen (N) and an extreme N supply may reduce bud fertility or fruit set (Winkler *et al.*, 1962; Khanduja & Balasubrahmanyam, 1972; Srinivasan & Mullins, 1981b; Mullins *et al.*, 1992; Vasconcelos *et al.*, 2009). The ratio between carbohydrates and nitrogen needs to be taken in account as indicators of growth and fruiting and viticultural practices must be managed to keep vegetative growth and fruiting in balance (Winkler *et al.*, 1962).

Phosphate is present in the xylem sap in a mineral form, but during flower differentiation and bud break it is found in an organic form (Mullins *et al.*, 1992). Optimum levels of phosphate will promote bud fertility (Vasconcelos *et al.*, 2009).

Environmental factors

Each position on a cane differs in relative fertility, which is influenced by changes in environmental conditions during the initiation and development of inflorescence primordia (Antcliff & Webster, 1955; Pratt, 1971; Khanduja & Balasubrahmanyam, 1972; Mullins *et al.*, 1992; Watt *et al.*, 2008). Conditions during these critical stages have some control over the potential size (number of flowers) of the inflorescence (Palma & Jackson, 1981; Watt *et al.*, 2008).

An association between sunlight and temperatures during fruit bud initiation and fertility the following season was first suggested by Antcliff & Webster (1955). According to Sommer *et al.* (2000) and Vasconcelos *et al.* (2009) light and temperature are both key factors that determine successful induction and initiation of inflorescence primordia.

Light

Light intensity is important during fruit bud formation in the renewal zone, in combination with high temperature, which are desirable for maximum fertility in the latent buds (Srinivasan & Mullins, 1981b; Williams, 2000; Sánchez & Dokoozlian, 2005; Vasconcelos *et al.*, 2009; Iland *et al.*, 2011). When severe shading is experienced, it can reduce the number as well as the size of the inflorescence primordia (Khanduja & Balasubrahmanyam, 1972; Srinivasan & Mullins, 1981b; Lavee, 1987; Mullins *et al.*, 1992; Vasconcelos *et al.*, 2009; Iland *et al.*, 2011).

Cultivars also differ in terms of light sensitivity to reach maximum fertility, namely Sultana reaches maximum fertility at one-third of full sunlight, compared to Flame Seedless where fertility will increase with increasing available light (Sánchez & Dokoozlian, 2005). Light requirements for each cultivar, as well as whether fertility can reach a saturation level in

response to solar radiation under field conditions, still need to be established (Sánchez & Dokoozlian, 2005).

Temperature

High temperatures are required to favour differentiation of the inflorescence primordia in grapes during summer and early autumn (Khanduja & Balasubrahmanyam, 1972; Srinivasan & Mullins, 1981b; Mullins *et al.*, 1992; Iland *et al.*, 2011). To induce a maximum number of inflorescence primordia, only a pulse of four to five hours of high temperatures is needed, which is also essential for the initiation of the second and third inflorescence in many cultivars (Srinivasan & Mullins, 1981b; Dunn & Martin, 2000).

The optimal temperature range at which fruit bud formation takes place is between 20°C and 35°C, although differences do occur among cultivars and geographical origins (Winkler *et al.*, 1962; Khanduja & Balasubrahmanyam, 1972; Srinivasan & Mullins, 1981b; Dunn & Martin, 2000; Sommer *et al.*, 2000; Sánchez & Dokoozlian, 2005). If temperatures were below the lower limit (20°C), cultivars such as Riesling and Shiraz showed a severe reduction in fertility (Buttrose, 1970; Sommer *et al.*, 2000). Smit (1970) (cited by Sommer *et al.*, 2000) found that higher temperatures during the initiation period increased the number of flowers per inflorescence for the cultivar Sultana. Palma & Jackson (1981) found a relationship between maximum temperature and flower number per shoot in the following season.

The results of Petrie & Clingeleffer (2005) indicated that the temperature immediately before and during bud break influences the number of flowers per inflorescence and that the effect of temperature on flower differentiation reduces as bud burst advances. May (1964) as cited by Vasconcelos *et al.* (2009) reported a decrease in the number of inflorescences with a temperature of 12°C shortly before and after bud break compared to a temperature of 25°C.

Water

Water stress has a major influence on inflorescence development (Winkler *et al.*, 1962; Srinivasan & Mullins, 1981b; Mullins *et al.*, 1992; Williams, 2000; Vasconcelos *et al.*, 2009). The most critical time for water stress is during the first four weeks following flowering (Winkler *et al.*, 1962). Water stress can influence bud fertility either directly by the amount of water available during cell division and cell enlargement or indirectly by means of its effect on the vines photosynthesis (Vasconcelos *et al.*, 2009).

Long periods of water stress negatively affect fertility of latent buds, which explains why rain-fed vines bear fewer fruitful buds than irrigated vines (Winkler *et al.*, 1962; Srinivasan & Mullins, 1981b; Mullins *et al.*, 1992; Iland *et al.*, 2011). This can differ in areas with irregular rainfall or in Mediterranean regions with winter rainfall and a dry growing season. Carbonneau & Casteran (1979) reported vigorous growth and decreased fertility are associated with excessive irrigation of Cabernet Sauvignon.

Viticultural practices

Canopy management

Canopy management is applied to obtain increased light interception, resulting in more fruitful buds and higher yields. The main aim is to reduce excessive canopy shading through certain techniques (Iland *et al.*, 2011). The techniques include trellis-training systems, shoot positioning, shoot orientation, shoot trimming, control of shoot number and spacing, leaf removal in fruiting zone and control of shoot vigour (tipping and topping of shoot tips) (Dry, 2000; Iland *et al.*, 2011).

Pruning

The position of the fertile buds on the cane, is one of the major factors determining the pruning system (see Table 2). Winter pruning, which can be done at any time between leaf fall and bud break, may affect bud break (Winkler *et al.*, 1962; Dunn & Martin, 2000). Applying appropriate pruning systems improves conditions for fruit bud formation for most cultivars by preventing over cropping (Winkler *et al.*, 1962).

Summer pruning (pruning during the post-harvest period) is a cultivation practice widely used by several table grape producers in South Africa. Producers believe that this practice will improve post-harvest reserve accumulation in the current season and bud fertility in the following season and also reduce the total time required for pruning.

Summer pruning is well documented for apples (Saure, 1987), but not for table grapes (Reynolds *et al.*, 2005). Summer pruning consists out of removing non-bearing shoots (Dokoozlian *et al.*, 1989), done pre- or post-harvest. By removing the non-bearing shoots, nutrients absorbed by and stored in the root system and other permanent structures of the vine, will be transported to the sinks (bunches), unnecessary reserves will not be stored during the winter and improved canopy microclimate is found (Reynolds *et al.*, 2005).

Regarding the impact of summer pruning on bud fertility of grapevines, varying research results are reported, whereas on defoliation research is well documented (Vasconcelos & Koblet, 1990; Dry, 2000; Reynolds *et al.*, 2005).

Rootstock and trellis systems

Sommer *et al.* (2000) stated that with vigorous rootstocks leading to more dense canopies may inhibit induction and initiation of the inflorescences and may cause a decline in the fertility. With the wrong rootstock, fertility can be influenced in a negative way (Winkler *et al.*, 1962; Khanduja & Balasubrahmanyam, 1972; Mullins *et al.*, 1992; Iland *et al.*, 2011).

Orth (1990b) mentioned that the combined effect of vigorous growth and GA₃ applications might be able to increase apical dominance, due to the excessive GA₃ concentrations in the plant.

Light exposure inside the canopy is more important for fertility than the direction in which the shoot grow (Vasconcelos *et al.*, 2009). Therefore with trellis systems and split canopies like the Gable trellis or Geneva Double Curtain system, bud fertility can be improved and an overall increase in productivity can be obtained due to improved light exposure (Winkler *et al.*, 1962; Srinivasan & Mullins, 1981b; Mullins *et al.*, 1992; Iland *et al.*, 2011).

Plant growth regulators

The use of PGRs has become a common used practice in the table grape industry (Iwahori *et al.*, 1968; Sachs & Weaver, 1968; Molitor *et al.*, 2012) (see section 2.3).

2.2.4.2 Methods for assessing grapevine fertility

Fertility can be assessed in the vineyard by counting the number of inflorescences after bud break to estimate the actual fertility (Swanepoel & Baard, 1988). Potential fertility can be determined in the winter by using two distinct methods (Sommer *et al.*, 2000).

The first method is stereomicroscope bud dissection, which have been used in Australia since 1950's in forecasting the Sultanina crop (Barnard, 1932; Khanduja & Abbas, 1973; Swanepoel & Baard, 1988; Sommer *et al.*, 2000; Iland *et al.*, 2011). A proportion of the primary buds are opened and buds containing inflorescence primordia are counted (Antcliff & Webster, 1955; Swanepoel & Baard, 1988; Iland *et al.*, 2011).

The second method, namely forced budding, is applied under controlled conditions in a glasshouse where single-node cuttings are evaluated, until inflorescences are visible or not (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).

The potential fertility only represents an estimate of the actual fertility which are expected in spring, but may not accurately represent the actual fertility in the vineyard (Khanduja & Abbas, 1973; Vasconcelos & Koblet, 1990).

With the use of these two methods to determine potential fertility, pruning methods can be adapted to obtain maximum yield in seasons where low fertility is expected and also to avoid over cropping of the vines in seasons where high fertility is expected (Antcliff & Webster, 1955; Khanduja & Abbas, 1973).

2.3 Overview of plant growth regulator use in table grape production

A 'plant hormone' is defined as (Durner, 2013): A naturally occurring organic substance produced by the plant, which at very low concentrations controls plant growth and development through effects on cell division, elongation and differentiation in the tissue of synthesis or elsewhere in the organism.

PGRs are a population of endogenous molecules and synthetic compounds with similar structures to the natural occurring plant hormones which plays an important role in regulating growth and development of plants (Roberts & Hooley, 1988; Korkutal *et al.*, 2008). Timing of PGR application to developing fruit is critical, for different stages of fruit development relate to changes in the endogenous levels of different growth regulators (Lavee, 1987).

In Table 3 the major plant hormones with their sites of biosynthesis, modes of translocation and general functions in horticultural physiology are summarised. In Table 4 the major plant PGRs used in table grape production, with their primary effects and recommended phenological stages of application are summarised.

The five main PGR groups are gibberellins (GA), cytokinins (CK), auxins, abscisic acid (ABA) and ethylene (Roberts & Hooley, 1988; Korkutal *et al.*, 2008; Durner, 2013). The focus of this study was on GA₃ and therefore only GA and specifically GA₃ is discussed in detail in this literature review.

Table 3 Major plant hormones, their sites of biosynthesis, modes of translocation and general functions in horticultural physiology (adapted from Durner (2013)).

Hormone	Primary site of biosynthesis	Primary mode of translocation	Primary function(s)
Auxin	Young meristematic tissue	Mass flow in phloem; polar transport	Cell elongation; vascular differentiation; root initiation; apical dominance; stimulates ethylene production
Cytokinin	Root tips; developing seeds	Xylem	Stimulates cell division; overcomes apical dominance; stimulates leaf blade growth; stimulates cell expansion
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
Ethylene	All living plant tissue	Diffusion	Promotes fruit ripening, senescence and abscission; promotes leaf abscission; promotes (<i>Ananas</i>) or delays (<i>Prunus</i>) flowering; promotes the production of female flowers; induces epinasty
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
Florigen	Leaf phloem	Phloem	Induces the transition of meristems from vegetative to reproductive
Brassinosteroids	Pollen, seeds and young vegetative tissue	Synthesized at site of action	Promotes organ elongation; inhibits root formation and growth; induces xylem differentiation; stimulates seed germination

Table 3 (cont.)

Hormone	Primary site of biosynthesis	Primary mode of translocation	Primary function(s)
Jasmonates	All living plant tissue; leaves; young developing fruit; cotyledons of germinated seeds	Synthesized at site of action; phloem; xylem	Induce tendrils coiling; inhibits general stem and root growth, photosynthesis, and seed germination; induces the production of storage proteins in tubers, bulbs and seeds; induces plant defence responses to insect and pathogen attack; increases production of secondary metabolites with the role in plant defences
Polyamines	All living plant cells especially actively dividing ones	Phloem; xylem	Enhances cell division; prevents mitotic senescence; delays leaf senescence; may help regulate flowering; inhibits ripening and senescence
Salicylic acid	Leaves	Mostly phloem	Signal in thermogenic plants; signalling hormone in plant resistance to pathogens; may be a signalling molecule for flowering

Table 4 Major PGRs with their primary effects and recommended phenological stages of application for table grapes (compiled from Roberts & Hooley (1988), Durner (2013), Van Der Merwe (2014) and Davies (2014).

Hormone or PGR	Primary effect	Recommended phenological stage of application
Auxin	Cluster elongation	5-10 cm shoot length
	Berry sizing	After set
	Colour development	Véraison
Cytokinin (CPPU)	Berry sizing	After set (7-10 mm berry diameter)
Gibberellin (GA ₃)	Cluster elongation	Top lateral of the inflorescence starts to separate from the rest of the inflorescence or when the inflorescence is about 80 mm in length
	Berry thinning	Flowering
	Berry sizing	After set (7-10 mm berry diameter)
Ethylene (Ethephon)	Colour development	Véraison
	Leaf senescence	Post-harvest in autumn
Abscisic acid (ABA)	Berry thinning	Flowering
	Colour development	Véraison

2.3.1 Gibberellin

Gibberellin (GA) was discovered when a study was carried out by a group of Japanese plant pathologists on the disease called 'foolish seedling', which made infected rice plants grow extremely tall (Roberts & Hooley, 1988). After a demonstration which was done in 1926 by

Kurosawa, a sterile culture filtrate was isolated from the disease, which was caused by a fungus known as *Gibberella fujikura* (Hooykaas *et al.*, 1999; Durner, 2013).

GA is a plant hormone that is found naturally in the plant and which regulates different metabolic processes such as growth and development (Graebe *et al.*, 1965; Hooykaas *et al.*, 1999; Roller, 2003; Teszlák *et al.*, 2005; Molitor *et al.*, 2012; Durner, 2013). GAs are found in growing meristematic tissues such as roots, shoot apical cells, young leaves, young fruits, and developing seeds (Roberts & Hooley, 1988; Durner, 2013). GA may be transported to developing seeds which do synthesize and metabolize GA (Roberts & Hooley, 1988). GA is also absorbed and translocated to young growing meristems and internodes resulting in stimulation of cell elongation (Weaver *et al.*, 1966).

“Gibberellin” and “gibberellin activity” are terms that are used to combine known GA and other GA-like compounds that have the same properties (Coombe, 1960; Roller, 2003). There are over 120 different GAs in grapes, but the most common form of GA in the grapes are GA₁, GA₃ and GA₂₀ (Graebe *et al.*, 1965; Roberts & Hooley, 1988; Garcia-Martinez, 1997; Hooykaas *et al.*, 1999; Hedden & Phillips, 2000; Durner, 2013). Though the structures of these GAs are similar, they are very different in their biological activity, due to their metabolism to active forms (Roberts & Hooley, 1988; Garcia-Martinez, 1997; Durner, 2013).

In seedless cultivars the gibberellin activity is high at early fruit set stage followed by a decrease after the fruit set stage and maintaining a rather low level until no activity is found after 25 days (Iwahori *et al.*, 1968).

GA₁ are active in stem elongation (Hooykaas *et al.*, 1999; Durner, 2013). GA₂₀ occur in unfolded leaflets and in tendrils confirming that the hormone move from leaflets to the upper stem where the bioactive GA₁ is formed (Hooykaas *et al.*, 1999). GA₃ is produced in the seeds, which is known where low biological activity is found, and also in the pulp of the berries (Wolf & Loubser, 1994). Numerous GAs have been isolated and characterised by Tudzynski (1999).

GA are tetracyclic diterpenoids that are derived from the terpenoid pathway and consist of four isoprene units (Graebe *et al.*, 1965; Garcia-Martinez, 1997; Tudzynski, 1999; Roller, 2003). GAs are derivatives of the *ent*-gibberellane skeleton and they have the same absolute configuration (Roberts & Hooley, 1988; Hooykaas *et al.*, 1999). All of the related compounds have some biological activity which share the same gibbane ring structure (Roberts & Hooley, 1988; Hooykaas *et al.*, 1999).

GA₃ (Figure 4) is the easiest and least expensive GA to extract from fungal cultures for commercial use and is mostly used in viticulture as a PGR that is involved in cell division and cell enlargement during berry development (Coombe, 1960; Sachs & Weaver, 1968; Harms & Oplinger, 1988; Hooykaas *et al.*, 1999; Roller, 2003; Molitor *et al.*, 2012; Özer *et al.*, 2012; Durner, 2013). Work done by Paleg *et al.* (1964) confirmed previous studies that GA₃ is more active than any of the other GAs tested.

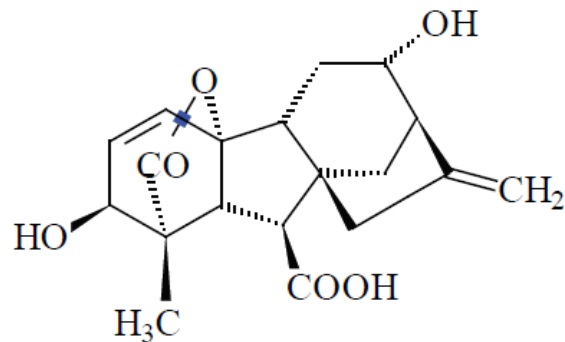


Figure 4 Chemical structure of GA₃ (Dimovska *et al.*, 2014)

In table grape production, there are three main objectives with GA₃ treatments, each requiring application at a specific phenological stage for the desired outcome, namely (Table 4): treatment for a stretching effect will be applied when the inflorescence length is at 8cm, treatment for a thinning effect will be applied at full bloom and treatments for a berry sizing effect will be applied at 7-10mm berry diameter. The specific flowering stage and berry size for GA₃ application differ between cultivars and the main aim of the treatment (Dokoozlian, 2000b; Christensen, 2000; Hed *et al.*, 2011; Molitor *et al.*, 2012; Van der Merwe, 2014).

Elgendy & Ahmed (2012) cited that the timing of the thinning application with GA₃ was extremely important and that the effectiveness of GA₃ on the bud depend on the physiological age of the buds. The thinning action also have a direct effect on the source-sink ratio in terms of less fruit, improved photosynthetic assimilation and also increase grape quality (Özer *et al.*, 2012).

GA₃ treatments also leads to increased petiole length, strengthening of the pedicel attachment (Motomura & Hori, 1978), decreased berry shatter (Retamales & Cooper, 1993; Özer *et al.*, 2012), less shot berries, enhanced shoot growth and decreased compactness clusters (Harms & Oplinger, 1988; Mullins *et al.*, 1992; Roller, 2003; Teszlák *et al.*, 2005).

GA₃ are mainly used in seedless grapes, compared to seeded cultivars where the use of GA₃ is limited to a few cultivars (Lavee, 1987; Mullins *et al.*, 1992).

GA₃ can be applied either by spraying the whole vine or by dipping the bunches in a solution at the recommended phenological stages (Weaver & Pool, 1971; Orth, 1990b). Applying GA₃ at different dosages, phenological stages and environmental conditions will have different outcomes (Mullins *et al.*, 1992; Molitor *et al.*, 2012).

2.3.1.1 GA₃ mechanism / Role in plant

In the plant, GA movement occurs over short distances by diffusion and over longer distances it occurs in the phloem, but it may also occur in the xylem and from cell to cell (Hooykaas *et al.*, 1999; Durner, 2013). The physiology behind the mechanism by which the

thinning effect after GA₃ treatment is obtained is still not fully understood, although several theories have been proposed, namely Dokoozlian (2000b):

(i) *The pollenicide hypothesis*: GA₃ acts as a pollenicide in seeded and seedless cultivars. This theory has already been eliminated from consideration due to results obtained by research work in Chile and California.

(ii) *The hormone balance hypothesis*: At flowering, with the thinning application, the endogenous balance of hormones in the berries are changed, which affects flowering or fruit abscission.

(iii) *The growth or nutrient competition hypothesis*: GA₃ encourages nutrient competition between flowers and shoots, or between flowers in a cluster.

2.3.1.2 Cluster elongation, stem elongation and compactness

Stem elongation treatments has been used extensively in seedless table grapes (Molitor *et al.*, 2012). It resulted in loosening the clusters by lengthening the stem framework (Christensen, 2000).

Dokoozlian (2000) also reported that this action on seedless table grapes have no significant affect as it only will hasten cluster growth but no effect on cluster length or compactness. Cluster or rachis elongation is cultivar dependent (Mullins *et al.*, 1992). If GA₃ are applied for this reason the optimal time is to spray onto the bunch stems when the bunches are a half to two thirds of their final length and will also help with preventing of excessive compactness (Coombe & Dry, 1992).

For over 50 years research has been done on the effect of pre-flowering and flowering GA₃ applications on vines and found that these applications can reduce the compactness as well as predisposition to bunch rot of several of the important grape cultivars in California (Hed *et al.*, 2011).

2.3.1.3 Berry thinning

The term *thinning* means the removal of flower clusters during flowering and of immature clusters or parts of a cluster after fruit set (Winkler *et al.*, 1962; Weaver & Pool, 1965; Coombe & Dry, 1992). The thinning action will strengthen the vine by limiting the crop load without shrinking the leaf area (Winkler *et al.*, 1962). By using PGRs for this action, it is considered as an amplification of the natural self-regulatory fruit abscission process (Bangerth, 2000).

Three different methods regarding thinning were developed namely flower-cluster thinning, cluster thinning and lastly berry thinning (Winkler *et al.*, 1962). For the purpose of this study only the latter thinning will be discussed.

Berry thinning can be done either by dipping the individual inflorescence into a solution, or by chemically spraying the vineyards with GA₃ and/or manually thinning on a later stage to

meet quality standards (Winkler *et al.*, 1962; Weaver & Pool, 1971; Orth, 1990a; Gil *et al.*, 1994).

Due to manual thinning being a very labour intensive and time consuming practice, an alternative was required by spraying clusters for the thinning effect with PGRs or chemicals (Winkler *et al.*, 1962; Myrianthousis & Hadjigiorgis, 1973; Orth, 1990a; Bangerth, 2000).

The choice of using GA₃ sprays for thinning became a common practice in seedless table grape cultivars (Dokoozlian & Peacock, 2001). Therefore the effectiveness for berry thinning differ from every situation in terms of differences in physiology or environmental factors which affect the berry set and the response to an exogenous regulator (Gil *et al.*, 1994; Dokoozlian & Peacock, 2001).

Elgendy & Ahmed (2012) cited that the timing of the thinning application with GA₃ was extremely important and that the effectiveness of GA₃ on the bud depend on the physiological age of the buds. According to Christensen (2000) the most effective results for the thinning application was found between 30 to 80 % full flowering, though the vineyard block may not all be on the same phenological stage, judgment must be based on the overall majority of flowers stage.

Berry thinning is an action that are being done at a very early stage to make space for the larger berries for when the berry sizing application is applied (Weaver & Pool, 1971; Jensen *et al.*, 1976; Orth, 1990a; Roller, 2003; Teszlák *et al.*, 2005). Making the bunches less compact before berry set improves the bunch structure, bunch size and quality (Winkler *et al.*, 1962; Sproule & Stannard, 1970; Özer *et al.*, 2012; Domingos *et al.*, 2016). By reducing the cluster compactness it makes the cluster easier to work with (Roller, 2003), therefore the levels of berry thinning must be applied in such a manner that the berry weight is not reduced and that the bunches do not became straggly (Özer *et al.*, 2012).

Dokoozlian (2000) cited that other studies have shown that late flowering thinning applications will increase the length and weight of ellipsoidal or cylindrical shape berries, for example Thompson Seedless will receive a thinning application near full flowering for this exact reason (Winkler *et al.*, 1962).

At a later stage manual thinning will be applied if the spraying application action was not successful. Manual thinning was economically feasible in the past, but due to increase in labour costs this method is no longer a first option in several cultivars (Winkler *et al.*, 1962; Myrianthousis & Hadjigiorgis, 1973; Orth, 1990a; Bangerth, 2000). Manual thinning result in more uniformed, medium sized, loose clusters of uniformly large, perfect berries and good texture (Özer *et al.*, 2012).

2.3.1.4 Berry sizing

Berry size is a genetically determined factor among table grape cultivars (Zoffoli *et al.*, 2009). Therefore GA₃ has been applied as a post-bloom spray since the 1960s to increase

the berry size of seedless berries (Lavee, 1958). Some other traditional ways of achieving this action is by practices of crop control or trunk girdling (Orth, 1990b; Mullins *et al.*, 1992).

GA₃ are applied to Prime and to other table grape cultivars after fruit set or post-bloom to increase the berry size (diameter and length), weight, and cluster length to achieve commercially acceptable fruit quality (Mullins *et al.*, 1992; Christensen, 2000; Dokoozlian, 2000b; Roller, 2003; Casanova *et al.*, 2009; Zoffoli *et al.*, 2009; Elgendy *et al.*, 2012; Özer *et al.*, 2012; Raban *et al.*, 2013).

The berry sizing application can be applied either by chemically spraying of the vineyards and/or manually dipping of bunches (Orth, 1990b; Coombe & Dry, 1992).

Not only is the phenological stage for berry sizing application important, but the optimal period of the application, rates, region, cultivar and endogenous and exogenous factors that affect the action is important (Weaver & McCune, 1958; Lavee, 1987; Orth, 1990b; Coombe & Dry, 1992; Mullins *et al.*, 1992; Wolf & Loubser, 1992; Dokoozlian, 2000b; Casanova *et al.*, 2009).

Berry growth is caused by cell division and cell enlargement during berry development (Coombe, 1960; Winkler *et al.*, 1962; Mullins *et al.*, 1992; Dokoozlian, 2000b; Roller, 2003; Molitor *et al.*, 2012; Özer *et al.*, 2012). In seedless berries the application of GA₃ increase the cell size that allows the berry to take up large amounts of water and sugars without changing the pressure potential (Casanova *et al.*, 2009). The mode of action consist of stimulation of cell elongation and division with a higher sugar and water intake into the cells (Ferrara *et al.*, 2014).

GA₃ will increase the berry size by inducing cell enlargement followed by a decrease in cell density (Ben-Arie *et al.*, 1998). When the thickness of the berries skin are reduces, the berries will enlarge thus stretching the skin and making it thinner (Ben-Arie *et al.*, 1998). GA₃ application will give the berries a greater length-to-width ratio (Sachs & Weaver, 1968; Retamles *et al.*, 1995). When seedless berries are treated with GA₃, a radical increase in growth rate is found, showing no lag phase and masking the well-known double sigmoid curve of berry development (Du Plessis, 2008; Raath, 2012; Sonnekus, 2015).

The industry often tend to overuse and misuse GA₃ applications in order to achieve the required berry size of the international markets (Zoffoli *et al.*, 2009). Number of applications can vary up to five (Casanova *et al.*, 2009). With too much GA₃ application, the down side is a delay in fruit ripening (Guelfat-Reich & Safran, 1973), increased berry shatter (Retamales & Cooper, 1993), cluster rigidity, and also an association with decrease in fertility the following year (Retamles *et al.*, 1995; Dokoozlian, 2000b; Raban *et al.*, 2013). All of these parameters are often cultivar dependent (Retamales & Cooper, 1993; Raban *et al.*, 2013). Even with too high concentrations of GA₃ the berries can become too large for commercial use (Winkler *et al.*, 1962).

According to South African Table grape Industry Guidelines for the preparation of Table grapes for export 2014/2015 (SATI, 2015), for most cultivars no more than three GA₃ applications are recommended to increase berry size as overexposure can affect the fruit quality, productivity and has disadvantages during storage (Zoffoli *et al.*, 2009). The idea of overexposure is so over marketed that it is missed at how little chemical is really required to achieve major fruit size and vine productivity benefits (Looney, 1993).

It was found in California, on Black Corinth in 1960's, that 2.5-5 ppm GA₃ applications at the phenological stage of 90% full flowering had the best results for increasing the berry size (Winkler *et al.*, 1962). If applied after fruit set, it can cause extensive damage to the buds that can influence the yield of the following season (Lavee, 1987).

2.3.1.5 Fruit quality and post-harvest quality after storage

The best quality table grapes comes from vineyards where the PGR management in the vineyard was applied correctly (Zoffoli *et al.*, 2009). Quality can be obtained by increasing the berry size and weight to make the grapes more commercially attractive (Lynn & Jensen, 1966; Roller, 2003).

Two main factors that determine the quality, is not only the size, colour and shape, but soluble solids and titratable acidity. These two factors has been reported by researchers cited by Roller (2003) to decrease and increase in numerous trials depending on the variables involved. Singh *et al.* (1978) reported that the firmness of the berries did increase after the grapes where treated with GA₃, which is important in storage and packaging proceedings.

Post-harvest quality of the table grapes can decline due to physical, physiological, or pathological factors that may occur in the vineyard or after harvest (Zoffoli *et al.*, 2009). Main defects that can be found is rachis shrivelling, skin browning, hairline cracking and *Botrytis cinerea* (Zoffoli *et al.*, 2009).

Zoffoli *et al.* (2009) reported that an overuse of GA₃ has increased the pedicel thickness but lowered the cuticle content which induced shatter and predisposed grapes to gray mold. According to Ben-Arie *et al.* (1998), they found that the GA₃ had an effect on the post-harvest life resulting in an increase of the rachis shrivelling.

2.3.1.6 Leaf area development

High vegetative vigour was found to be associated with high endogenous levels of gibberellins and with this high levels the buds on the shoot contain double the amount of free GA than normally-growing shoots (Lavee, 1987). Due to the vigorous growth the lower buds on the vigour's shoots underwent an abortion of the central main winter buds of the eyes and resulted in a reduction of fertility (Lavee, 1987).

According to Weaver *et al.* (1966) foliar application of GA to the vine resulted in the effect that intact internodes and leaves were poor paths for entry of GA, and that the movement was mainly active in the growing shoots tips and young internodes gave a better response than older ones.

According to Weaver *et al.* (1966) a foliar application of GA to the vine resulted in the effect that intact internodes and leaves resulted in poor entry paths for GA, whereas in growing shoots tips and young internodes, active movement was found and gave a better entry response for GA.

2.3.1.7 GA₃ and CPPU

It has been reported by Zoffoli *et al.* (2009) that Thompson Seedless gave larger berries with the GA₃ + CPPU applications however an over application of GA₃ + CPPU has delayed TSS accumulation. Retamles *et al.* (1994) reported that combination of these two PGRs had a greater berry weight than GA₃ alone and more compact bunches. CPPU alone do not have the same result as GA₃ alone (Retamles *et al.*, 1995). As cited by Strydom (2013) the combination of GA₃ and CPPU improved the berry firmness.

In the study that Reynolds *et al.* (1992) conducted on seedless cultivars showed that CPPU in combination with GA had no beneficial or synergistic effects which differs from Nickell's work (cited by Reynolds *et al.*, 1992). CK's can also be used in combination with GA₃ to avoid poor berry set, if it is applied at the proper timing (Motomura & Hori, 1978; Shiozaki *et al.*, 2014).

2.4 Comparing different plant growth regulator application methods for thinning and/or berry sizing in table grape production

2.4.1 Spray application

Over the past few years spray application methods became one of the most talk about topics in terms of improving spray efficiency of agrochemicals. The main focus is to find the most effective method of improving spray deposition and reducing drift during the application. The main advantage of spraying is the meaningful reduction in time needed for the application, namely a reduction factor of 64 comparing to dipping of bunches (Kolasinska *et al.*, 2008).

2.4.1.1 Coverage, water volumes

Table grape canopies can be very dense and therefore the level of coverage achieved in the canopies may be affected by the type of sprayer, operating parameters, weather conditions, cultivar, growth stage and trellis system (Wise *et al.*, 2010). One of the main parameters is the volume of water that is used to carry the active ingredient to the target and very little is known about the variation in water volumes that affects target coverage in grape canopies (Wise *et al.*, 2010).

Wise *et al.* (2010) conducted a study on the effect of water volume on the ability of vineyard sprays to effectively cover grape clusters, found that water volume did affect the quality of the pesticide deposition by the air blast sprayer (conventional spray) and concluded that the coverage of the target can be optimized for an air blast sprayer by selecting the appropriate water volume.

2.4.1.2 Conventional

The most commonly used spray pumps for conventional spraying, are conventional air-assisted (mist-blower) sprayers with an arc-shaped spray boom and an axial-flow fan, or pneumatic sprayers with air shear nozzles and a centrifugal fan (Pascuzzi & Cerruto, 2015). According to Pascuzzi & Cerruto (2015) these specific machines must be adjusted correctly to avoid non-uniform deposition, over dosage, off-target spray and environmental pollution like drift and run-off.

It was found by Jahannama *et al.* (1999) that a large portion of the spray was lost by airborne drifts of droplets away from the crop or target organ, due to rapid gravitational settling of large droplets on the soil underneath. According to studies conducted at the Universities of California, Georgia, Illinois and Chile only 15-20% of the spray from conventional or air blast sprayers ends up on the plants, while nearly 60% of the chemical goes wasted onto the ground and less than 3% ends up on the undersides of leaves.

Studies done on grapevines indicated that the air blast of conventional sprays can cause leaves to bend backwards against each other, thereby creating a wall where the foliage cannot readily be penetrated by the spray stream (Furness & Pinczewski, 1985). Higher volumes of lower velocity air produced better coverage than lower volumes of higher velocity air for a given energy input and thus the nature of the airstream produced has a significant effect on coverage obtained (Randal, 1971, as cited by Furness & Pinczewski, 1985).

With the Cima Blitz 50 mist-blower, which was used in this study, excellent coverage is obtained, which is associated with the high air speed that ensures penetration into dense canopies, carrying the active ingredient application into the target area and depositing spray evenly over the upper and lower leaf surfaces. One major advantage of the Cima mist-blower is that volumes from 250L/ha up to 1000L/ha can be applied (Electrostatic spraying system, www.iandmsmith.com/upload/ElectrostaticSprayersforGrapeGrowersNEW.pdf).

2.4.1.3 Ultra-low volume or Electrostatic spray

The electrostatic spray pump (ESS) is designed to use low volume mixtures and to produce droplets with a volume median diameter (VMD) ranging between 30 to 50 μ m, that may be electrostatically charged by induction using an electrode inside each nozzle (Pascuzzi & Cerruto, 2015). The ESS may meet requirements to improve the overall deposition and distribution on foliage and also reduce spray drift (Jahannama *et al.*, 1999; Pascuzzi & Cerruto, 2015).

Farooq *et al.* (2010) proposed the theory that with the ESS the electrostatic charge in the droplets causes attraction between droplets and the target and assists in the deposition. After the release from a sprayer, droplets in the spray cloud will interact with the surrounding air, while it is traveling to the target, thus the interaction can be summarized as mass, heat and the momentum transfer between droplets and the surrounding air (Farooq *et al.*, 2010). It seems that the most significant forces acting on spray droplets during the travel time between spray pump and targets are gravity, resistance and strain. Therefore the electrostatic force must be strong enough to overcome these forces, to ensure that the droplets are still within a certain distance from the target (Farooq *et al.*, 2010).

According to Law (1989) three basic requirements are needed for a successful ESS application: firstly the generation and electrification of spray droplets, secondly the droplet transport to the area of the target and lastly is the deposition of droplets on the target (Farooq *et al.*, 2010). Benefits of ESS use are: reduction in active ingredients which enter the ecosystem, enhancement of droplet deposition and also to obtain even coverage of complex targets (Jahannama *et al.*, 1999; Law, 2001). For table grape vineyards ESS sprays must consist of charged droplets of conductive liquids which are forced into three-dimensional canopies (Pascuzzi & Cerruto, 2015).

Pascuzzi & Cerruto (2015) compared an ESS to a conventional sprayer and concluded that with the ESS it is possible to ensure deposits comparable with those achievable using conventional sprayers. Bode (1981) and Hislop (1987), as cited by Welsh *et al.* (2000) confirmed that conventional equipment is not as efficient as some reduced-volume equipment, such as the ESS. With the ESS, the active ingredient mass applied which is being transferred onto the target organs are increased, which is not generally found with conventional spraying methods (Jahannama *et al.*, 1999).

With the ESS systems the amount of chemical applied can be reduced by half and the amount of water by 20 times. Even at half the chemical rate and a twentieth of the water volume, the ESS system deliver 3-times more product onto grapes inside the bunch, compared than a conventional sprayer. The reduction in chemical dosages is also beneficial for PGR applications (Electrostatic spraying system, www.iandmsmith.com/upload/ElectrostaticSprayersforGrapeGrowersNEW.pdf).

The ESS sprayers use the MaxCharge nozzle, which is specially designed to create very small sized droplets with a large electrical charge (Farooq *et al.*, 2010). The combination of high electrical charge and optimum droplet size (and weight) causes the spray to move against gravity and wrap around leaves to deposit onto leaf undersides (Pascuzzi & Cerruto, 2015).

The droplet size spectrum for the MaxCharge nozzle and that of a typical high-pressure conventional nozzle are compared in Figure 5. The average droplet size for the MaxCharge is 35 microns and the spectrum is very narrow. The conventional spray nozzle produces an average droplet of about 280 microns and the spectrum is very wide. Figure 5 also shows

why smaller droplets give better coverage — 1000 spray droplets of 30 microns from an ESS equals the volume of a single 300-micron droplet from a conventional sprayer.

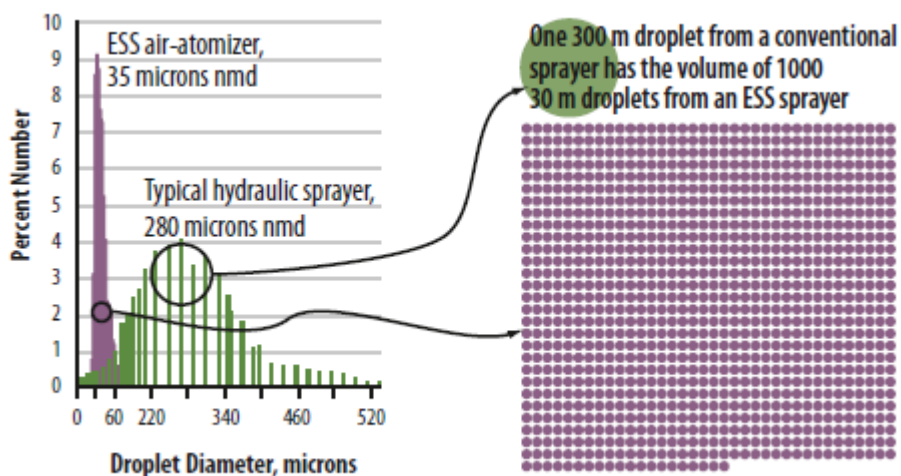


Figure 5 Droplet size of an electrostatic spray pump (Electrostatic spraying system, www.iandmsmith.com/upload/ElectrostaticSprayersforGrapeGrowersNEW.pdf)

With the ESS used in this study, a volume of ± 72 L/ha was applied. It is environmental friendly and compared to conventional mist blowers, gives a four-time better coverage on leaves, a six times better coverage on grape clusters and results in a nine times reduction in soil deposition (Electrostatic spraying system, www.iandmsmith.com/upload/ElectrostaticSprayersforGrapeGrowersNEW.pdf).

2.4.2 Dipping of bunches

Dipping of bunches was and is still the best application method for obtaining complete and uniform coverage of bunches (Kolasinska *et al.*, 2008). With dipping of bunches much more workers and time per block is needed to complete this action compared to a conventional spraying methods. Due to increased production costs, table grape producers are trying to find alternative application methods to replace dipping of bunches.

2.4.3 Recommendations for best practices to follow based on current knowledge

Over the years producers have been struggling with environmental and economic reasons demanding them to use less chemicals resulting in the strive towards finding alternative application methods while still maintaining effectiveness (Welsh *et al.*, 2000). An application method must not only improve the droplet-deposition efficiency, but also the spatial distribution of the deposited droplets throughout the canopy (Law, 2001). According to Sasaki *et al.* (2013) when the ESS is used correctly, it will provide advantages over the conventional spray, however many factors could affect the system efficiency.

The final decision regarding whether to spray conventionally at high or low volumes, or with an ESS, or by dipping bunches, will depend on the action that needs to be achieved, the

amount of money available to be spent on a spray pump, as well as availability of labour, if dipping is applied.

2.5 The effect of plant growth regulator treatments for thinning and berry sizing on table grape return fertility

The effect of plant growth regulator treatments on return fertility has been investigated by few researchers and limited published research results regarding this topic is available.

2.5.1 Gibberellic acid (GA₃)

Published research results regarding the effect of GA₃ treatments on return fertility are limited to a few cultivars. Several researchers reported that GA₃ treatments had a negative effect on return fertility and their main findings can be summarised as follows:

- Weaver and McCune (1959, cited by Lavee et al., 1993) found that growth of grapevine shoots was enhanced with GA₃ applications and GA₃ treatments that was applied early post-flowering led to a reduction of yield in the following season.
- Weaver (1960) reported that GA₃ application for yield in a specific year decreased shoot and cluster count in the following year.
- Jawanda *et al.* (1974) found that over application of GA₃ negatively affected bud fertility of Thompson Seedless in the following season
- According to Ziv *et al.*, 1981, and Harrell and Williams, 1987, as cited by Mullins *et al.* (1992) GA₃ applications can cause bud necrosis, which can lead to decreased fertility in the next season.
- Where GA₃ was applied to field grown vines, the formation of inflorescences was inhibited and the number of flowers produced per inflorescence was also reduced in the following season (Palma & Jackson, 1989).
- Orth (1990b) compared GA₃ spraying and dipping applications on Muscat Seedless and reported a reduction in the number of inflorescence in the season following treatment for treatments where the entire vine was sprayed.
- Coombe & Dry (1992b) recommended that when the canopy are being sprayed with GA₃, high dosages must be avoided, because the next season's bunch number may be reduced.
- GA₃ applications applied at flowering resulted in reduced fertility the following year (Mullins *et al.*, 1992).
- Peacock (1998) reported for Ruby Seedless that high rates of GA₃ applied at berry set caused a decrease in fertility the following year.

- GA₃ stretching applications have shown to have the most negative effect on the fertility the following year, due to the application being applied early in spring when flower primordia are being initiated (Christensen, 2000).
- In California where GA₃ had been used over a long term, table grape growers found a decline in vine fertility (Dokoozlian, 2000b). According to Dokoozlian (2000b) the berry sizing application are believed to be responsible for this, due to the high rates applied. However, when GA₃ is applied at fruit set the effect on bud fertility are variable and are highly dependent upon cultivar and season (Dokoozlian, 2000b).
- Dokoozlian & Peacock (2001) found with Crimson Seedless where GA₃ was applied at 80% flowering at concentrations between 0 to 25 g/ha GA₃, that where ≥ 6.25 g/ha GA₃ was applied at flowering, a significant reduction was found for cluster number per vine for two seasons after the first application.
- With Riesling where GA₃ was applied at 500L/ha and a concentration of 50 ppm during flowering (50-80% capfall), Weyand & Schultz (2004) also found a reduction in bud fertility for two seasons after the first application.
- According to Korkutal *et al.* (2008), extreme GA₃ dosages can negatively affect the crop load of the current season, as well as the next season.
- In a study done by Hed *et al.* (2011) on Chardonnay and Vignoles grapes, with the aim to reduce bunch compactness by applying GA₃ as a pre-flowering or as a full-to-late flowering application with rates not exceeding 25ppm, no negative effects of GA₃ were found in terms of yield loss and thus they concluded that the negative effects of GA₃ applications appear to be associated with much higher rates than those tested in their study, and that cultivar and application time play a major role in the effect of GA₃.
- Elgendy & Ahmed (2012) stated that high concentrations of GA₃ applied during crucial phenological stages during which clusters of the following year are formed, may cause a significant decrease in fertility.
- Molitor *et al.* (2012) made an assumption that when GA₃ is applied during the crucial time of 5-7 weeks after bud break, when the processes of initiation and differentiation have commenced, GA₃ has a direct or indirect effect on the differentiation process, favouring the development of more tendrils and less inflorescences, and also that pre-flowering applications may have an impact on inflorescence size.

2.5.2 CPPU (Forchlorfenuron)

CPPU in combination with GA₃ for berry sizing (normal GA₃ timings) did not reduce the return fertility more than GA₃ alone (Retamales *et al.*, 1995).

2.5.3 Ethephon

According to Shulman *et al.* (1980) when ethephon was applied after fruit-set, a reduction in the annual amount of wood and a slight reduction in the canes diameter were found, but the following season's potential spurs were not reduced by the treatments. Ethephon does not affect the vines fertility when it is applied at the recommended rate (Peacock, 2010).

2.6 Conclusions

Guidelines regarding the timing of viticultural practices or chemical treatments are often linked to grapevine phenological stages. The timing of bud break has a major influence on the course of the subsequent vegetative growth and reproductive development. Genetic, morphological and physiological factors, as well as cultivation practices affect grapevine fertility.

The potential yield of the next season is already determined during the current season, during initiation and differentiation of inflorescence primordia that take place from between two weeks before flowering, until about 10 weeks after flowering in the current season. Individual flower parts will only differentiate after recommencement of growth in the next spring.

In grapevines, three types of set occur, namely normal set, stimulative parthenocarpy and stenospermocarpy. The majority of current commercially important table grape cultivars set through stenospermocarpy, for example Prime, Crimson Seedless, Sultanina, Sugraone, Regal Seedless, Flame Seedless, Midnight Seedless and Sable Seedless.

To meet market requirements regarding table grape bunch size and compactness, as well as berry size, colour, flavour, texture and firmness, viticultural practices including the use of PGRs are applied. The five main PGR groups are gibberellins (GA), cytokinins (CK), auxins, abscisic acid (ABA) and ethylene. In the table grape industry, GA₃ is one of the most used PGR's to thin out clusters, decrease berry set and increase berry size, especially in seedless table grape cultivars that naturally set compact bunches with small berries.

There are several reports from the industry that decreased bud fertility is linked to GA₃ treatment, but very few published research results are available to support these practical observations. Some negative consequences of GA₃ treatments that have been reported by researchers are that GA₃ application delays maturity, increases cluster rigidity and berry shatter and also reduce the following season's bud fertility. It has been reported that if the recommended dosages are exceeded, a decline in fertility was observed.

GA₃ applications for thinning and sizing can be applied by bunch dipping or various spray application techniques. There are limited published scientific results available regarding the effect of different GA₃ application techniques on return fertility of grapevines and specifically of table grapes. The majority of published studies compared bunch dipping with conventional spraying and did not include different spray application techniques and spray volumes.

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Chapter 3

MATERIALS AND METHODS

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3.1 Experimental vineyard

The experiment was conducted over two seasons (2014/2015 and 2015/2016) on a 15-year-old *Vitis vinifera* L. cv. Prime vineyard grafted onto Ramsey rootstock on the farm Laborans in Paarl. The vines were spaced 3 m x 1.5 m and were trained onto a gable trellis system, with the rows orientated in an East-West direction. The vines were irrigated by means of scheduled micro-irrigation. Visually the vigour of the block is considered moderate. The soil type is clay loam and results of a soil analysis of the block, which was done on the 31st of March 2015, is shown in Addendum B. According to Raath (personal communication), the soil has a slightly high pH (pH_{KCl} of 6.2), e.g. higher than the optimal pH_{KCl} of 5.6. Based on this analysis, all macro and micro elements levels were within the prescribed norms (Van Schoor *et al.*, 2000). Viticultural practices were applied as recommended for the production of export quality Prime table grapes (Van der Merwe, 2014). The experimental vines were selected for uniformity. Vines were pruned to nine node fruiting canes with eight to ten canes allocated per vine.

3.1.1 Weather data

Weather data for 2013/2014, 2014/2015 and 2015/2016 season was obtained from a weather station, Môrewag, approximately within 4km from the experimental blocks (Lat 33° 40' 43.8"S, Long 18° 56' 33.4"E) (Source: Ileaf: www.ileaf.co.za). The Berg River Valley is a winter rainfall area with an average rainfall per year of 772mm, the region often experiences rain in the summer, and with an average maximum temperature of 25°C (Wettergreen & Avenant, 2016).

Long term weather data of the average monthly minimum and maximum temperatures, daily rainfall is displayed in Figure 6, heat unit accumulation (base temperature of 10°C) is displayed in Figure 7 and cold unit accumulation is displayed in Figure 8, both as Infruitec or Daily Positive Chill Unit (DPCU) accumulation (Linsley-Noakes and Allan (1994), as cited by (Luedeling, 2012)) as well as Richardson or Utah units (Richardson et al (1974), as cited by (Luedeling, 2012)) from July 2014 up until June 2016, for the Paarl are during the period which the trial was conducted (Source: Ileaf: www.ileaf.co.za).

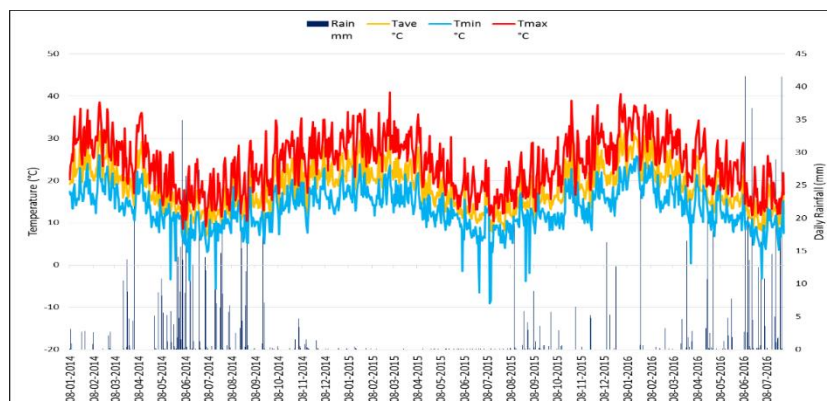


Figure 6 Average monthly minimum and maximum temperatures as well as daily rainfall for Paarl, Berg River Valley, for 2014/2015 and 2015/2016 season (Source: Ileaf: www.ileaf.co.za).

Over the three seasons, similar trends in minimum and maximum temperatures were found (Figure 6). However, minimum and maximum temperatures were lower in October, November and December of the 2014/2015 season than in the 2015/2016 season.

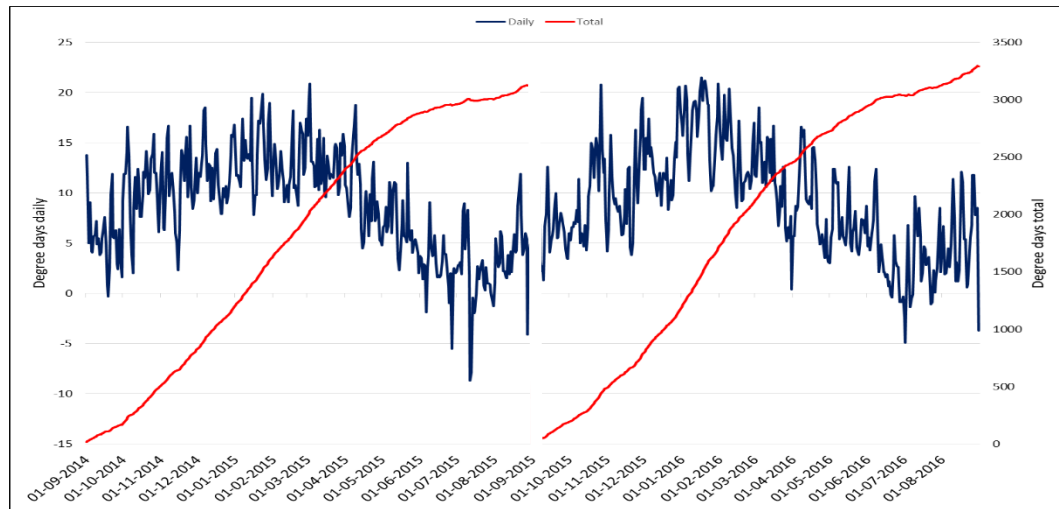


Figure 7 Heat unit accumulation (Degree days) for Paarl, Berg River Valley, for 2014/2015 and 2015/2016 season (Source: Ileaf: www.ileaf.co.za).

More rainfall occurred in the 2015/2016 season over the period mentioned above compared to the 2014/2015 season. During 2014/2015 harvest month (December), average of 0.02mm rainfall occurred and during 2015/2016 harvest month (December), average of 1.1mm rainfall occurred. In both seasons of the trial, no rainfall occurred during the application period.

According to Avenant & Avenant (2014), long term weather data indicates that the Paarl area of the Berg River Valley receives more than 400 chill units (Infruited units) during the period 1 May to 31 August, which is sufficient to obtain an even bud break. They also found that Prime has a lower chilling requirement (50 Infruited units) compared to Sultana and Sugraone. According to the cold unit accumulation (Figure 8) Paarl received sufficient cold units during May to August of all three seasons. During all three seasons, as part of standard viticultural practices applied on the farm, hydrogen cyanamide (2.5%) was applied as rest breaking agent (at three to four weeks before normal bud break) for obtaining even bud break.

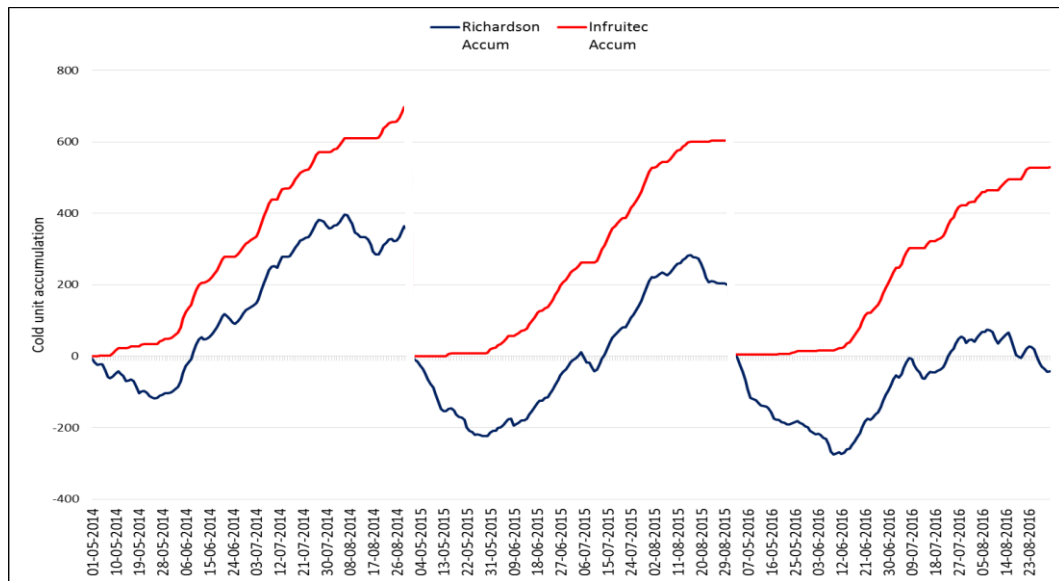


Figure 8 Cold unit accumulation for Paarl, Berg River Valley, for 2013/2014, 2014/2015 and 2015/2016 season (Source: Ileaf: www.ileaf.co.za).

3.1.2 Phenology

In the 2014/2015 season bud break was reached one week before the 2015/2016 season, and full bloom was five days earlier compared to the 2015/2016 season. The rest of the phenological development for both seasons followed a similar trend. In Table 5 the phenological stages for the 2014/2015 season and 2015/2016 seasons are presented and in Table 6 temperature conditions from one day before to one day after bud break, from one day before to one day after full bloom, as well as for the ripening period are presented.

Table 5 Phenological stages of Prime in the return fertility project at Laborans for both seasons.

Phenological stage	2014/2015 season	2015/2016 season
Bud break	20/08/2014	27/08/2015
Full bloom	15/10/2014	20/10/2015
Véraison	18/11/2014	22/11/2015
Harvest	17/12/2014	21/12/2015

Table 6 Temperature conditions one day before to one day after bud break and full bloom as well as during ripening at Laborans for both seasons (Source: Ileaf: www.ileaf.co.za).

Phenological stage	Date	Minimum (°C)	Maximum (°C)	Average (°C)	Average Relative Humidity (%)
Bud break 2014	19/08/2014	12.3	15.7	13.4	84.9
	20/08/2014	8.2	12.2	11.3	91.5
	21/08/2014	7.9	14.3	10.7	87.3
Bud break 2015	26/08/2015	9.3	22.6	14.7	81.1
	27/08/2015	11.9	18.6	14.8	86.1
	28/08/2015	-3.9	15.7	13	83
Full bloom 2014	14/10/2014	14.2	28.8	22.6	45.3
	15/10/2014	12.8	22.3	17.2	72.7
	16/10/2014	13.3	26.2	16.6	64.5
Full bloom 2015	19/10/2015	20.3	29.7	25	50.1
	20/10/2015	18.1	31.1	23.6	61.4
	21/10/2015	17.3	25.6	20.5	64.6
Ripening 2014 (Véraison – Harvest)	18/11/2014 – 17/12/2014	17	28.5	22.4	53
Ripening 2015 (Véraison – Harvest)	22/11/2015 – 21/12/2015	16.3	28.8	22.3	55

3.2 Experimental layout

The experimental block (Figure 9) comprised of 18 rows where each row consisted of 15 experimental units, with each unit containing five vines. Each experimental plot was allocated over three rows and three experimental units. The central unit in the central row of each experimental plot was the data experimental unit. All the field sampling was done in the data experimental unit.

The experimental design used was randomized with six treatments and each treatment had five replications. The experimental design contained 30 experimental plots, where each experimental plot consisted of nine experimental units with 5 vines each (45 vines per plot).

Row no.	Treatment no. (Colour code)														
	Vak	Vak	Vak	Vak	Vak	Vak	Vak	Vak	Vak	Vak	Vak	Vak	Vak	Vak	Vak
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
115	1	1	1	2	2	2	3	3	3	5	5	5	4	4	4
116	1	1	1	2	2	2	3	3	3	5	5	5	4	4	4
117	1	1	1	2	2	2	3	3	3	5	5	5	4	4	4
118	5	5	5	6	6	6	4	4	4	2	2	2	1	1	1
119	5	5	5	6	6	6	4	4	4	2	2	2	1	1	1
120	5	5	5	6	6	6	4	4	4	2	2	2	1	1	1
121	2	2	2	1	1	1	4	4	4	6	6	6	3	3	3
122	2	2	2	1	1	1	4	4	4	6	6	6	3	3	3
123	2	2	2	1	1	1	4	4	4	6	6	6	3	3	3
124	3	3	3	5	5	5	2	2	2	1	1	1	4	4	4
125	3	3	3	5	5	5	2	2	2	1	1	1	4	4	4
126	3	3	3	5	5	5	2	2	2	1	1	1	4	4	4
127	4	4	4	6	6	6	3	3	3	5	5	5	2	2	2
128	4	4	4	6	6	6	3	3	3	5	5	5	2	2	2
129	4	4	4	6	6	6	3	3	3	5	5	5	2	2	2
130	6	6	6	5	5	5	1	1	1	3	3	3	6	6	6
131	6	6	6	5	5	5	1	1	1	3	3	3	6	6	6
132	6	6	6	5	5	5	1	1	1	3	3	3	6	6	6

Figure 9 Experimental layout of Prime in the return fertility project at Laborans.

3.3 Statistical procedures

The data were subjected to an appropriate analysis of variance (ANOVA). The Shapiro-Wilk's test was performed on the standardized residuals to test for deviations from normality (Shapiro & Wilk, 1965). In cases where significant deviation from normality, and it was due to skewness, outliers were removed until it was normal or symmetric distributed (Glass *et al.*, 1972). Student's t-LSD (Least significant difference) were calculated at a 5% significance level to compare means of significant source effects. All the data analysis was performed with SAS version 9.3 statistical software (SAS Institute, 1999). Tables and graphs of means were done in Excel 2013.

3.4 Treatments

Thinning and berry sizing treatments were applied according to commercial concentrations recommended for Prime (Van Der Merwe, 2014). In this experiment, GA₃ application methods and different application volumes per hectare were evaluated (Table 7). In Addendum A, the calculations for the GA₃ application rate for thinning and berry sizing treatments for the different application volumes are shown.

Two GA₃ treatments were applied to the vineyard during two different phenological stages. The first application was the thinning treatment, which was applied at 80-100% FB (on 18 October 2014 for the first season (2014/2015) and 23 October 2015 for the second season (2015/2016)).

The second application was the berry sizing treatment, which was applied when the berries were at 7-8 mm diameter (on 1 November 2014 for the first season (2014/2015) and 3 November 2015 for the second season (2015/2016)).

Six treatments were applied (Table 7): Treatment 1 (NoThin + Dip (Control)), comprised of a no thinning application, followed by the berry sizing treatment applied by dipping (15 ppm GA₃). Treatment 2 (Thin + Dip) comprised a conventional thinning spray application (2 g GA₃/ha), followed by a berry sizing treatment applied by dipping (15 ppm GA₃). Treatment 3 (Thin + 250 L/ha), Treatment 4 (Thin + 500 L/ha) and Treatment 5 (Thin + 1000 L/ha) comprised conventional thinning spray application (5 g ProGibb/ha applied in 1000 L/ha) with a mist blower (Agrico 1500 L, 1000 L/ha – 4x green Albus nozzle/side, 45 sec/50 m rpm), followed by berry sizing treatments (15 g GA₃/ha) applied by spraying with a mist blower (Cima Blitz Model A.T50S2.15.L11, 1500 L/ha, Figure 10b) with spray volumes of 250 L/ha, 500 L/ha and 1000 L/ha respectively. Treatment 6 (Thin + ESS) comprised a conventional thinning spray, followed by a berry sizing treatment, applied at 72 L/ha with an electrostatic spray pump (ESS 150RB, 378 L, 85 L/ha, 51 sec/50 m rpm, Figure 10a).

Table 7 Treatments that were applied on Prime in the return fertility project at Laborans.

Treatment no.	Treatment Code	Treatment Colour code	Thinning application (1000 L/ha; 2 ppm GA ₃ ; 2 g GA ₃ /ha; 5 g ProGibb/ha)		Berry sizing application (15 g GA ₃ /ha; 37.5 g ProGibb/ha)	
			Application technique and volume	Phenological Stage	Application technique and volume	Phenological Stage
1	NoThin + Dip (Control)	RED	No thinning		Dip	7-8 mm berry diameter
2	Thin + Dip	WHITE RED	Spray conv. 1000 L/ha	80-100% Full bloom	Dip	
3	Thin + 250 L/ha	WHITE YELLOW			Spray conv. 250 L/ha	
4	Thin + 500 L/ha	WHITE BLACK			Spray conv. 500 L/ha	
5	Thin + 1000 L/ha	WHITE BLUE			Spray conv. 1000 L/ha	
6	Thin + ESS	WHITE GREEN			ESS (72 L/ha)	



Figure 10 Berry sizing application methods; a) Electro static spray pump, b) Mist blower spray pump (Photo by Rovic Leers, 2014)

The thinning treatment followed no order in applying the treatment due to same concentration. The berry sizing treatments were applied in the following order: Treatment 5, Treatment 4, Treatment 3 and Treatment 6, from the highest to the lowest volume. It was done in this order to avoid the risk of contamination between treatments. The dipping treatments were applied at the same time as the berry sizing treatments which were being applied by spray pumps.

In Figure 11 application of berry sizing treatments is illustrated: both sides of the nozzles were open where both sides of the canopy row had to be treated. With the outside rows, only one side's nozzles were open and directed to the row that required that specific application, whereas the other side's nozzles were kept closed.

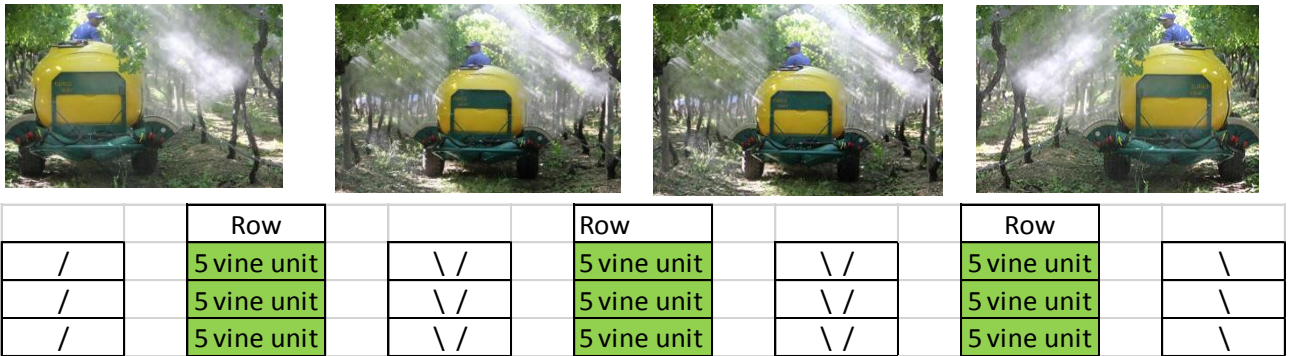


Figure 11 Spray application over an experimental plot (Photo by Rovic Leers, 2014)

Legend:
 / = One side of nozzles open, directed to canopy, other side closed
 \ = One side of nozzles open, directed to canopy, other side closed
 > = Both nozzles open, directed to canopy

The dipping treatment (Figure 12) was first made up in a water container, containing the solution, at the same concentration as the spraying application for Treatment 5. The solution was transferred into smaller containers (Figure 12a) before application to allow labourers to work easier during the application. The whole bunch was dipped into the container (Figure 12b).



Figure 12 Dipping method; a) Small container used for dipping, b) Dipping of whole bunch in container.

During all spray applications, the nearby data experimental units were protected with thick plastic sheets (Figure 13), to prevent contamination.



Figure 13 Plastic sheets that were used during spray to shield untreated vines (Photo by E. Avenant, 2014)

3.4.1 Marking of bunches according to phenological stage

In both seasons, before the thinning application was applied, 15 inflorescences per data experimental unit were marked according to a phenological stage on that specific day in the experimental vineyard. In the 2014/2015 season, ten inflorescences per data experimental unit were marked with red insulation tape to indicate the phenological stage 80-100% FB (Figure 14a), whereas five inflorescences were marked with blue insulation tape indicating the phenological stage 10% set (Figure 14b). In the 2015/2016 season five inflorescences per data experimental units were marked with red insulation tape to indicate the phenological stage 80-100% FB, five inflorescences were marked with blue insulation tape indicating the phenological stage 10% set and five inflorescences were marked with green insulation tape indicating the phenological stage 100% set (Figure 14c).

No manual bunch preparation actions (removing of small berries, shortening clusters) were applied to these marked bunches and no berry sampling were done from them. The natural state of these clusters were preserved for evaluation of bunch structure and compactness at harvest.

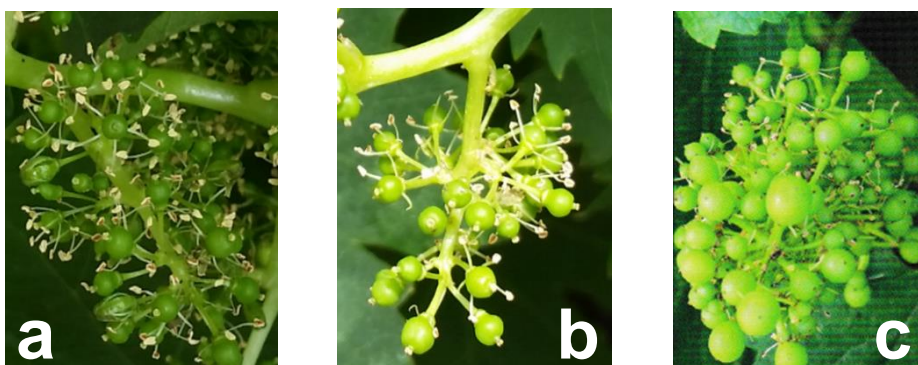


Figure 14 Phenological stages of bunches during thinning application. a) 80-100% Full-bloom (FB) bunch (Photo by E. Avenant, 2015), b) 10% Set bunch (Photo by E. Avenant, 2015), c) 100% set bunch (Iland *et al.*, 2011)

3.4.2 Application of fluorescent product to assess spray coverage

During berry sizing applications, applications with water containing a fluorescent product (Ruco-Blanc ADE, 100L/x) were also done using the same application techniques and volumes as indicated for GA₃ in Table 7. The fluorescent product applications were done in another part of the same commercial block. It was not done in the experimental plots due to the risk of interaction between the fluorescent product and GA₃ which could have affected data. The fluorescent product was applied in water only (no GA₃ added).

Shoots, leaves and bunches sprayed with fluorescent product were evaluated during the evening of the same day to visually assess coverage obtained with the different application methods (volumes) (Figure 15). Brink's (2005) method for visual assessment of fluorescent pigment were used. Due to the project being on a commercial table grape farm no shoots, leaves or bunches could be removed for image processing and analysis under laboratory conditions. Images obtained during visual assessment can be used to explain certain results obtained.



Figure 15 Fluorescent spray evaluated during the night
(Photo by Rovic Leers, 2014)

3.5 Manual thinning: timing experiment

Before any sampling could take place all undesired bunches were removed on 7 November 2014 in the 2014/2015 season and on 4 November 2015 in the 2015/2016 season. Undesired bunches include bunches that are too small, lagging behind the specific phenological stage, as well as damaged bunches.

After the removal of these bunches, manual labour was used to remove all small berries. The manual thinning out action was done on 24 November 2014 in the 2014/2015 season and on 18 November in the 2015/2016 season.

Two farm workers worked together on one data experimental unit, each on one side. The labourer's total time to remove small berries from all bunches in the data experimental unit was measured by using a stop watch.

3.6 Pre-harvest evaluation

3.6.1 Field sampling

Berry sampling was done on a weekly basis for monitoring berry development and ripening from 10 November to 17 December 2014, and from 09 November to 21 December 2015 respectively for the two seasons. With each collection date 50 berries of each data experimental unit were collected for determining total soluble solids (TSS), pH and titratable acid (TA). In the data experimental unit 25 berries on each side of the vines were collected (five berries on each side of each vine). Berries were collected randomly across each data experimental unit.

In 2014/2015 season from 1 December 2014, 80 berries were collected and in 2015/2016 season from 30 November 2015, 80 berries were collected. Eight berries on each side of each vine were collected, of which 50 berries were used for determining TSS, pH and TA. The additional 30 berries were used for organic acid analysis.

3.6.2 Berry development and ripening

After each collection date the 30 samples were taken back to the laboratory. Berry length and diameter of 50 berries per sample were measured with a digital calliper. After these measurements, the total sample mass was determined with a three-decimal digital scale (Precisa, Type. 280-9826, PAG Oerlikon AG, Zurich, Switzerland), whereafter the sample was homogenised (liquidised) with a household blender and separated from materials other than clear juice. The TSS (°Brix) was measured with a digital pocket refractometer (Atago PAL-1, Tokyo, Japan). A volume of 50ml clear juice was measured out with a 50ml glass pipette and poured into a 100ml measuring glass holder, which was placed in the rotor of an automatic titration device (Metrohm 785 DMP Titrino, Herisau, Switzerland) connected to a bench pH meter (Crison Basic 20 with Crison 5531 PT1000 electrode, Barcelona, Spain) for the measurement of TA (g/L) and pH. The Metrohm was calibrated each time before placing the samples in the rotor at pH 7 and pH 4. The TA was determined using sodium hydroxide at a concentration of 0.33%.

Prime has a low acid level at harvest maturity, therefore sugar alone is the best parameter for measuring the optimum maturity (Sonego *et al.*, 2002). Prime is harvested at a minimum soluble solids level of 14% (DAAF, 2012), where it has a good organoleptic quality (Sonego *et al.*, 2002), making the optimal maturity for Prime if harvested on the sugars alone at 16°Brix (Van Der Merwe, 2014; SATI, 2015).

3.6.3 Organic acid

The organic acid (malic acid) was determined in October 2015 and in January 2016. This measurement was conducted on berries obtained from berry sampling from véraison up until harvest of the 2014/2015 season and 2015/2016 season. Frozen berry samples (n=30; -20°C) were defrosted, weighed, and homogenised using a household blender and separated from materials other than clear juice, where after 50ml clear juice was transferred into 50ml falcon tubes for analyses. The analysis was done in an ISO credited controlled laboratory area at Vinlab,

Stellenbosch, South Africa. Analysis of organic acid were measured using an enzymatic test with L-Malate-dehydrogenase (L-MDH) and Glutamate-Oxalacetate-Transaminase (GOT). The method was performed at 70°C, using 340nm filter. The values obtained from this method were expressed as gram per liter.

3.6.4 Bunch structure

Before harvest, in both the 2014/2015 and 2015/2016 seasons, the bunch length (Figure 16a) and diameter of the rachis (Figure 16b) were measured in the vineyard. The bunch length, from the first lateral to the last berry, was measured by using a custom-made tool designed by J.H. Avenant. The length was measured with a measuring tape and the diameter of the rachis was measured with a digital calliper 1cm above the first lateral.

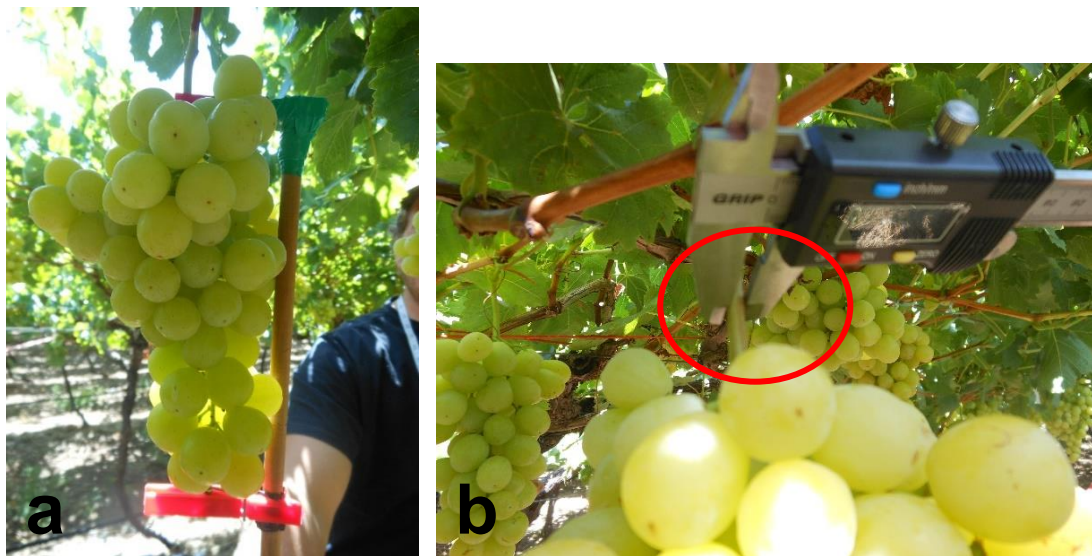


Figure 16 Bunch structure measurements in vineyard; a) Measuring bunch length with custom-made tool, b) Measuring rachis diameter

3.7 Harvest evaluation

The field experiment was harvested when the grapes reached the minimum export maturity of 16° Brix on 17 December 2014 for the first season and on 22 December 2015 for the second season. The grapes were packed according to South African export standards and placed in cold storage for six weeks at -0.5°C to 2°C, followed by one week at 7.5°C. After cold storage, various grape quality parameters were evaluated, according to standard methods of ARC Infruitec-Nietvoorbij, Plant Protection and Viticulture Division.

3.7.1 Yield and Pack out

In the pack house, total yield per data experimental unit was determined and grapes were separated into four classes (based on berry size), namely: XL (>20mm), L (>18mm), R (>15mm) and Cull (<15mm). Bunch size and compactness were also assessed to distinguish between

export quality, local quality and cull. The total mass and number of clusters in each category were determined for each replication.

Within each class, the total number and total mass of marked clusters (red/blue/green) were determined. The marked clusters of each data experimental unit were placed together to be analysed further. On the marked bunches the rachis, the distance from above the first lateral to just above the fifth lateral was measured. The first four laterals of each marked cluster were removed and placed in a clear marked bag for further measurements in the laboratory.

3.7.2 Bunch structure

Bunch structure measurements were done according to the standard protocol applied by ARC Infruitec-Nietvoorbij, Plant Protection and Viticulture Division. On each sample of four laterals (Figure 17) collected during harvest (see 3.7.1) the following measurements were done to evaluate bunch compactness and determine berry size distribution:

Firstly, all the shot berries and berries smaller than 15 mm were removed from the four laterals and counted separately (Figure 18). Thereafter all the berries left on all four laterals were cut off as close to the lateral as possible and counted (Figure 19).

The total length of the four laterals were measured with a measuring tape (Figure 20). Thereafter each lateral's diameter was measured with a digital calliper (Figure 21). The pedicel diameter of 16 randomly selected normal berries (>15 mm) were measured (Figure 22). Thereafter the total mass of all the normal berries (>15 mm) were determined (Figure 23).

After measuring all the samples for each data experimental unit, 80 berries were randomly selected for determining TSS, TA, pH and organic acid as described in section 3.6.2 and section 3.6.3.



Figure 17 Four laterals that were removed from marked bunches during harvest (Photo by R. van der Vyver, 2014)



Figure 18 Removing berries smaller than 15mm and shot berries from the four laterals (Photo by R. van der Vyver, 2014)



Figure 19 Removing of berries larger than 15mm (Photo by R. van der Vyver, 2014)



Figure 20 Measuring the total length of the four laterals (Photo by R. van der Vyver, 2014)



Figure 21 Measuring the diameter of each of the four laterals (Photo by R. van der Vyver, 2014)



Figure 22 Measuring the pedicel diameter of 16 normal (>15mm) berries (Photo by R. van der Vyver, 2014)



Figure 23 Determining total mass of all the normal (>15mm) berries (Photo by R. van der Vyver, 2014)

3.8 Pruning measurements

During full dormancy (June 2015 and June 2016) all the vines in the experimental unit were pruned to half long beares and the dormant canes were weighed. The yield to pruning mass ratio (Ravaz index) is used as indicator of the reproductive to vegetative growth relationship in the grapevine (Iland *et al.* 2011).

3.9 Post-harvest evaluation)

During the 2014/2015 harvest for each replication, two 4.5 kg cartons were packed and during the 2015/2016 harvest for each replication, one 4.5 kg carton were packed according to export guidelines and stored for six weeks at -0.5°C and for one week at 7.5°C before post cold storage evaluation was conducted. After cold storage, ±2 kg grapes from each carton were evaluated for post-harvest quality.

These grapes were evaluated for the following: stem condition, stem brittleness, berry firmness, decay, loose berry, berry split, SO₂ damage and browning (Table 8). The method was adapted from Avenant & Avenant (2006) and Zoffoli *et al.* (2009).

Table 8 Categories for post-cold storage quality evaluation

Categories		
stem condition	*1	
stem brittleness	*2	
berry firmness	*3	
decay (g)		
mass (g) loose berry	-SO ₂	+SO ₂
mass (g) loose berry (Shake)	-SO ₂	+SO ₂
mass (g) berry split	-SO ₂	+SO ₂
mass (g) SO ₂ damage	Pedicel	Surface
mass (g) browning	External	Internal

Number code	Scale
*1	1 (very good, green + hydrated) to 5 (very poor, brown + desiccated)
*2	1 (not brittle) to 5 (very brittle)
*3	1 (turgid) to 5 (flaccid)

3.10 Bud fertility of the experimental block

Fertility was determined according to three different methods, namely forced budding in glasshouse, bud dissection and actual fertility in the vineyard. The experimental block was pruned with canes (nine buds per cane) and therefore fertility was assessed from bud position one to nine. Dormant shoots were used for both the forced budding and bud dissections methods.

3.10.1 Potential fertility

3.10.1.1 Plant material

In the 2013/2014 season 40 canes (20 canes for forced budding and 20 canes for bud dissection) were randomly collected on 31 July 2014 in the experimental vineyard to assess the fertility before commencement of the project.

In June 2015 two representative canes from each replicate of all six treatments were collected (60 canes in total) for forced budding and bud dissection.

3.10.1.2 Forced budding in glasshouse

In the 2013/2014 season 180 single-node cuttings (4-5 cm long) were prepared. On each collection date in the 2014/2015 season and 2015/2016 season 270 single-node cuttings (4-5 cm long) were prepared. The fertility was only determined from bud position one to nine for each cane.

In the 2013/2014 season 20 canes were stored in plastic bags at 4°C for six weeks. After storage each cane was cut up into single-node cuttings, which were placed in water trays (tap water) in a glasshouse at 25°C (Palma & Jackson, 1989). Experimental errors did occur during the first trial. Bud break occurred but no inflorescences were noted. Possible reasons for this were that the canes were stored for too long in cold storage and that they started to dry out.

In the 2014/2015 and 2015/2016 season, the plant material as explained above (see section 3.10.1.1) were collected. Plant material was collected and were taken directly to the glasshouse for forced budding at 25°C, where each cane was cut up into single-node cuttings and placed in water trays (Figure 24a) (Palma & Jackson, 1989).

Bud break for each collection date was noted every four to five days for a period of 160 days. Bud break was indicated by the presence of visible green tissue (Figure 25a) appearing at the tip of the bud (Shulman *et al.*, 1983; Palma & Jackson, 1989; Dunn & Martin, 2000).

The cuttings were kept in the water trays until inflorescences were noticeable in open buds (Figure 25b) (Palma & Jackson, 1989). After recording the inflorescence, the cuttings were removed (Figure 24b).

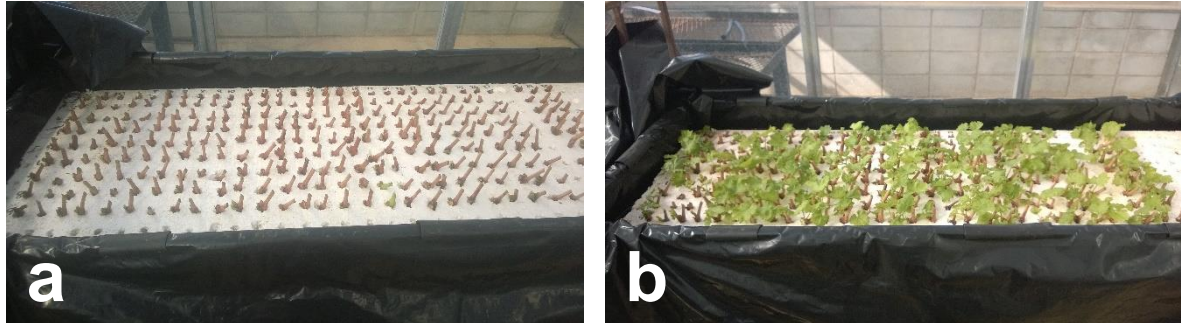


Figure 24 Forced budding in glasshouse; a) Tray with dormant buds, b) Tray after four weeks, budded buds can be seen.

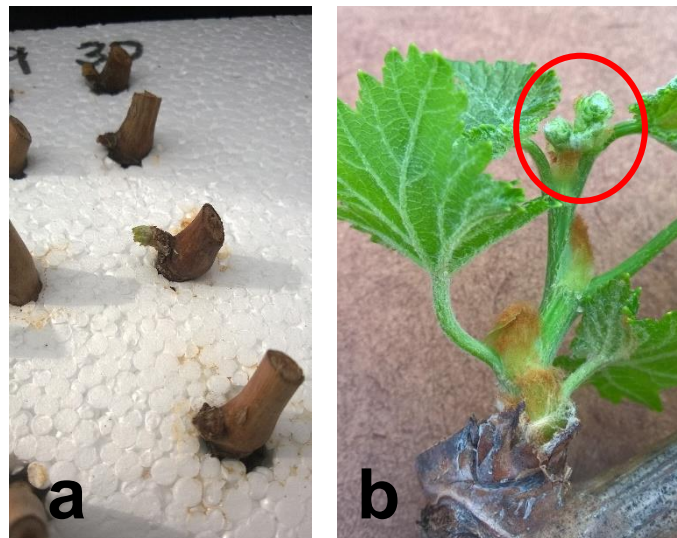


Figure 25 a) Early stage of budding detection, b) Inflorescence detected

3.10.1.3 Bud dissection

Bud dissections were done at ARC Infruitec-Nietvoorbij, Plant Protection and Viticulture Division, Stellenbosch. Before bud dissections were done, the internode length of each cane was recorded (in both 2014/2015 and 2015/2016) and the diameter of each internode was also measured in 2015/2016. Thereafter the canes were cut up into single node cuttings. Each bud was evaluated under a stereomicroscope, where the number of inflorescence primordia was determined and noted. Bud mite status was also determined.

The method for bud dissection as described by Swanepoel, J.J. and Baard (1988) was used. Firstly, the hard cork that covers the wool was removed (Figure 26a). After the hard cork were removed the wool also needs to be removed. With very precise handling the leaves need to be folded open to detect the inflorescence. Inflorescences are easily noticeable due to the lobes that

usually are present on the primordia (Swanepoel & Baard, 1988). To be considered an inflorescence primordia, three noticeable lobes must be clearly visible under the stereomicroscope as described by Srinivasan and Mullins (1981) and by Swanepoel and Baard (1988). After the inflorescences were noted and recorded, it was removed (Figure 26b).

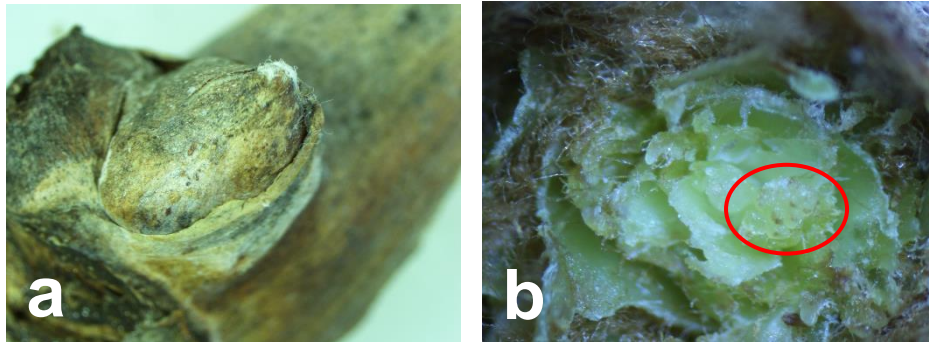


Figure 26 Bud dissection; a) closed dormant bud, b) Bud that is opened, red ring indicates the inflorescence

3.10.2 Actual Fertility

Before the project commenced, the actual fertility was evaluated on 20 canes, on the same vines allocated for potential fertility (see section 3.10.1.1), from bud position one to nine, just before crop control. In the 2014/2015 season two shoots per data experimental unit were evaluated from bud position one to nine before crop control. It was noted whether the bud did develop, as well as the structure/organ originating from it (vegetative shoot, reproductive shoot or inflorescence).

The actual fertility after the second season's treatment will not be presented in the thesis, because it can only be determined in November 2016, while the proposed thesis submission date is end of September 2016. Actual fertility determined in November 2016 will be included in the next research progress report of the project.

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Chapter 4

RESULTS AND DISCUSSION

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Introduction

In this chapter results regarding fertility, manual thinning, berry development and ripening, yield, bunch structure and post-harvest quality, as affected by the different treatments are presented.

4.2 Fertility

4.2.1 Potential and actual fertility before the trial commenced

4.2.1.1 Potential fertility (Bud dissection) and actual fertility

Potential fertility before the trial commenced (determined through bud dissections) revealed that at least one inflorescence primordia per node position was found from node no. 4 to no. 9 on the cane and an average of 1.01 over all node positions, which indicates an acceptable level of fertility (Table 9). According to Iland *et al.* (2011) the compound bud has the potential to form at least one inflorescence. Total of 8.40 inflorescence primordia per 9 node cane was found. The actual fertility, expressed as total bunches per cane was 6.53 (Table 9), $\pm 22\%$ lower than the potential fertility. The results indicate a difference between potential and actual fertility. This corresponds to the results of Vasconcelos & Koblet (1990) that the potential fertility may not accurately represent the actual fertility in the vineyard, as well as Sommer *et al.* (2000) who reported that in their trials the potential fertility values were always higher than the actual fertility.

Table 9 Potential and actual fertility of Prime at Laborans in 2014, before commencement of the project.

Potential fertility (June 2014) (Bud dissection results - no. of inflorescence primordia)		Actual fertility (November 2014) (no. of bunches)	
Node position	Mean per node position	Node position	Mean
1	0.81	1	0.22
2	0.88	2	0.25
3	0.72	3	0.72
4	1.14	4	0.85
5	1.00	5	0.92
6	1.03	6	0.97
7	1.09	7	0.95
8	1.29	8	0.80
9	1.13	9	0.82
Total (per 9 node cane)	8.40	Total (per 9 node cane)	6.53
Average potential fertility (inflorescence primordia per node position)	1.01	Average actual fertility (bunches per node position)	0.72
		Total (bunches per vine)	30.7

Results of both assessment methods (Table 9), indicated that node bud positions no. 1 to no. 3 had a lower potential and actual fertility than node positions no. 4 to no. 9. This confirms that the pruning method of half long bearers applied in the experimental block, as part of the standard production practices applied in all commercial Prime blocks on Laborans, is the correct pruning method for this cultivar and area, as recommended by Van der Merwe (2014).

Possible reasons for the difference between potential (June 2014) and actual fertility (November 2014) are: with bud dissection, every compound bud that might have contained inflorescence primordia, from bud position no. 1 to no. 9 was evaluated and could be seen under the microscope, without knowing whether or not it will bud and the final differentiation of inflorescences and individual flowers will completely take place. The final differentiation of inflorescence primordia into inflorescences with fully developed individual flowers takes place between bud break and flowering and are affected by several environmental conditions and cultivation practices, as explained in section 2.2.4.1 in Chapter 2.

With actual fertility, all shoots that developed after bud break are visually assessed and the actual number of bunches present are noted. Therefore, actual fertility is more reliable than potential fertility to estimate yield. Potential fertility can be used in decision making regarding appropriate pruning systems for a specific cultivar, for example in this particular case, if the producer made his/her decision solely on the available cultivar description and available industry cultivation guidelines that “Prime is a very fertile cultivar that can be spur pruned or with half long bearers, depending on the growing area” (Van Der Merwe, 2014) and decided to spur prune, it could have resulted in a lower yield than what they did obtain with the 9 node canes that they did use.

4.2.2 Potential and actual fertility after treatments were applied

Potential fertility and actual fertility after treatments were applied were assessed to determine whether fertility was affected by GA₃ treatments (Table 10). During assessment of potential and actual fertility, bud break percentage was also measured to determine whether bud break was affected by GA₃, as well as whether commercially acceptable levels of bud break (>80%, according to Dokoozlian & Williams (1995) and Dokoozlian (1999)) were obtained. The bud break% determined through forced budding in June 2015 and June 2016, as well as through assessment in the vineyard (October 2015) did not differ significantly between treatments and was above 80% for all treatments (Table 10).

Commercially acceptable levels of bud break%, were thus obtained in this trial in both seasons. This differs from the results of Orth (1990b) (cv. Muscat Seedless in the Berg River Valley) that spraying the whole vine with GA₃ (10ppm at FB, followed by 20ppm at 10 days after FB), led to a reduced bud break in the following season (36% compared to 75%+ for dipping and control treatments). In this trial the standard recommended GA₃ dosages and number of applications for Prime was applied (1 x 2ppm at 80-100% FB and 1 x 15ppm at 7-

8mm berry diameter, see Chapter 3.4). The lower GA₃ dosages used in this trial could explain why bud break was not negatively affected.

4.2.2.1 Potential fertility (Forced budding)

The average potential fertility over all treatments assessed in June 2016 was lower than in June 2015 (Table 10).

In June 2015, significant differences were found between treatments (Table 10). Treatment 1 had a significantly higher potential fertility and Treatment 6 had a significantly lower potential fertility compared to the other treatments. In June 2016, no significant differences were found between treatments (Table 10), although Treatment 6 again had the lowest potential fertility.

Possible reasons for the high potential fertility obtained with Treatment 1 in June 2015, is that no part of the canopy was sprayed with GA₃, only bunches were treated with a dipping application. This corresponds to the results of Orth (1990b) that dipping of bunches with GA₃ contributed to maintaining higher fertility compared to spraying the entire vine. Regarding Treatment 6 it is suggested that the lower application volume and smaller droplet size obtained with the ESS are associated with lower fertility, possibly due to more effective coverage obtained on not only the bunches (the target organs for berry sizing treatments), but also on the shoots and buds and that GA₃ applied to the buds had a negative effect on potential fertility.

The GA₃ thinning treatments in this trial were applied during the flowering stage, when initiation of inflorescence primordia for the next season's crop were taking place. Molitor *et al.* (2012) reported that when GA₃ is applied during the crucial time of five to seven weeks after bud break when the process of inflorescence primordia initiation and differentiation commenced, GA₃ has a direct or indirect influence on the differentiation process favouring the development of more tendrils and less inflorescences. In this trial, the GA₃ berry sizing treatments of both seasons (2014/2015 and 2015/2016) were done at 6-8mm berry size, when the inflorescence primordia differentiation for the next season's crop had already commenced and thus spray applications reaching the buds, might have affected this process negatively.

Table 10 Potential return fertility and actual return fertility of Prime at Laborans, after the 1st season's treatments (applied in Oct-Nov 2014) and after the 2nd season's treatments (applied in Oct-Nov 2015)

No.	Treatment	Forced budding				Bud dissection		Assessment in vineyard		
		Bud break		Potential fertility (no. of bunches)		Potential fertility (no. of inflorescence primordia)		Bud break	Actual fertility (no. of bunches)	
		Jun-15	Jun-16	Jun-15	Jun-16	Jun-15	Jun-16	Oct-15	Nov-15	
		(%)	(%)	(per sprouted bud)	(per sprouted bud)	(average over all node positions)	(average over all node positions)	(%)	(per vine)	(per 9 node cane)
1	NoThin+Dip (Control)	95.6 a	93.3 a	1.03 a	0.52 a	1.30 a	1.06 a	98.9 a	40.2 a	6.70 a
2	Thin+Dip	86.7 a	97.8 a	0.67 ab	0.72 a	0.98 a	1.06 a	95.5 b	40.1 a	6.68 a
3	Thin+250L/ha	95.6 a	95.6 a	0.82 ab	0.72 a	1.02 a	0.76 ab	100 a	30.4 b	5.07 b
4	Thin+500L/ha	95.6 a	95.6 a	0.69 ab	0.73 a	1.07 a	0.91 a	100 a	40.0 a	6.67 a
5	Thin+1000L/ha	97.8 a	91.1 a	0.66 ab	0.77 a	0.83 a	0.48 b	100 a	41.6 a	6.93 a
6	Thin+ESS	93.3 a	88.9 a	0.61 b	0.39 a	0.82 a	0.94 a	98.9 a	34.0 ab	5.67 ab
	Means	94.1 x	93.7 x	0.75 x	0.65 x	0.99	0.87	98.9	37.7	6.30
	LSD _p =0.05	11.2	13.6	0.42	0.52	0.52	0.39	2.31	9.57	1.59

Comparing treatments within columns, values with different letters (of the range a, b, c) are significantly different at a 5% significance level

Comparing seasons within rows, values with different letters (of the range x, y, z) are significantly different at a 5% significance level

4.2.2.2 Potential fertility (Bud dissection)

A comparison of bud dissection results from before the trial commenced (Tabel 9), with results obtained after the first season of treatment (June 2015) as well as after the second season of treatment (June 2016) (Table 10), indicates that the average potential fertility over all treatments decreased.

Regarding potential fertility assessed in June 2015, no significant differences were found between treatments (Table 10). In June 2016, the potential fertility of Treatment 5 was significantly lower compared to all other treatments, except Treatment 3. This result did not correspond with the previous seasons' bud dissection results and also did not follow the same pattern than the June 2016 forced budding results. A possible explanation for this difference may be that an experimental error might have occurred, namely that during the bud dissection in June 2016, some inflorescence primordia could have been incorrectly classified as leaf primordia or even been miscounted.

4.2.2.3 Actual fertility

A comparison of actual fertility expressed as total number of bunches per cane before the trial commenced (November 2014) (Tabel 9), with results obtained after the first season of treatment (November 2015) (Table 10), indicates that the average actual fertility over all treatments decreased from 6.53 to 6.30 (a decrease of 3.5%).

This corresponds to the results of Weaver (1960), Weaver & Pool (1971), Swanepoel & Baard (1988), Orth (1990b), Lavee *et al.* (1993), Peacock (1998), Christensen (2000), Dokoozlian (2000), Sommer *et al.* (2000), Dokoozlian & Peacock (2001), Weyand & Schultz (2005) and Iland *et al.* (2011) that the application of GA₃ in a specific season did decrease the cluster count in the following season. Hed *et al.* (2011) reported that the return fertility was not negatively affected and came to the conclusion that the negative effects of GA₃ applications appear to be associated with much higher rates (25ppm) than those tested in their study and that timing of application and cultivar play a major role in the effect of GA₃.

Regarding actual fertility in November 2015, Treatments 3 and 6 had the lowest values (only significant for Treatment 3), which indicated the same trend as found for the potential fertility. There is a tendency that the lower application volumes are associated with lower fertility, possibly due to a more effective coverage obtained on not only the bunches (the target organs), but also on the shoots and buds.

Based on the potential fertility results obtained in June 2015, namely 0.75 bunches per sprouted bud with forced budding and 0.99 inflorescence primordia per bud position with bud dissection, an actual fertility of 6.75 bunches per cane and 8.91 bunches per cane was expected. The actual fertility in November 2015 was 6.30 bunches per cane, which was 6.6% lower than expected based on forced budding results and 29.29% lower based on bud

dissection results. Thus, similar to the 2014/2015 season, the actual fertility was lower than potential fertility in the 2015/2016 season.

Although actual fertility expressed as bunches per cane was lower in November 2015 than in November 2014, the total number of bunches per vine present before crop control was applied in November 2015 was 37.7, compared to 30.7 in November 2014. This can be ascribed to allocation of more canes during winter pruning of July 2015, when 10 canes were allocated to all vines with sufficient vigour, compared to eight canes allocated per vine during winter pruning in July 2014.

The difference between potential fertility and actual fertility corresponds to results of Swanepoel & Baard (1988) and Palma & Jackson (1989). Final differentiation of inflorescence primordia into inflorescences with fully developed individual flowers takes place between bud break and flowering and are affected by several environmental conditions and cultivation practices, as explained in section 2.2.4.1 in Chapter 2.

Cultivation practices including irrigation and fertilisation were applied according to standard recommendations for the cultivar and therefore water and nutrient supply were adequate. Weather conditions, specifically temperature might have affected the final differentiation process. The results of Petrie & Clingeffer (2005) indicated that temperature immediately before and during bud break influences the number of flowers per inflorescence and that the effect of temperature on flower differentiation diminishes as bud burst advances.

May (1964) as cited by Vasconcelos *et al.* (2009) reported a decrease in the number of inflorescences with a temperature of 12°C shortly before and after bud break compared to a temperature of 25°C. In 2014 the average minimum temperature for one day before to one day after bud break ranged between 7.9 and 12.3°C, while in 2015 it ranged between -3.9 and 11.9°C, which might have affected inflorescence development negatively (see section 3.1.2 in Chapter 3, Table 6).

Accurate assessment of actual fertility is important both in research and commercial table grape production. Khanduja & Abbas (1973) stated that an early assessment of fertility is not reliable for estimating of final yield. Therefore, to obtain accurate research data and reliable crop estimates, it is important to assess actual fertility when bunches are clearly visible, before crop control is applied.

4.3 Manual thinning (timing)

Time required for manual thinning differed significantly between treatments in both seasons (Table 11). Two possible reasons for this difference between seasons could be: (i) due to low temperatures and higher relative humidity during flowering (see section 3.1.2 in Chapter 3, Table 6), causing setting of more shot berries in 2014/2015; and (ii) not exactly the same team of workers was used for doing the manual thinning in both seasons. The 2015/2016 season had more experienced workers for this specific action. Thus the discussion of results is focused on comparing treatments within a season rather than comparing seasons.

Table 11 Time required for manual thinning of Prime at Laborans 2014/2015 and 2016/2016 seasons

No.	Treatment	Thinning time			
		2014/2015		2015/2016	
		per bunch# (min)	per ha* (h)	per bunch# (min)	per ha* (h)
1	NoThin+Dip (Control)	02:20 a	2578	01:02 ab	1149
2	Thin+Dip	01:24 b	1547	00:52 b	942
3	Thin+250L/ha	01:40 ab	1758	01:10 a	1295
4	Thin+500L/ha	02:00 ab	2238	01:10 a	1287
5	Thin+1000L/ha	01:18 b	1442	00:54 ab	1011
6	Thin+ESS	02:06 ab	2331	01:14 a	1329
	Means	01:48	1982	01:03	1169
	LSD _{p=0.05}	0.81		0.30	

#measured time

*calculated time based on: 30 bunches/vine x 2222 vines/ha = 66 660 bunches/ha

Comparing treatments within columns, values with different letters are significantly different at a 5% significance level

In both seasons, the same trend was found, where Treatment 1 required the longest time spent per ha (Table 11). Treatment 1 had the highest percentage of small berries, which can be ascribed to the fact that no thinning treatment was applied (Table 15).

In the 2014/2015 season Treatment 2 and 5 required significantly the least time compared to Treatment 1 and in the 2015/2016 season Treatment 2 and 5 required the least time, with no significant difference between the other treatments (Table 11). It was observed that Treatment 2 had the largest berries and an even berry size distribution, probably due to the high-volume thinning application, followed up with a full coverage cluster dipping for berry sizing. Berry size classification of marked bunches is presented in section 4.6.

Practical implementation of Treatment 2 in commercial table grape production requires availability of sufficient labour and this is practically applied by several producers in the industry where they have practical experience of a decline in fertility after GA₃ spray applications (Mouton, B., 2016, personal communication, du Toit, F., 2016, personal communication, van Rensburg, D., 2016, personal communication).

Since no significant difference in time required for manual thinning was found between the different spray treatments (application volumes), the conclusion can be made that the “best” method for application will depend on the effect on return fertility.

No scientific publications regarding time and costs required for manual thinning of table grapes could be found. Therefore, results of this trial are compared to three commercial scenarios (see section 1.1 in Chapter 1, Table 1). The thinning and sizing applications for these scenarios were applied according to standard practises, namely as in Treatment 5

(Thin+1000L/ha). The hourly compensation rate differs between regions and commercial producer. Therefore, the minimum labour cost per hour according to the Department of Labour Agriculture was used for cost calculations presented in Table 12.

Table 12 Time required and cost of manual thinning of Prime at Laborans for the 2014/2015 and 2015/2016 seasons.

No.	Treatment	Year	Bunch mass (g)	*Hours/ha	Hourly rate	R/ha	R/bunch
1	NoThin+Dip (Control)	2014	357	2578	R12.41	R31 992	R0.48
1	NoThin+Dip (Control)	2015	396	1149	R13.37	R15 362	R0.23
2	Thin+Dip	2014	379	1547	R12.41	R19 198	R0.29
2	Thin+Dip	2015	438	942	R13.37	R12 595	R0.19
5	Thin+1000L/ha	2014	334	1442	R12.41	R17 895	R0.27
5	Thin+1000L/ha	2015	374	1011	R13.37	R13 517	R0.20

*calculated time based on: 30 bunches/vine x 2222 vines/ha = 66 660 bunches/ha

In both seasons, Treatment 1 required the most hours and consequently had the highest cost, verifying the need for chemical thinning of Prime, to save labour cost. Time and cost of manual thinning of Prime using Treatment 2, can be up to 40% lower than with Treatment 1. Time required and cost for Treatments 2 and 5 ranged from 942 to 2578 hours and R12 595 and R31 992, which were in line with the time and cost required for the commercial Prime blocks in Scenario's 1 and 2 (see section 1.1 in Chapter 1, Table 1).

Comparing the results from Prime in this trial, as well as the commercial Prime blocks in Scenario's 1 and 2 with Scenario 3 (a less labour intensive cultivar, Crimson Seedless), emphasizes how labour intensive and costly manual thinning of Prime is. Time and cost of manual thinning of Prime can be up to 300% higher compared to Crimson Seedless.

4.4 Berry development and ripening

Data for both seasons are included in each of the graphs presented in Figure 27 to Figure 32.

The 2015/2016 season was similar to the 2014/2015 season, where the berry development (Figure 27, Figure 28 and Figure 29) from berry sizing treatment application until harvest, as well as ripening patterns (Figure 30, Figure 31 and Figure 32) from véraison until harvest followed the expected patterns for the cultivar. Berry sampling dates will be referred to as days after full bloom (DAFB) throughout this chapter. Full bloom dates are presented in section 3.1.2 in Chapter 3, Table 5.

4.4.1 Berry diameter and length

In both seasons, the change in berry diameter from 27 DAFB until harvest followed a similar pattern for all treatments and no significant difference between treatments was found for five of the six sampling dates (Figure 27). Berry diameter and length of all treatments reached a plateau after 55 DAFB. At 62 DAFB Treatment 4 of the 2015/2016 season showed a steep

increase in width. This can be due to an experimental error: on the day of sampling, the person who assisted with the sampling, may have sampled differently than the student working on the project, it seems that the largest berries were selected for sampling.

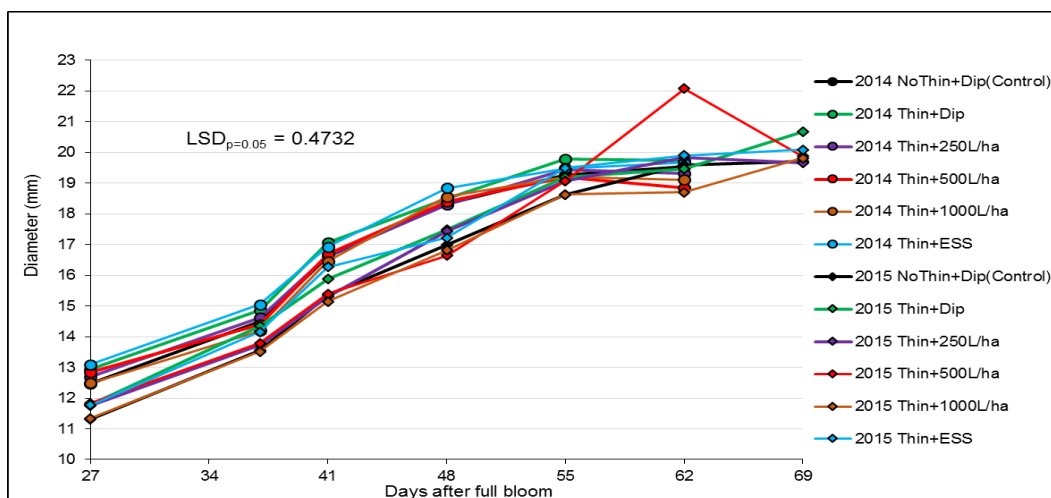


Figure 27 Changes in berry diameter during the 2014/2015 and 2015/2016 seasons of Prime at Laborans.

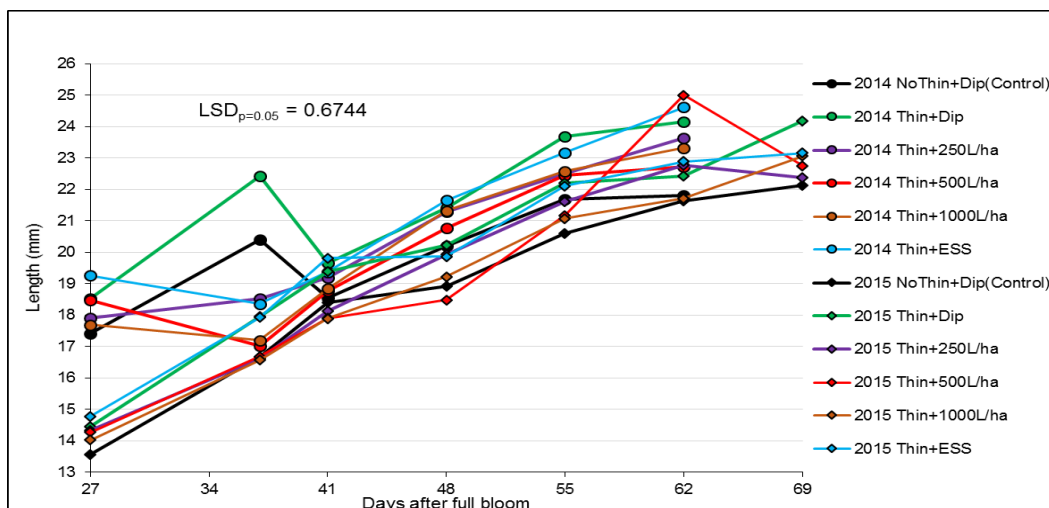


Figure 28 Changes in berry length during the 2014/2015 and 2015/2016 seasons of Prime at Laborans.

As the berries started to increase in width, they also increased in length. In both seasons, the change in berry length from 27 DAFB until harvest followed a similar pattern for all treatments (Figure 28). Berry length of all treatments reached a plateau after 55 DAFB. The 2015/2016 season followed a more even pattern compared to the 2014/2015 season. This occurrence in the 2014/2015 season can be explained as follows: berries were sampled at random over the whole cluster. Manual thinning for the 2014/2015 season was done on 41 DAFB and in the 2015/2016 season on 34 DAFB, making bunches in the 2015/2016 season more uniform at a very earlier stage than in the 2014/2015 season. This supports results

obtained that berries which were sampled for the 2014/2015 season between 27 and 41 DAFB were of varying sizes, compared to a more uniform size in the 2015/2016 season.

Results in Figure 27 and Figure 28 indicate that the berries were longer than they were wide on each sampling date, this pattern corresponds with results reported in other studies (Sproule & Stannard, 1970; Casanova *et al.*, 2009; Zoffoli *et al.*, 2009; Abu-Zahra, 2013).

4.4.2 Mass

In both seasons, the change in berry mass from 27 DAFB until harvest followed a similar pattern for all treatments and no significant difference between treatments was found for five of the six sampling dates (Figure 29). A rapid increase in berry mass was found from 34 DAFB till 62 DAFB, followed by a plateaued from after 62 DAFB.

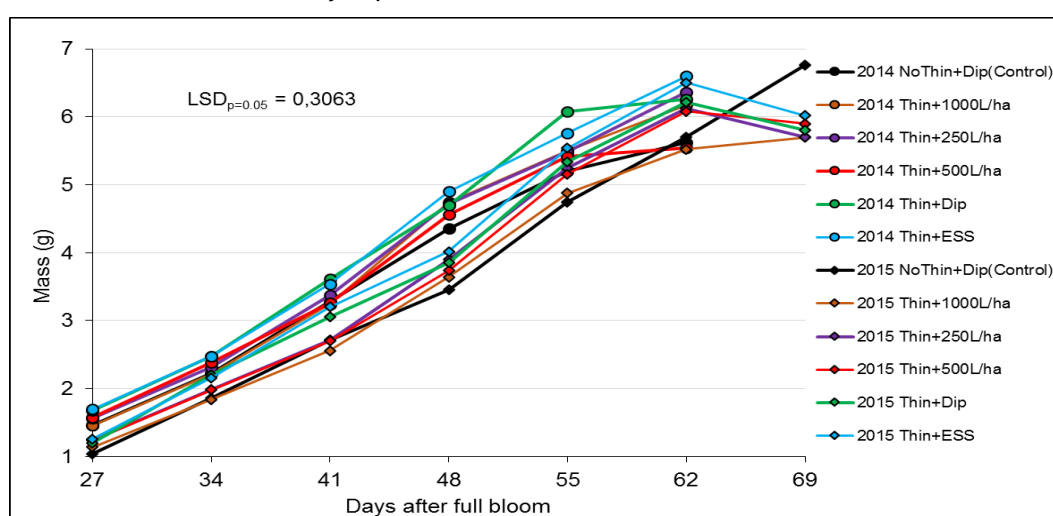


Figure 29 Changes in berry mass during the 2014/2015 and 2015/2016 seasons of Prime at Laborans.

The pattern found in this trial was also reported in numerous studies (Hrazdina *et al.*, 1984; Casanova *et al.*, 2009; Sonnekus, 2015). The berry mass did not show a clear double sigmoidal curve which is typical of berry development which is described by Coombe & Hale (1973). The phenomenon was also reported by Raath (2012) and Sonnekus (2015) explaining that the lack of a lag stage can be ascribed to Prime being an early ripening cultivar with a short development period from set to post véraison.

4.4.3 Total Soluble Solids

In each of the seasons, the change in TSS from 27 DAFB until harvest followed a similar pattern for all treatments (Figure 30). The 2015/2016 season was harvested at a lower TSS (Figure 30). Due to the project being situated on a commercial farm and the grapes in the experimental block were also used for commercial packing, grapes of each experimental unit had to be harvested on the same day as the block's commercial harvest date in both

seasons. For export requirements, Prime's optimum maturity is at 16°B (Van Der Merwe, 2014).

In the 2014/2015 season TSS showed a more rapid increase compared to the 2015/2016 season. Temperatures conditions during the ripening period were similar for both seasons (see section 3.1.2 in Chapter 3, Table 6). A possible reason for the lower TSS in the 2015/2016 season could be due the 16.3mm and 5.3mm rainfall which were experience on the 12th (51 DAFB) and 16th (55 DAFB) of December 2015 respectively (see section 3.1.1 in Chapter 3, Figure 6). It was found by Dreier *et al.* (2000) that when the berries take up a vast amount of water, a dilution effect occurs, resulting in a lower TSS value, while the berries keep enlarging.

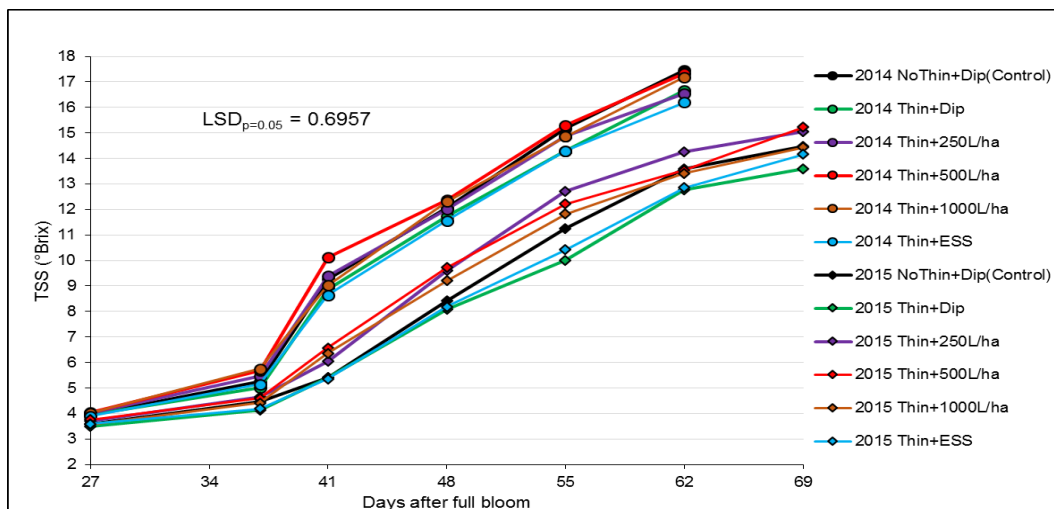


Figure 30 Changes in berry total soluble solids during the 2014/2015 and 2015/2016 seasons of Prime at Laborans.

The pattern which was found in both seasons showed a short lag stage in 2014/2015 season and none in 2015/2016 season, which is similar to patterns reported in other studies which was conducted on berries of early ripening cultivars (Coombe, 1980; Robinson & Davies, 2000; Raath, 2012; Sonnekus, 2015). Other than these studies similar patterns were found by Coombe (1992), Muñoz-Robredo & Robledo (2011) and Zoffoli *et al.* (2009).

4.4.4 Titratable acids

In each of the seasons, the change in TA from 34 DAFB until harvest followed a similar pattern for all treatments and no significant difference between treatments was found for each sampling dates (Figure 31). The TA started at a high concentration than decreased up to harvest, this occurrence was also reported by other studies (Harris *et al.*, 1968; Hrazdina *et al.*, 1984; Liu *et al.*, 2006; Sonnekus, 2015).

The 2014/2015 season started with a much lower TA than the 2015/2016 season, which can be ascribed to the fact that the Metrohm gave an experimental error on 27 DAFB, as the machine was yet not calibrated by an expert. At 34 DAFB the TA pattern started to show a similar pattern as obtained in 2015/2016 season. Between 34 DAFB up until 55 DAFB the

2014/2015 season TA decrease at a faster rate compared to the 2015/2016 season. The 2015/2016 season's pattern was more comparable to the pattern Sonnekus (2015) reported for Prime.

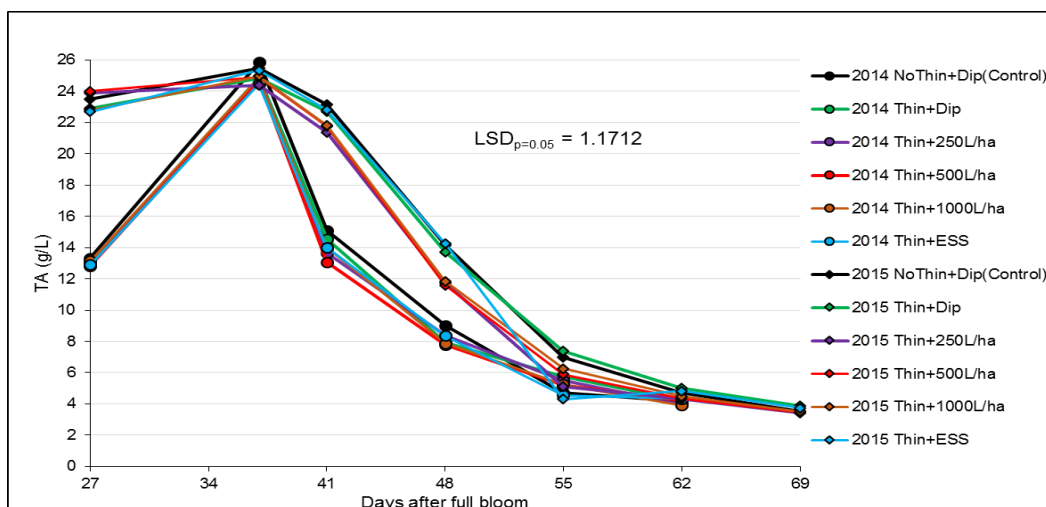


Figure 31 Changes in berry titratable acids during the 2014/2015 and 2015/2016 seasons of Prime at Laborans.

According to a study which was conducted by Liu *et al.* (2006) on ninety-eight cultivars (wine and table grape cultivars), a TA of 5.8g/L were obtained for table grapes. Sonnekus (2015) found in the one year TA values of 5.88g/L whereas in another year it was lower (3.59g/L) than the levels given by Liu *et al.* (2006). In this study the TA at harvest in the 2014/2015 season was 3.36g/L and in 2015/2016 the TA was 3.59g/L which corresponds with the values reported by Sonnekus (2015) second season.

4.4.5 Malic acid

In each of the seasons, the change in malic acid concentration from 27 DAFB until harvest followed a similar pattern for all treatments and no significant difference between treatments was found for three of the four sampling dates (Figure 32).

The average MA concentration were higher in the 2014/2015 season compared to the 2015/2016 season. MA makes out a small portion of the total TA, therefore a similar pattern was found. According to Hrazdina *et al.* (1984) an increase is found in MA concentration in the first growing stage followed by a decline rapidly thereafter due to MA being metabolised, diluted or transformed during ripening. A similar pattern was also reported in other studies (Iland & Coombe, 1988; Coombe, 1992; Gutiérrez-Granda & Morrison, 1992; Rusjan, 2010; Sonnekus, 2015).

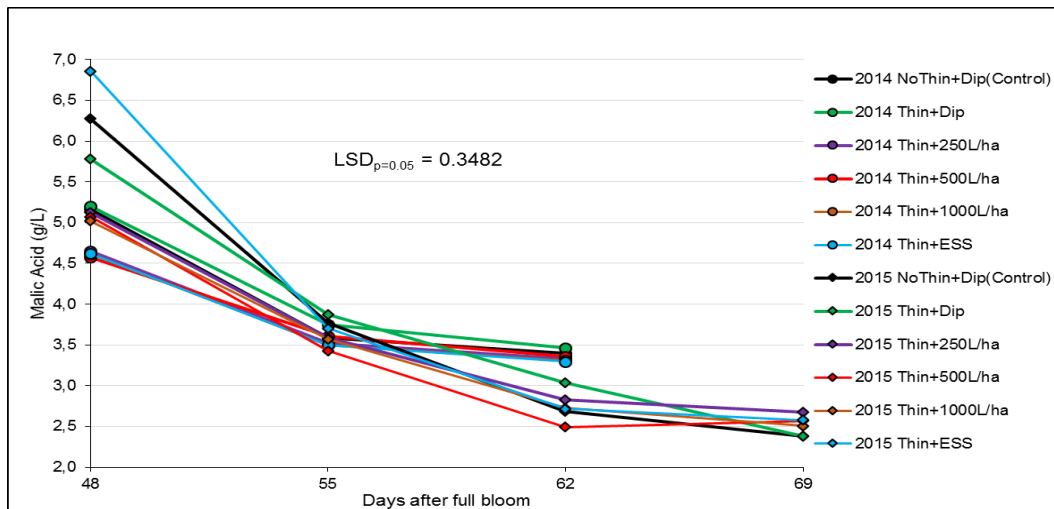


Figure 32 Changes in berry malic acid during the 2014/2015 and 2015/2016 seasons of Prime at Laborans.

4.5 Yield

The 2015/2016 season had an overall higher yield at harvest than the 2014/2015 season, in contrast to the lower potential and actual fertility obtained in June 2015 and November 2015 (see 4.2.2, Table 10).

The crop load in 2015/2016 season (37 bunches per vine before crop control and 34 bunches per vine after crop control) was higher than in 2014/2015 season (30 bunches per vine before crop control and 28 bunches per vine after crop control). This can be ascribed to allocation of more canes during winter pruning of July 2015, when 10 canes were allocated to all vines with sufficient vigour, compared to eight canes allocated per vine during winter pruning in July 2014.

Regarding yield, no significant difference was found between treatments in the 2014/2015 season, whereas in the 2015/2016 season significant differences were found (Table 13). Compared to Treatment 2, Treatments 3 and 6 had the lowest values (only significant for Treatment 3), which indicated the same trend as found for the potential fertility (June 2015) and actual fertility (November 2015) (see 4.2.2, Table 10).

The bunch mass for the 2015/2016 season was higher than in 2014/2015 season, but no significant difference was found in 2014/2015 season. In the 2015/2016 season Treatment 5 had a significant higher bunch mass compared to Treatment 6 (Table 13). There was no significant difference in bunch mass between the other treatments. The bunch mass of all treatments was higher than the minimum required for export (250g) (DAAF, 2012).

Table 13 Yield, bunch mass and berry mass of Prime at Laborans at harvest for the 2014/2015 and 2015/2016 season.

No.	Treatment	2014/2015			2015/2016		
		Yield (kg/vine)	Bunch mass (g)	Berry mass (g)	Yield (kg/vine)	Bunch mass (g)	Berry mass (g)
1	NoThin+Dip (Control)	11 a	357 a	5.6 b	15 ab	396 ab	5.8 b
2	Thin+Dip	10 a	379 a	6.2 a	17 a	438 ab	6.8 a
3	Thin+250L/ha	11 a	361 a	6.4 a	12 b	409 ab	6.0 b
4	Thin+500L/ha	10 a	373 a	5.6 b	15 ab	399 ab	5.9 b
5	Thin+1000L/ha	9 a	334 a	6.4 a	15 ab	374 b	6.0 b
6	Thin+ESS	11 a	339 a	6.8 a	14 ab	457 a	6.0 b
	Means	10 y	357 Y	6.16 x	15 x	412 X	6.13 x
	LSD _{p=0.05}	3.21	70.7	0.68	5.01	69.0	0.68

Comparing treatments within columns, values with different letters (of the range a, b, c) are significantly different at a 5% significance level

Comparing seasons within rows, values with different letters (of the range x, y, z) are significantly different at a 5% significance level

In the 2014/2015 season, no significant difference was found between the treatments for berry mass (Table 13). In the 2015/2016 season berry mass of Treatment 2 was significantly higher compared to Treatment 3 and 4. As expected, for both seasons, the smallest berry mass was obtained in Treatment 1 where no thinning was applied.

In Table 14 the pruning mass and Ravaz index are represented.

Table 14 Pruning mass and Ravaz index of Prime at Laborans for the 2014/2015 and 2015/2016 season.

No.	Treatment	2014/2015		2015/2016	
		Pruning mass (kg/experimental unit)	Ravaz Index (ratio)	Pruning mass (kg/experimental unit)	Ravaz Index (ratio)
1	NoThin+Dip (Control)	8.93 a	6 a	9.38 a	8 ab
2	Thin+Dip	9.44 a	5 a	8.78 a	10 a
3	Thin+250L/ha	10.2 a	6 a	10.98 a	5 b
4	Thin+500L/ha	9.94 a	5 a	10.94 a	7 ab
5	Thin+1000L/ha	9.71 a	5 a	10.18 a	7 ab
6	Thin+ESS	9.00 a	7 a	9.52 a	8 ab
	Means	9.54 x	6 Y	9.96 x	8 X
	LSD _{p=0.05}	2.10	2.32	2.31	3.04

Comparing treatments within columns, values with different letters (of the range a, b, c) are significantly different at a 5% significance level

Comparing seasons within rows, values with different letters (of the range x, y, z) are significantly different at a 5% significance level

In both seasons the pruning mass did not differ significantly between treatments. Only in the 2015/2016 season the Ravaz index of Treatments 2 and 3 differed significantly which can be ascribed to the significant difference between the two treatments regarding yield (see Table 13).

According to Vasconcelos *et al.* (2009) excessive vigour have been associated with a decrease in fertility or poor fertility. According to Reynolds (2001), Kliewer and Dokoozlian (2001) and Smart (2001), as cited by Iland *et al.* (2011) the yield to pruning mass ratio (Ravaz index) is used as indicator of the reproductive to vegetative growth relationship in the grapevine. A Ravaz index of between 3 and 4 was reported for vigorous Festival Seedless table grapes (Avenant, 1998). Research done on wine grapes found the most optimal balance between growth and yield where yield to pruning mass ratios ranged from 4 to 10 (Iland *et al.*, 2011). The Ravaz index of all the treatments was within this optimal range.

4.6 Berry size classification of marked bunches

Regarding the berry size classification in the 2014/2015 season, there were few significant differences between Treatment 1 and the other treatments, but between spray applications treatments only one significant difference was found (Table 15), therefore in the 2015/2016 season berry size classification was only done for the three extreme treatments (Treatment 1, 2 and 5) (Table 16).

4.6.1 2014/2015 season

The 80-100% FB Treatment 5 had a significantly lower percentage normal berries than Treatment 2 and 3, while there was no significant difference between the other treatments (Table 15). The 80-100% FB Treatment 5 had a significantly higher percentage shot berries than Treatment 1 and 2, while there was no significant difference between the other treatments (Table 15).

The 10% set Treatment 1 had a significantly lower percentage normal berries than the other treatments, while there was no significant difference between the other treatments (Table 15). The 10% set Treatment 1 had a significantly higher percentage small berries than the other treatments, there were no significant difference between the other treatments (Table 15).

These results indicate that a more even berry size distribution was obtained with the 80-100% FB thinning application, although a higher berry mass was obtained with the 10% set application, which corresponds to the results of Lynn & Jensen (1966) with Thompson Seedless which was sprayed at 70% FB with 10ppm GA₃.

4.6.2 2015/2016 season

The 100% set Treatment 1 had a significant lower berry mass than Treatment 2, while there was no significant difference between the other treatments (Table 16).

Similar to the 2014/2015 season, higher berry mass were obtained with the thinning applications applied at the later stages (10% set and 100% set), which corresponds to the findings of Orth (1990), that larger berry size was obtained with later bloom application (100% FB).

Table 15 Berry size classification of marked bunches for the 2014/2015 season of Prime at Laborans at harvest.

No.	Treatment	Normal Berry mass (g)		% Normal berries		% Small berries		% Shot berries	
		Phenological stage of thinning application							
		80-100% FB*	10% set	80-100% FB*	10% set	80-100% FB*	10% set	80-100% FB*	10% set
1	NoThin+Dip (Control)	3.27 a	4.53 a	79.6 ab	64.5 b	11.6 a	23.1 a	8.77 b	12.4 a
2	Thin+Dip	3.37 a	5.12 a	85.1 a	78.1 a	7.18 a	10.3 b	7.68 b	11.6 a
3	Thin+250L/ha	3.24 a	5.30 a	81.7 a	74.6 ab	8.68 a	13.8 b	9.60 ab	11.6 a
4	Thin+500L/ha	3.46 a	5.79 a	80.3 ab	78.0 a	10.8 a	7.53 b	8.97 ab	14.5 a
5	Thin+1000L/ha	2.98 a	5.67 a	73.7 b	75.5 a	13.3 a	11.7 b	13.0 a	12.8 a
6	Thin+ESS	3.30 a	5.82 a	78.5 ab	77.8 a	10.5 a	11.2 b	11.0 ab	11.0 a
	Means	3.27	5.37	79.8	74.8	10.3	12.9	9.82	12.3
	LSD _{p=0.05}	0.80	1.82	7.84	10.5	6.14	6.74	4.09	8.02

* FB: Full Bloom

Comparing treatments within columns, values with different letters are significantly different at a 5% significance level

Table 16 Berry size classification of marked bunches for the 2015/2016 season of Prime at Laborans at harvest.

No.	Treatment	Normal Berry mass (g)			% Normal berries			% Small berries			% Shot berries		
		Phenological stage of thinning application											
		80-100% FB*	10% set	100% set	80-100% FB*	10% set	100% set	80-100% FB*	10% set	100% set	80-100% FB*	10% set	100% set
1	NoThin+Dip (Control)	3.27 a	4.02 a	3.62 b	84.0 a	91.5 a	80.4 a	14.4 a	7.16 a	16.4 a	1.52 a	1.35 a	3.15 a
2	Thin+Dip	2.89 a	4.18 a	5.76 a	82.2 a	89.5 a	87.5 a	15.6 a	9.07 a	10.8 a	2.23 a	1.40 a	1.70 a
5	Thin+1000L/ha	2.81 a	3.76 a	4.21 ab	84.7 a	87.5 a	87.6 a	13.9 a	11.3 a	11.0 a	1.45 a	1.13 a	1.43 a
	Means	2.99	3.99	4.53	83.7	89.5	85.2	14.6	9.19	12.8	1.76	1.29	2.09
	LSD _{p=0.05}	0.99	1.14	1.69	7.22	4.50	8.67	7.69	4.56	8.55	2.85	1.28	1.40

* FB: Full Bloom

Comparing treatments within columns, values with different letters are significantly different at a 5% significance level

4.7 Bunch structure

Regarding the bunch structure in the 2014/2015 season, there were few significant differences between treatments. Between spray applications treatments only two significant differences were found (Table 17), therefore in the 2015/2016 season bunch structure was only done for the three extreme treatments (Treatment 1, 2 and 5) (Table 18).

4.7.1 2014/2015 season

The 80-100% FB Treatment 3 to 5 had significantly the smallest lateral diameter compared to Treatment 1, while there was no significant difference between the other treatments (Table 17). The 80-100% FB Treatment 6 had significantly the largest pedicel diameter compared to Treatment 4 and 5, while there was no significant difference between the other treatments. The increase in diameter and length described below can be ascribed to improved cell division or cell enlargement in reaction to the GA₃ treatments.

The 10% set Treatment 4 and 6 had significantly the longest lateral length compared to Treatment 2, while there was no significant difference between the other treatments (Table 17). For the 10% set Treatments 1, 4 and 5 pedicel diameter was significantly smaller compared to Treatments 2, 3 and 6.

Bunch length and Distance from Lateral 1 to 5 indicate that a less compact bunch structure was obtained with the 80-100% FB thinning application supporting the results obtained regarding the berry size distribution (see 4.6.1, Table 15). In contrast of the results, if the bunch is too compact, it will mean that more time needs to be allocated to manual thinning as well as to the quality aspect.

4.7.2 2015/2016 season

The 80-100% FB Treatment 2 had a significantly shorter Distance from Lateral 1 to 5 compared to Treatment 1, while there was no significant difference between the other treatments (Table 18). The 10% set Treatment 1 had a significantly shorter Distance from Lateral 1 to 5 compared to Treatment 2, while there was no significant difference between the other treatments (Table 18). Similar to the 2014/2015 season, shorter bunch length and Distance from Lateral 1 to 5 were obtained with the thinning applications applied at the later stages (10% set and 100% set). For both seasons the later flowering stage thinning application (10% set) was associated with a thicker rachis, which corresponds to Raban *et al.* (2013).

In this trial, detailed measurements were performed for bunch structure of Prime, as opposed to more subjective assessments reported/done by several other researchers. Few published research results could be found regarding detailed bunch structure measurements. Hanni *et al.* (2012) evaluated tightness of bunches by twisting bunches and allocating a percentage. Lynn & Jensen (1966) measured the average number of berries per

cluster, Pérez & Gómez (2000) measured pedicel diameter, Casanova *et al.* (2009) measured the pedicel length and diameter, Zoffoli *et al.* (2009) measured the thickness of the pedicel, main and lateral rachis and lastly Molitor *et al.* (2012) measured cluster length at different numbers of unfolded leaves up until full bloom.

4.8 Bunch compactness

In Table 19 and Table 20 the bunch compactness expressed as the number of berries per cm total lateral length is presented for the Treatments 1, 2 and 5.

4.8.1 2014/2015 season

The number of Total Berries/cm Total lateral length, as well as the number of Normal Berries/cm Total lateral length of the 80-100% FB Treatment 1 (no thinning application) was significantly higher compared to Treatment 2. Which indicate that the bunches of the Treatment 1 were more compact than the bunches of Treatment 2, which links to results reported for bunch structure measurements in Table 17 and how important the thinning application is.

The 80-100% FB Treatment 1 had a significantly higher number of Small Berries/cm Total lateral length compared to the other two treatments, which can be linked to the longer time required for manual thinning of this cultivar in this trial (see 4.3, Table 11). Both the 80-100% FB and 10% set Treatment 5 had a significantly higher number of Small and Shot Berries/cm Total lateral length compared to the other two treatments.

4.8.2 2015/2016 season

The number of Total Berries/cm Total lateral length did not differ significantly between treatments and phenological stages, although Treatment 1 had the highest ratio for all three phenological stages.

The number of Normal Berries/cm Total lateral length of the 10% set Treatment 1 was significantly higher compared to Treatments 2 and 5, indicating that the bunches of Treatment 1 were more compact than the bunches of Treatment 2.

Bunch compactness expressed as the number of berries per cm total lateral length indicate that a less compact bunch was obtained with the 80-100% FB thinning application. These results indicate that a more even berry size distribution was obtained with the 80-100% FB thinning application. In contrast of the results, if the bunch is too compact, it will mean that more time needs to be allocated to manual thinning as well as to the quality aspect.

Table 17 Bunch structure of marked bunches for the 2014/2015 season of Prime at Laborans.

No.	Treatment	Rachis diameter (mm)		Bunch length (mm)		Lateral 1-5 distance (mm)		Lateral diameter 1-4 (mm)		Total lateral length (mm)		Berry pedicel diameter (mm)	
		Phenological stage of thinning application											
		80-100% FB*	10% set	80-100% FB*	10% set	80-100% FB*	10% set	80-100% FB*	10% set	80-100% FB*	10% set	80-100% FB*	10% set
1	NoThin+Dip (Control)	4.15 a	4.76 a	189 a	185 a	51.3 a	46.6 a	3.83 a	2.48 a	187 a	233 ab	1.23 ab	1.03 b
2	Thin+Dip	4.08 a	4.74 a	192 a	177 a	52.4 a	47.7 a	2.14 ab	2.37 a	206 a	202 b	1.29 ab	1.23 a
3	Thin+250L/ha	3.90 a	4.92 a	187 a	188 a	53.9 a	45.2 a	2.08 b	2.38 a	200 a	236 ab	1.23 ab	1.24 a
4	Thin+500L/ha	3.99 a	4.83 a	120 a	188 a	50.8 a	44.5 a	2.02 b	2.46 a	212 a	249 a	1.05 b	1.02 b
5	Thin+1000L/ha	4.06 a	5.03 a	206 a	173 a	51.0 a	45.3 a	1.90 b	2.30 a	183 a	220 ab	1.01 b	0.97 b
6	Thin+ESS	3.93 a	4.89 a	187 a	195 a	54.2 a	47.7 a	2.15 ab	2.49 a	196 a	250 a	1.46 a	1.20 a
	Means	4.02	4.86	194	184	52.3	46.2	2.35	2.41	203	231	1.21	1.12
	LSD _{p=0.05}	0.42	0.67	51.4	32.1	4.8	5.49	1.75	0.34	44.1	45.48	0.30	0.16

* FB: Full Bloom

Comparing treatments within columns, values with different letters are significantly different at a 5% significance level

Table 18 Bunch structure of marked bunches for the 2015/2016 season of Prime at Laborans.

No.	Treatment	Rachis diameter (mm)			Bunch length (mm)			Lateral 1-5 distance (mm)			Total lateral length (mm)		
		Phenological stage of thinning application											
		80-100% FB*	10% set	100% set	80-100% FB*	10% set	100% set	80-100% FB*	10% set	100% set	80-100% FB*	10% set	100% set
1	NoThin+Dip (Control)	4.15 a	4.54 a	4.44 a	203 a	194 a	185 a	61.7 a	50.0 b	50.2 a	219 a	221 a	207 a
2	Thin+Dip	4.06 a	4.40 a	4.49 a	204 a	194 a	198 a	54.1 b	55.2 a	52.3 a	204 a	215 a	222 a
5	Thin+1000L/ha	4.15 a	4.32 a	4.73 a	193 a	183 a	187 a	56.4 ab	53.1 ab	49.7 a	212 a	218 a	220 a
	Means	4.12	4.42	4.55	200	190	190	57.4	52.8	50.7	212	218	216
	LSD _{p=0.05}	0.44	0.48	0.44	32.35	22.6	16.1	6.47	4.86	5.98	40.5	43	33.4

* FB: Full Bloom

Comparing treatments within columns, values with different letters are significantly different at a 5% significance level

Table 19 Bunch compactness of marked bunches for the 2014/2015 season of Prime at Laborans.

No.	Treatment	no. Total Berries/ cm Total lateral length		no. Normal Berries/ cm Total lateral length		no. Small Berries/ cm Total lateral length		no. Shot Berries/ cm Total lateral length	
		Phenological stage of thinning application							
		80-100% FB*	10% set	80-100% FB*	10% set	80-100% FB*	10% set	80-100% FB*	10% set
1	NoThin+Dip (Control)	2.34 a	3.10 a	1.83 a	2.01 a	0.31 a	0.69 a	0.24 b	0.38 b
2	Thin+Dip	1.99 b	3.17 a	1.52 b	2.44 a	0.22 a	0.37 b	0.26 b	0.37 b
5	Thin+1000L/ha	2.31 ab	3.38 a	1.57 ab	2.51 a	0.32 a	0.41 b	0.42 a	0.45 a
	Means	2.23	3.22	1.64	2.33	0.28	0.49	0.31	0.40
	LSD _{p=0.05}	0.36	0.46	0.27	0.43	0.11	0.16	0.13	0.06

* FB: Full Bloom

Comparing treatments within columns, values with different letters are significantly different at a 5% significance level

Table 20 Bunch compactness of marked bunches for the 2015/2016 season of Prime at Laborans.

No.	Treatment	no. Total Berries/ cm Total lateral length			no. Normal Berries/ cm Total lateral length			no. Small Berries/ cm Total lateral length			no. Shot Berries/ cm Total lateral length		
		Phenological stage of thinning application											
		80-100% FB*	10% set	100% set	80-100% FB*	10% set	100% set	80-100% FB*	10% set	100% set	80-100% FB*	10% set	100% set
1	NoThin+Dip (Control)	1.85 a	1.88 a	2.45 a	1.48 a	2.68 a	1.91 a	0.33 a	0.17 a	0.46 a	0.04 a	0.03 a	0.08 a
2	Thin+Dip	1.53 a	1.81 a	2.33 a	1.24 a	1.61 b	2.01 a	0.26 a	0.17 a	0.27 a	0.03 a	0.03 a	0.05 a
5	Thin+1000L/ha	1.49 a	1.76 a	1.93 a	1.25 a	1.50 b	1.60 a	0.22 a	0.24 a	0.29 a	0.03 a	0.02 a	0.04 a
	Means	1.63	1.82	2.24	1.32	1.60	1.84	0.27	0.19	0.34	0.30	0.03	0.05
	LSD _{p=0.05}	0.36	0.46	0.71	0.27	0.43	0.46	0.11	0.16	0.41	0.11	0.06	0.07

* FB: Full Bloom

Comparing treatments within columns, values with different letters are significantly different at a 5% significance level

4.9 Post-harvest quality

Berry quality defects, including decay, berry split and SO₂ pedicel damage showed significant differences in the 2014/2015 season (Table 21). Stem brittleness, decay, SO₂ pedicel damage and internal browning showed significant differences in the 2015/2016 season (Table 22). Although some significant differences were found, the effects did not practically impact on the marketability of the grapes as specified by Perishable Products Export Control Board (PPECB) standards for export (DAAF, 2012). Prime is a cultivar that is very sensitive to browning, but the post-harvest quality results indicated that the percentage browning was negligible little and was not a negative factor.

Table 21 Post-harvest quality of Prime at Laborans for the 2014/2015 season.

No.	Treatment	Stem Condition	Stem Brittleness	Berry Firmness	% Decay	% Loose berry		% Berry split		% SO ₂ Damage		% Browning	
						-SO ₂ *	+SO ₂ **	-SO ₂ *	+SO ₂ **	Pedicel	Surface	External	Internal
1	NoThin+Dip (Control)	2.10 a	2.30 a	2.20 a	0.24 b	0.10 a	0.06 a	1.05 ab	0.48 a	0.00 d	0.10 a	0.40 a	0.36 a
2	Thin+Dip	2.50 a	2.40 a	2.20 a	0.33 ab	0.09 a	0.00 a	1.50 ab	0.34 a	0.14 c	0.06 a	0.37 a	0.36 a
3	Thin+250L/ha	2.30 a	2.30 a	2.50 a	0.53 a	0.25 a	0.10 a	1.13 ab	0.61 a	0.00 d	0.24 a	0.31 a	0.81 a
4	Thin+500L/ha	2.20 a	2.30 a	2.10 a	0.38 ab	0.13 a	0.00 a	2.59 a	0.25 a	0.29 b	0.37 a	1.49 a	0.47 a
5	Thin+1000L/ha	2.26 a	2.36 a	2.36 a	0.30 ab	0.08 a	0.25 a	1.43 ab	0.41 a	0.00 d	0.13 a	0.23 a	0.43 a
6	Thin+ESS	2.20 a	2.10 a	2.30 a	0.32 ab	0.14 a	0.17 a	0.96 b	0.38 a	1.58 a	0.15 a	0.51 a	0.22 a
	Means	2.26 x	2.29 x	2.28 x	0.34 y	0.13 y	0.14	1.44 x	0.41	0.67	0.17	0.51 x	0.44 y
	LSD _{p=0.05}	0.41	0.55	0.40	0.24	0.25	1.08	1.55	0.40	0.12	0.39	1.60	0.89

* Without SO₂ damage** With SO₂ damage

Comparing treatments within columns, values with different letters (of the range a, b, c) are significantly different at a 5% significance level

Comparing seasons within rows, values with different letters (of the range x, y, z) are significantly different at a 5% significance level

Table 22 Post-harvest quality of Prime at Laborans for the 2015/2016 season.

No.	Treatment	Stem Condition	Stem Brittleness	Berry Firmness	% Decay	% Loose berry		% Berry split		% SO ₂ Damage		% Browning	
						-SO ₂ *	+SO ₂ **	-SO ₂ *	+SO ₂ **	Pedicel	Surface	External	Internal
1	NoThin+Dip (Control)	2.4 a	1.8 a	1.8 a	2.04 a	0.51 b	0.11 a	0.49 a	0.48 a	0.14 c	0.10 a	1.01 a	0.42 b
2	Thin+Dip	1.8 a	1.4 ab	1.6 a	1.13 a	0.29 b	0.23 a	0.85 a	0.44 a	0.00 d	0.06 a	0.87 a	0.52 ab
3	Thin+250L/ha	1.8 a	1.2 ab	1.6 a	1.78 a	0.39 b	0.10 a	0.23 a	0.61 a	0.00 d	0.24 a	0.77 a	1.48 a
4	Thin+500L/ha	1.8 a	1.4 ab	1.8 a	4.12 a	1.47 a	0.00 a	0.22 a	0.25 a	0.29 b	0.37 a	1.71 a	1.23 ab
5	Thin+1000L/ha	1.6 a	1.0 b	1.8 a	1.92 a	0.40 b	0.22 a	0.37 a	0.37 a	0.00 d	0.13 a	1.17 a	0.89 ab
6	Thin+ESS	1.8 a	1.0 b	1.6 a	1.70 a	0.39 b	0.17 a	1.42 a	0.38 a	1.58 a	0.15 a	1.32 a	0.39 b
	Means	1.87 y	1.3 y	1.7 y	2.16 x	0.57 x	0.17	0.66 x	0.42	0.67	0.17	1.15 x	0.81 x
	LSD _{p=0.05}	1.05	0.65	0.92	3.99	0.85	0.46	1.76	0.38	0.12	0.38	1.61	0.99

* Without SO₂ damage** With SO₂ damage

Comparing treatments within columns, values with different letters (of the range a, b, c) are significantly different at a 5% significance level

Comparing seasons within rows, values with different letters (of the range x, y, z) are significantly different at a 5% significance level

4.10 Summary

The bud break% determined through forced budding in June 2015 and June 2016, as well as through assessment in the vineyard in November 2015 did not differ significantly between treatments and was above 80% for all treatments. Commercially acceptable levels of bud break were obtained in both seasons.

The potential and actual fertility decreased over the two seasons. In June 2015 Treatment 1 had a significantly higher potential fertility and Treatment 6 had a significantly lower potential fertility compared to the other treatments. In June 2016, no significant differences were found between treatments, although Treatment 6 had the lowest potential fertility again. It is suggested that Treatment 3 and 6 with a lower application volumes and smaller droplet size are associated with lower fertility, possibly due to more effective coverage obtained on bunches (the target organs for berry sizing treatments), but also on the shoots and buds and that GA₃ applied to the buds had a negative effect on potential fertility. This was also reflected by the actual fertility and yield obtained in the November 2015, that Treatment 2 compared to Treatments 3 and 6 had the lowest yield (only significant for Treatment 3).

In both seasons the pruning mass did not differ significantly between treatments. The Ravaz index of all the treatments was within the optimal range of 4 to 10.

Regarding manual thinning in both seasons, Treatment 1 required the longest time spent per ha and Treatment 2 and 5 required significantly the least time, which can be ascribed to the larger berry size and % normal berries obtained with Treatment 2. No significant difference were found between the different spray applications (volumes). Therefore, the “best” method for application will depend on the effect on return fertility.

In both seasons, Treatment 1 required the most hours and consequently had the highest cost, verifying the need for chemical thinning of Prime, to save labour cost. Time and cost of manual thinning of Prime using Treatment 2, can be up to 40% lower than with Treatment 1. Time required and cost for Treatments 2 and 5 ranged from 942 to 2578 hours and R12 595 and R31 992, which were in line with the time and cost required for the commercial Prime blocks.

Berry juice composition (berry diameter and length, TSS, pH, TA and MA) was not negatively affected by any of the treatments. The expected berry development and ripening patterns were found. Although a few significant differences were found regarding post-harvest quality, it did not practically impact on the marketability of the grapes.

Regarding the bunch structure in the 2014/2015 season, there were few significant differences between treatments. The 80-100% FB Bunch length and Distance from Lateral 1 to 5 indicate that a less compact bunch structure was obtained. These results indicate that a more even berry size distribution was obtained with the 80-100% FB thinning application. A higher berry mass was obtained with the thinning applications applied at the later stages

(10% set and 100% set), as well as shorter bunch length and Distance from Lateral 1 to 5 were obtained with the thinning applications applied at the later stages.

The number of Total Berries/cm Total lateral length, as well as the number of Normal Berries/cm Total lateral length of the 80-100% FB Treatment 1 was significantly higher compared to Treatment 2, indicating that the bunches of the Treatment 1 were more compact than the bunches of Treatment 2. The 80-100% FB Treatment 1 had a significantly higher number of Small Berries/cm Total lateral length compared to the other two treatments which can be linked to the longer time required for manual thinning of this cultivar.

The 80-100% FB bunch compactness expressed as the number of berries per cm total lateral length indicate that a less compact bunch structure was obtained. These results indicate that a more even berry size distribution was obtained with the 80-100% FB thinning application.

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Chapter 5

GENERAL CONCLUSION AND RECOMMENDATIONS

CHAPTER 5: GENERAL CONCLUSIONS AND RECOMMENDATIONS

The results of this study contribute to the available scientific published results regarding the effect of GA₃ application methods (volumes) on fertility of table grapes. Based on the results after the first two seasons of the trial, the following are recommended regarding identifying GA₃ application methods and volumes for effective thinning and sizing treatments of table grapes without negatively affecting fertility:

- Treatment 2 (Chemical thinning with a standard GA₃ spray application, followed by a GA₃ dipping treatment for berry sizing) had the largest berries, less compact bunches and even berry size distribution. This treatment also required the least time for manual thinning.
- Practical implementation of Treatment 2 (Chemical thinning with a standard GA₃ spray application, followed by a GA₃ dipping treatment for berry sizing) in commercial table grape production requires availability of sufficient labour and this is practically applied by several producers in the industry where they have experience of a decline in fertility after GA₃ spray applications
- Current available results indicate that the lower spray application volumes, Treatment 3 (250 L/ha) and Treatment 6 (ESS 72 L/ha) were associated with a decrease in fertility, while with Treatment 4 (500 L/ha) no indication of a negative effect on fertility was found. Therefore, repetition of the trial is needed to verify these results and to investigate whether the 500 L/ha spray application volume could be used instead of the current standard industry practice of using 1000 L/ha for the majority PGR and other spray applications. Using an application volume of 500 L/ha instead of 1000 L/ha will have several practical and economic benefits, in terms of more hectares being sprayed with a one tank mix, decreasing the water foot print, as well as the carbon foot print.

Although the trial was conducted for two full seasons, the November 2015 harvest was the first harvest where the actual fertility as affected by treatments applied in the first season of the trial could be assessed. It is recommended to repeat the trial for at least one more season to verify results obtained, test repeatability and verify whether trends that were found could over time develop into significant differences.

To address research gaps/ questions regarding return fertility that still need to be investigated, the following are recommended for continuation of the existing trial and/ or new similar trials:

- Good results were obtained with dipping bunches compared to spraying application for berry sizing. Therefore, is recommended to also include an extra treatment where

bunches are dipped for thinning to compare to the conventional thinning spray with regards to return fertility.

- It is recommended to include a treatment where certain vines in the trial is not be sprayed with a rest breaking agent (hydrogen cyanamide) to eliminate the possible effect of hydrogen cyanamide on bud break of vines. This was not possible in this study, due to the trial being situated on a commercial farm.
- In future studies the relationship between starch content and fertility could be investigated.
- Bud dissection and forced budding results must only be used as an indication for potential fertility, due to final differentiation of bunch primordia into bunches and individual flowers that takes place between bud break and flowering and which could be affected by environmental conditions and cultivation practices during the period.
- Accurate assessment of actual fertility is important both in research and commercial table grape production. Early assessment of fertility is not reliable for estimating of final yield. Therefore, to obtain accurate research data and reliable crop estimates, it is important to assess actual fertility when bunches are clearly visible, before crop control is applied.

ADDENDUM A

Calculations for thinning and berry sizing treatments

Thinning calculations

Concentration GA₃ of the product (ProGibb = 40% GA₃) that was used = 1 ppm in 1000L tank/ha

$$\frac{1\text{mg}}{\text{kg}} \times \frac{100 \text{ product}}{40 \text{ GA}_3}$$

$$= 2.5 \text{ mg/kg}$$

$$= 2.5 \text{ mg/1L} = 1 \text{ ppm}$$

$$\therefore 2.5 \text{ g/1L} = 1000 \text{ ppm}$$

$$\therefore 2.5 \text{ g/1000L} \quad (1 \text{ ppm})$$

$$\therefore 5 \text{ g/1000L} \quad (2 \text{ ppm})$$

Berry sizing calculations

Concentration GA₃ of the product (ProGibb = 40% GA₃) that was used = 15 ppm in 1000L tank/ha

$$\frac{15\text{mg}}{\text{kg}} \times \frac{100 \text{ product}}{40 \text{ GA}_3}$$

$$= 37.5 \text{ mg/kg}$$

$$= 37.5 \text{ mg/1L} = 1 \text{ ppm}$$

$$\therefore 37.5 \text{ g/1L} = 1000 \text{ ppm}$$

$$\therefore 37.5 \text{ g/1000L} \quad (1 \text{ ppm})$$

	1000L/ha @ 15 ppm	500L/ha @ 15 ppm	250L/ha @ 15 ppm	ESS (72)L/ha @ 15 ppm
g/100L	3.75 g/100L			
g/200L	7.5g * 1	7.5 g * 2	7.5 g * 4	
	7.5 g/200L	15 g/200L	30 g/200L	7.5 g/14L

ADDENDUM B

GRONDONTLEDINGSVERSLAG / SOIL ANALYSIS REPORT

Reg. Nr. : 96/17268/07 BTW / VAT Reg. No. : 4040162804

JD KIRSTEN EDMS BPK
LABORANS

SGS Kaap Laboratoriums

Datum : 31 / 03 / 2015

Verw. Nr. : PVDM37

Posbus 927, Somerset-Wes 7129

Vertw. : Peppie v/d Merwe

Tel : +27 21 852 7899

Faks : +27 21 851 5319

Lab. Nr.	Mons. Nr.	Land	% C	% GF	cm Diepte	Tekstuur			Ohms	pH (KCl)	H+ cmo(+)/kg		P	Makro-elemente in mg / kg						T-Wde	S-Wde	Base-Vers.	Mikro-elemente mg / kg						
						% Klei	% Slik	% Sand			KCl	K2SO4		K	Ca	Mg	Na	S	Cl				Cu	Zn	Mn	B			
C15-153-5	19	90/1	1.20	59		-			1294	6.3			73 Bray I	170 0.44	981 4.91	106 0.87	44 0.19				6.4	6.4	100	6.05	25.34	33.3	0.45		
KI <= Tekstuur													7	77	14	3													
C15-153-6	20	90/2	1.68	48		-			1234	6.1			67 Bray I	166 0.42	1063 5.32	136 1.11	49 0.21				7.1	7.1	100	6.86	23.85	24.7	0.36		
KI <= Tekstuur													6	75	16	3													
C15-153-7	21	90/3	2.96	55		-			7938	6.2			53 Bray I	25 0.06	144 0.72	16 0.13	5 0.02				0.9	0.9	100	9.91	39.98	51.8	0.44		
KI <= Tekstuur													7	77	14	2													
C15-153-8	22	91/1	1.60	43		-			1524	6.0			20 Bray I	119 0.30	1001 5.01	157 1.29	48 0.21				6.8	6.8	100	6.90	20.69	31.4	0.60		
KI <= Tekstuur													5	74	19	3													
C15-153-9	23	91/2	0.88	43		-			1296	5.8			15 Bray I	126 0.32	733 3.66	118 0.97	57 0.25				5.2	5.2	100	4.35	17.05	18.7	0.74		
KI <= Tekstuur													6	70	19	5													
C15-153-10	24	91/3	1.00	51		-			1315	5.9			19 Bray I	122 0.31	830 4.15	129 1.05	57 0.25				5.8	5.8	100	4.76	12.46	25.0	0.45		
KI <= Tekstuur													5	72	18	4													
C15-153-11	25	91/4	0.64	65		-			1289	5.1		0.58	20 Bray I	118 0.30	504 2.52	93 0.76	60 0.26				4.4	3.8	87	3.54	10.54	7.7	0.36		
KI <= Tekstuur													7	57	17	6													
C15-153-12	26	91/5	0.80	60		-			1337	5.5		0.54	19 Bray I	125 0.32	701 3.51	114 0.93	55 0.24				5.5	5.0	90	5.55	8.32	13.2	0.46		
KI <= Tekstuur													6	63	17	4													

This Laboratory participates in one or more disciplines of the AgriLASA's Control Scheme. A certificate of participation and performance is available at www.agrilasa.co.za

ab

ADDENDUM C

Grondregstelling

Naam: JD Kirsten
Adres: Laborans



Yara Africa Fertilizer Us
YARA CAPE
Lambrecht Street, Huguenot
7546 Paarl, South Africa
www.yara.co.za

Datum: 2/4/2015
Agent J.M.P. van der Merwe

Monster nr.	Blok	Diepte cm	% Klip	Kalk/Gips ton / ha			Maxifos kg/ha	KCl kg/ha	K ₂ SO ₄ kg/ha	MgSO ₄ kg/ha
				Kalsiet	Dolomiet	Gips				
1	80/1	40	30	2.5						
2	80/2	40	50	1.5		0.5				
3	80/3	40	35	2.0						
4	81/1	40	40	1.0	0.5					
5	81/2	40	30	2.0	0.5					
6	82/1	40	45							
7	82/2	40	30							
8	83	40	40			1.0				
9	84	40	65	1.5						
10	85/1	40	40							
11	85/2	40	55	1.0						
12	86	40	55			1.0				
13	87/1	40	60							
14	87/2	40	60			1.0				
15	87/3	40	60				70			
16	87/4	40	55				40			
17	89/1	40	45	3.0						
18	89/2	40	55				110			
19	90/1	40	60							
20	90/2	40	50							
21	90/3	40	55							
22	91/1	40	45				205			
23	91/2	40	45			1.0	325			
24	91/3	40	50			1.0	215			
25	91/4	40	65	1.5		1.0	200			
26	91/5	40	60	1.0		1.0	215			
27										
28										
29										
30										
31										
32										

Aanbeveling deur:
Pieter Botha, MSc. Agric
Cand.Sci.Nat. 100219/13
Sel: 0795140459