Factors affecting the predisposition of 'Cabernet Sauvignon' grapevines (*Vitis vinifera* L.) to the physiological disorder, bunch stem necrosis.

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

in

Plant Physiology

at

Massey University, Palmerston North, New Zealand.

Andrea Hilary Pickering

2006
Abstract

Bunch stem necrosis (BSN) is a physiological disorder in grapes. It results in shrivelled berries with poor quality attributes such that wine produced from grapes with high BSN incidence is of compromised quality. Past research has proposed many different hypotheses to explain the disorder. Literature indicates that conditions during certain stages of development may predispose berries to BSN but results are not consistent as to which stage is the critical one or which factors have the most impact. This study was designed to resolve these points of uncertainty. Treatments that either enhanced or decreased vine vigour, or manipulated the light environment around the fruit zone were applied to field grown ‘Cabernet Sauvignon’ vines over three seasons. Treatments included root pruning, heading back of canes by 50%, laying down a reflective mulch and two 50% shade treatments applied for three weeks either pre- or post-full bloom (FB). A strong positive correlation was found between vine vigour and the incidence of BSN. Three weeks post-FB, during both the current and previous season, was identified as the critical period within which factors predispose bunches to BSN. Plant growth regulators, including GA₃, IAA and NPA, were applied to bunches on a different group of field grown vines immediately after FB. Application of GA₃ during the critical period, tended to reduce the incidence of BSN, while the effects of IAA and NPA application were less clear and require further research. In a controlled environment (CE) trial, pot-grown vines were placed in CE rooms during one of three development stages. Results showed that treatments applied during the critical three-week period after FB increased the incidence of BSN three fold compared with no change in BSN incidence for vines that were placed in the CE rooms immediately prior to FB or prior to veraison. Collective results from these studies clearly demonstrate that the period immediately following FB is the most critical time in the predisposition of bunches to BSN. It is suggested that competitive dominance of vegetative growth over the developing inflorescence and bunch for assimilates and/or nutrients may be the predisposing factor/s influencing this disorder.
I would like to dedicate this thesis to my grandfather, Dr Owen Haylock (1923 – 2002). Without him in my life, the desire and motivation to even start this thesis would not have been there. Sadly he died before he could see me start this study, but his memory has been with me throughout the four years of this research.
Acknowledgements

So many people and organisations have helped me over the last four years, without who I would not have accomplished what I have. I would like to thank as many of them as I can.

The most important person I would like to thank is my chief supervisor, Prof. Ian Warrington. Without his knowledge, guidance, understanding, patience, and motivation I would not have managed to undertake such a large project or even completed this thesis. I was extremely privileged to have him as my supervisor for the entire course of study and to also have him mucking in, out in the field, with spade, secators, pen and paper.

Secondly, my co-supervisor Dr David Woolley, who I have had many a long discussion with, that have helped me immensely with formulating and clarifying many of my ideas. Thank you for stepping in when you were needed.

Also, Drs Steve McArtney and Jens Wunche, whose knowledge and help was greatly appreciated even though their time associated with my study was not as long as was originally planned. Thank you to Dr Siva Ganesh, who guided me through so much of my statistics and who, without his help, and I would have been lost in a statistical world. Also, Dr Damian Martin, who got the ball rolling for this study and assisted me to gain my research funding.

Thanks to my two main helpers, Jake Bixley and Ben van Hooijdonk, who assisted in data collection from my field trial. The extensive amount of data that I collected, and sadly have not been able to report all of, would not have been possible without these two.

Thank you so much to AGMARDT for my doctoral scholarship that kept me fed and housed through out my study, and Winegrowers New Zealand, who with out their financial support this study would never have been started. Also, thanks to Extenday Ltd, who provided the Extenday mulch used in this study.
Acknowledgements

My thanks also for the grape vines, analyses and extensive help I received from all the people at Pernod-Ricard, especially David Werry, who guided me in my viticultural knowledge, George who looked after my field vines so well, and Adam for putting up with a student on his block. Also, Justin, Teresa and the others in the lab who tirelessly analysed my juice samples for me.

To all my colleagues and friends at HortResearch and Massey University, especially at the PGU, INR and The Soils Department, whose help and advice has been fantastic, thank you. Thank you also to HortResearch for allowing me to go on leave without pay, while still allowing me to have an office and use equipment throughout my study.

And finally thanks to my friends and family who have put up with me over the last four years. My grandmother for her love and occasional financial support, my mother for helping me with sample taking and spending many an hour proof reading my thesis, and especially my husband, Phil, who has stuck by me through my bad moods, occasional desire to chuck it all in and late nights at the computer (and the desire to kill the thing). He has supported me not just emotionally but has been there as a sounding board for ideas, helped collect data, written computer programs to help with my data, and built approximately five computers for me, as I killed them off one by one.

To all of you, and so many other people I have not been able to thank as it would take a second volume, thank you so much for what you have done for me.
Extended Thesis Summary

Bunch stem necrosis (BSN) is a physiological disorder in grapes that results in unripe shrivelled berries with poor quality attributes. This includes the Brix concentration remaining low, while titratable acidity (TA) remains high. Visual symptoms include not only the shrivelled or flacid appearance of the berries, but necrosis of the rachis, peduncle or pedicels. Symptoms usually occur soon after 100% veraison has been reached and progressively worsens until harvest. Wine produced from grapes with a high proportion of BSN is consequently low in quality and therefore the disorder is of concern to the industry. Past research results are conflicting, with many different hypotheses being proposed to explain the disorder. Although symptoms are not exhibited until after veraison, the literature indicates that conditions during certain stages in development may predispose berries to BSN. However, the literature is not consistent as to which stage is the critical one or which factors have most impact on the disorder. This study was designed to try and identify this possible critical stage in berry development and the factors that impact on the severity of BSN.

For two seasons 50% shade cloth was applied to field grown ‘Cabernet Sauvignon’ vines. One group received shade for three weeks prior to full bloom (FB) with a second group receiving shade for three weeks immediately following FB. Assessments including juice analyses and non-destructively estimating BSN incidence from veraison to harvest were carried out. Raw BSN incidence data were adjusted for days after 50% veraison and a common Brix/TA ratio in order to accommodate any differences in maturity among treatments and compare the incidence of the disorder across all three seasons where BSN incidence was assessed. In Season Two, plant growth regulator treatments, which included GA\(_4\) (50 mg l\(^{-1}\)), IAA (200 mg l\(^{-1}\)) and NPA (200 mg l\(^{-1}\)), along with a control, were applied to bunches on a different group of field grown ‘Cabernet Sauvignon’ vines immediately after FB. This was in combination with canopy manipulation treatments of removing laterals from vines and retaining the laterals, that were also carried out immediately after FB and continued through the growing season. In a controlled environment (CE) trial, potted ‘Cabernet Sauvignon’
vines were placed in CE rooms for three weeks during one of three stages: immediately prior to FB, immediately after FB and for three weeks prior to veraison. The controlled environment room conditions were set at 23/11°C day/night temperatures for the entire length of the experiment. Day length was 16 hours with an 8 hour night. Photosynthetic photon flux (PPF) was set at 600 ± 15 µmol m⁻² s⁻¹, which is considered to be close to optimum for photosynthesis on grapevines. Contrasting relative humidity (RH) conditions (40 and 80% RH) were included within the treatments.

Shade prior to FB tended to reduce BSN incidence in both seasons in which the shade cloth was applied (Figure A). Assessments carried out in the third season, where shade cloth was not applied, found no change in BSN incidence. Adjusting the data for days after 50% veraison and the maturity ratio made no difference to any of the conclusions drawn for all three seasons.

Shade immediately after FB did not significantly affect BSN incidence in the first season, although there was some indication that it may increase the disorder. In the second season shade applied immediately after FB significantly increased the incidence...
of BSN and in the third season assessments showed that shade applied after FB in the previous season significantly increased the incidence of BSN (Figure A). When the data were adjusted for days after 50% veraison and the maturity ratio similar conclusions could be drawn.

Various treatments that affected the vegetative growth of vines were also applied to ‘Cabernet Sauvignon’ vines during Seasons One and Two. These included root pruning, which was carried out during the winter months in Season One, heading back of the canes by 50% and the application of a reflective mulch, Extenday™, both of which were applied in Seasons One and Two. A second group of vines were root pruned in Season Three. Point quadrat analysis was carried out on these treatments, as well as the two shade treatments, during Seasons One and Two. Leaf layer number was determined to be a good measure of vine vigour and was also significantly correlated with the incidence of BSN (Figure B). Although point quadrat measures were carried out from FB to harvest, it was determined that the measurement approximately three weeks after FB represented the differences in vigour among treatments after FB the best. It was therefore this assessment which was used. The correlation demonstrated that treatments that reduced vine vigour after FB reduced the incidence of BSN.

![Figure B: Correlation between leaf layer number approximately three weeks after FB and BSN incidence (%) across all three seasons. Y=4.6x - 4.21, R² = 0.65, p=0.0085.](image-url)
Application of GA₃ increased berry size, and consequently bunch weight, delayed maturity and tended to reduce the incidence of BSN (Figure C). Compared to control bunches, application of IAA and NPA tended to reduce BSN incidence in vines where laterals were removed, but not in vines where they were retained. However, BSN incidence in IAA and NPA treated bunches tended not to be different between vines with laterals removed or retained (Figure C). Therefore, any difference in BSN incidence that these plant growth regulator treated bunches had compared to control bunches, may have been due to an increase in BSN incidence of control bunches on vines with laterals removed. Further research is therefore required to determine the effect of NPA and IAA on BSN incidence and the mechanisms involved.

![Figure C](image_url)

**Figure C:** BSN incidence from 100% veraison to harvest in Season Two for canopy manipulated vines of laterals retained (control) and laterals removed and plant growth regulator dipped bunches of NPA, GA₃, IAA and control.

In the controlled environment (CE) studies, vines that were placed in the CE rooms immediately prior to FB and prior to veraison did not demonstrate a difference in BSN incidence compared to control vines. However, vines that were placed in the CE rooms after FB had an approximate three fold increase in BSN incidence compared to all other vines (Table A). Relative humidity did not impact on the disorder.
Table A: Mean BSN incidence (%) for bunches from vines placed in the CE rooms at either one of three stages. Stage One – pre-FB, Stage Two – post-FB, Stage Three – pre-veraison. Means for vines not placed in the CE rooms also included (control).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>One</td>
<td>8.3</td>
<td>24.5 b</td>
<td>26.5 b</td>
</tr>
<tr>
<td>Two</td>
<td>38.8</td>
<td>69.4 a</td>
<td>76.0 a</td>
</tr>
<tr>
<td>Three</td>
<td>6.9</td>
<td>15.6 b</td>
<td>27.0 b</td>
</tr>
<tr>
<td>Control</td>
<td>4.5</td>
<td>14.3*</td>
<td>24.2*</td>
</tr>
</tbody>
</table>

Means within a column with a different letter are significantly different from each other at P≤0.15 (LSMeans, SAS).

*Control means not used in statistical analysis

Results from these studies clearly demonstrate that the period immediately following FB is the most critical time in the predisposition of bunches to BSN. Conditions during this time not only affected the incidence of BSN in bunches of the current season, but also in the following season. It is suggested that competitive dominance of vegetative growth over the developing inflorescence and bunch for assimilates and/or nutrients at this development stage may be the predisposing factor/s. Therefore management techniques that reduce vegetative growth during the time immediately after FB, such as root pruning during dormancy, can reduce the incidence of BSN.
Table of Contents

Abstract ii
Acknowledgements iv
Extended Thesis Summary vi
Table of Contents xi
List of Figures xiv
List of Tables xix
List of Plates xxii
List of Appendices xxiii
List ofAbbreviations xxiv

1 Literature Review 1
  1.1 History 1
  1.2 Taxonomy 1
  1.3 Physiology 2
     1.3.1 Inflorescence 2
     1.3.2 Flower 4
     1.3.3 Pollination 5
     1.3.4 Berry growth and veraison 5
     1.3.5 Ripening 7
        1.3.5.1 Cation accumulation 9
        1.3.5.2 Plant growth substances 10
  1.4 BSN 10
     1.4.1 Symptoms 12
     1.4.2 Causes 17
        1.4.2.1 Environmental 17
        1.4.2.2 Mineral nutrition in vine and berry 19
        1.4.2.3 Plant growth regulators 25
        1.4.2.4 Cultivars and rootstocks 25
        1.4.2.5 Canopy development 25
        1.4.2.6 Xylem development 27
     1.4.3 Application of substances 28
        1.4.3.1 Potassium 28
        1.4.3.2 Calcium 28
        1.4.3.3 Magnesium 29
        1.4.3.4 Nitrogen 30
        1.4.3.5 Plant growth regulators 30
  1.5 Summary 32

2 General Materials and Methods 35
  2.1 Vine vigour 35
     2.1.1 Point quadrat 35
     2.1.2 Dormant canopy measurements 35
  2.2 Veraison 36
  2.3 Analyses of berry juice 36
     2.3.1 Brix concentration 37
     2.3.2 Titratable acidity and pH 37
     2.3.3 Acid and cation determination 37
  2.4 Harvest 37
  2.5 Seed number and weight 38
  2.6 Bunch stem necrosis (BSN) 39
  2.7 Data handling and statistical analyses 39
     2.7.1 Data handling 39
     2.7.2 Experimental design 40
        2.7.2.1 Field trial 40
        2.7.2.2 Controlled environment room experiments 41
     2.7.3 Statistical analyses 41
        2.7.3.1 Test statistics 42
Table of Contents

2.7.3.2 Treatment comparison testing 42

3 Vigour and Light Effects on BSN 44

3.1 Introduction 44

3.2 Materials and Methods 48

3.2.1 Plant material 48

3.2.2 Treatments 48

3.2.3 Statistical analyses 51

3.2.4 Assessments 51

3.2.4.1 Vine vigour 51

3.2.4.2 Veraison scores 52

3.2.4.3 Cation analyses 52

3.2.4.4 Gas exchange measurements 55

3.2.4.5 Harvest measurements 56

3.2.4.6 Seed number and weight 56

3.2.4.7 BSN incidence 56

3.3 Results 59

3.3.1 Vine vigour 59

3.3.1.1 Point quadrat 59

3.3.1.2 Dormant canopy measurements 73

3.3.2 Veraison 82

3.3.2.1 Season One 82

3.3.2.2 Season Two 85

3.3.2.3 Season Three 88

3.3.3 Petiole, rachis and berry cation analyses 91

3.3.3.1 Leaf petiole cation concentrations 91

3.3.3.2 Berry cation concentrations 94

3.3.3.3 Rachis cation concentration 99

3.3.4 Juice analyses 103

3.3.4.1 Brix 103

3.3.4.2 Titratable Acidity (TA) 106

3.3.4.3 Maturity ratio – Brix/TA 108

3.3.4.4 Calcium concentration 109

3.3.4.5 Magnesium concentration 111

3.3.4.6 Potassium concentration 112

3.3.4.7 Ammonium concentration 114

3.3.4.8 pH 116

3.3.4.9 Malic acid 118

3.3.4.10 Tartaric acid 119

3.3.5 Gas exchange 121

3.3.5.1 Season One 121

3.3.5.2 Season Two 121

3.3.6 Harvest 124

3.3.6.1 Bunch weight 124

3.3.6.2 Bunch length 126

3.3.6.3 Bunch width 126

3.3.6.4 Peduncle diameter 127

3.3.6.5 Total number of berries per bunch 128

3.3.6.6 Mean healthy berry weight 128

3.3.7 Seed number and seed weight 129

3.3.8 Bunch stem necrosis 130

3.3.8.1 Season One 130

3.3.8.2 Season Two 134

3.3.8.3 Season Three 139

3.4 Discussion 147

3.4.1 Vegetative growth 147

3.4.1.1 Vegetative growth and BSN 155

3.4.2 Berry quality 161

3.4.3 Root growth 167

3.4.4 Nutrient analyses 169

3.4.5 Xylem development 172
Table of Contents

3.4.6 Plant growth regulators 172
3.4.7 Summary 173

4 Source-Sink Relationships and BSN 175
4.1 Introduction 175
4.2 Materials and Methods 180
  4.2.1 Plant material 180
  4.2.2 Treatments
    4.2.2.1 Canopy manipulation 180
    4.2.2.2 Plant growth regulators 180
  4.2.3 Statistical analyses 181
  4.2.4 Measurements
    4.2.4.1 Vigour 181
    4.2.4.2 Veraison development 182
    4.2.4.3 Berry juice analyses 182
    4.2.4.4 Harvest measurements 182
    4.2.4.5 Bunch stem necrosis 183

4.3 Results 184
  4.3.1 Vigour
    4.3.1.1 Point quadrat analyses 184
    4.3.1.2 Dormant canopy measurements 184
  4.3.2 Veraison development 185
  4.3.3 Berry juice analyses 188
  4.3.4 Harvest measurements 190
  4.3.5 Seed number and seed weight 191
  4.3.6 BSN incidence 191

4.4 Discussion 194
  4.4.1 Plant growth regulators 199

5 Controlled Environment Studies 211
5.1 Introduction 211
5.2 Materials and Methods
  5.2.1 Plant material 214
  5.2.2 Experimental design 215
  5.2.3 Leaf area and growing points 217
  5.2.4 Photosynthesis 218
  5.2.5 Sap flow measurements 218
  5.2.6 BSN incidence 219

5.3 Results 220
  5.3.1 Leaf area 220
  5.3.2 Growing point number (GPN) 222
  5.3.3 Photosynthesis 226
  5.3.4 Sap flow
    5.3.4.1 Peduncle sap flow 228
    5.3.4.2 Cane sap flow 235
  5.3.5 Bunch stem necrosis 240

5.4 Discussion 244
  5.4.1 Canopy development 244
  5.4.2 Photosynthesis measurements 245
  5.4.3 Sap flow 247
  5.4.4 BSN 255

6 General Discussion and Conclusions 261
6.1 Critical timing 261
6.2 Nutrient 264
6.3 Plant growth regulators 267
6.4 Future research 271

7 Appendices 275
8 References 291
Figure A: BSN incidence (%) over three seasons of control vines and vines treated with 50% shade cloth applied for three weeks prior to FB and three weeks post-FB. Shade treatments were only applied in Seasons One and Two. Values are adjusted to a common maturity ratio of 1.8 in order to compare across all three seasons.

Figure B: Correlation between leaf layer number approximately three weeks after FB and BSN incidence (%) across all three seasons. \(Y = 4.6x - 4.21, R^2 = 0.65, p=0.0085\).

Figure C: BSN incidence from 100% veraison to harvest in Season Two for canopy manipulated vines of laterals retained (control) and laterals removed and plant growth regulator dipped bunches of NPA, GA3, IAA and control.

Figure 1: Stylised drawing of a grape inflorescence/bunch showing the parts: peduncle, rachis and pedicel.

Figure 2: Central Diagram: Appearance of berries at 10-day intervals revealing the two successive sigmoidal growth curves of a grape berry, designated ‘berry formation’ and ‘berry ripening’. Three generalised x-axes are shown – days after flowering, approximate juice °Brix values during ripening, and developmental growth stages using modified E-L system. The key growth stages and the approximate timing of the accumulation of major solutes are shown. At bottom: Scale drawings of anatomical features in the longitudinal sections of developing grape seeds at days 4, 14, 28, 42 and 98 days after flowering. Figure and caption from Dry and Coombe, (2004).

Figure 3: Time line indicating physiological stages, dates when treatments were applied, and dates of measurements and assessments carried out on field grown ‘Cabernet Sauvignon’ vines during Seasons One (2002/2003), Two (2003/2004) and Three (2004/2005). Note that not all factors were measured in all seasons.

Figure 4: Mean leaf layer number (LLN) of vines for main treatment for Seasons One and Two.

Figure 5: Leaf layer number (LLN) assessments carried out in Seasons One, Two and Three. Control B and root pruned B are treatments that were applied in Season Three. All other treatments were applied in Season One. LLN values for each of the three years have been superimposed on a common date scale. F = approximate flowering date, V = approximate 50% veraison date.

Figure 6: Mean percentage gaps (PG) within vine canopy area of main treatments for Seasons One and Two. F = 50% flowering, V = 50% veraison, H = harvest date.

Figure 7: Percentage gaps (PG) for Seasons One, Two and Three. Control B and root pruned B are treatments that were applied in Season Three. All other treatments were applied in Season One. PG values for each of the three years have been superimposed on a common date scale. F = approximate flowering date, V = approximate 50% veraison date.

Figure 8: Mean percent interior leaves (PIL) within vine canopy area for main treatments for Seasons One and Two. F = 50% flowering, V = 50% veraison, H = harvest date.

Figure 9: Percent Interior Leaves (PIL) for Seasons One, Two and Three. Control B and root pruned B are treatments that were applied in Season Three. All other treatments were applied in Season One. PIL values for each of the three years have been superimposed on a common date scale. F = approximate flowering date, V = approximate 50% veraison date.

Figure 10: Effective cane number (ECN) for Seasons One and Two. Statistical analyses carried out on logECN in Season Two. Season One LSD = 1.63, Season Two (logECN) LSD = 0.168.
List of Figures

Figure 11: Pruning Weights (PW) for Seasons One and Two. Season One LSD = 0.324, Season Two LSD = 0.509.

Figure 12: Mean Effective Cane Pruning Weights (ECW) for Seasons One and Two. Season One LSD = 0.231, Season Two LSD = 0.202.

Figure 13: Veraison development for Season One for treatments applied to Cabernet Sauvignon vines in the field.

Figure 14: Veraison development for Season Two for treatments applied to ‘Cabernet Sauvignon’ vines in the field.

Figure 15: Veraison development for Season Three for treatments applied to ‘Cabernet Sauvignon’ vines in the field.

Figure 16: Veraison development in Season Three for Season One and Season Three root pruned ‘Cabernet Sauvignon’ vines with their controls.

Figure 17: Mean petiole magnesium concentration (mg 1⁻¹) for each treatment in Season One. LSD, for comparison among treatment means at post-veraison sampling time, was derived using SAS 8.2.

Figure 18: Mean petiole calcium concentration (mg 1⁻¹) for each treatment in Season One. LSD, for comparison among treatment means at post-veraison sampling time, derived using SAS 8.2.

Figure 19: Mean petiole potassium concentration (mg 1⁻¹) for each treatment in Season One. LSD, for comparison among treatment means at post-veraison sampling time, derived using SAS 8.2.

Figure 20: Mean berry magnesium concentration (mg 1⁻¹) from dried samples taken pre-veraison in Season One. LSD, for comparison among treatment means, was derived using SAS 8.2.

Figure 21: Mean berry calcium concentration (mg 1⁻¹) from dried samples taken pre-veraison in Season One. LSD, for comparison among treatment means, derived using SAS 8.2.

Figure 22: Mean berry potassium concentration (mg 1⁻¹) from dried samples taken pre-veraison in Season One. LSD, for comparison among treatment means, derived using SAS 8.2.

Figure 23: Mean rachis magnesium concentration (mg 1⁻¹) from dried samples taken pre-veraison in Season One. LSD, for comparison among treatment means, derived using SAS 8.2.

Figure 24: Mean rachis calcium concentration (mg 1⁻¹) from dried samples taken pre-veraison in Season One. LSD, for comparison among treatment means, derived using SAS 8.2.

Figure 25: Mean rachis potassium concentration (mg 1⁻¹) from dried samples taken pre-veraison in Season One. LSD, for comparison among treatment means, derived using SAS 8.2.

Figure 26: Mean Brix concentrations of treatments for six weeks following 100% veraison to harvest in Season One.

Figure 27: Mean Brix concentrations of treatments from one to five weeks after 100% veraison in Season Two.

Figure 28: Mean titratable acidity (g 1⁻¹) values of treatments for six weeks following 100% veraison to harvest in Season One.

Figure 29: Mean titratable acidity (g 1⁻¹) values of treatments from one to five weeks after 100% veraison in Season Two.

Figure 30: Mean maturity ratio of treatments for six weeks following 100% veraison to harvest in Season One.
List of Figures

Figure 31: Mean maturity ratio of treatments from one to five weeks after 100% veraison in Season Two.

Figure 32: Mean calcium concentration (mg l^{-1}) of treatments for six weeks following 100% veraison to harvest in Season One.

Figure 33: Mean calcium concentration (mg l^{-1}) of treatments from one to five weeks after 100% veraison in Season Two.

Figure 34: Magnesium concentration (mg l^{-1}) of treatments from 100% veraison to six weeks after in Season One.

Figure 35: Mean magnesium concentration (mg l^{-1}) of treatments from one to five weeks after 100% veraison in Season Two.

Figure 36: Potassium concentration (mg l^{-1}) of treatments for six weeks following 100% veraison to harvest in Season One.

Figure 37: Mean potassium concentration (mg l^{-1}) of treatments from one to five weeks after 100% veraison in Season Two.

Figure 38: Ammonium concentration (mg l^{-1}) of treatments for the six weeks from 100% veraison to harvest in Season One.

Figure 39: Mean ammonium concentration (mg l^{-1}) of treatments from one to five weeks after 100% veraison in Season Two.

Figure 40: pH of treatments for the six weeks from 100% veraison to harvest in Season One.

Figure 41: Mean pH of treatments one to five weeks after 100% veraison in Season Two.

Figure 42: Malic acid concentration (mg l^{-1}) of treatments from 100% veraison to six weeks after in Season One.

Figure 43: Mean malic acid concentration (mg l^{-1}) of treatments one to five weeks after 100% veraison in Season Two.

Figure 44: Tartaric acid concentration (mg l^{-1}) of treatments from 100% veraison to six weeks after in Season One.

Figure 45: Mean tartaric acid concentration (mg l^{-1}) for treatments one to five weeks after 100% veraison in Season Two.

Figure 46: Raw, veraison adjusted and standard maturity adjusted BSN incidence (%) of all treatments in Season One at the second assessment.

Figure 47: BSN incidence (%) in Season Two from 100% veraison to harvest.

Figure 48: Raw, veraison adjusted and standard maturity adjusted BSN incidence (%) of all treatments in Season Two at harvest.

Figure 49: Progression of BSN incidence of all treatments in Season Three from 100% veraison to harvest.

Figure 50: Raw, veraison adjusted and standard maturity adjusted BSN incidence (%) of all treatments in Season Three one week prior to commercial harvest. * adjustment was unable to be made due to juice analyses not being carried out on these treatments.

Figure 51: BSN incidence (%) from 100% veraison to one week before commercial harvest in Season Three. LSD bars are at 5% significance level.
List of Figures

Figure 52: BSN incidence (%) of the main treatments adjusted to a common maturity ratio of 1.8 in three seasons. Note: root pruning was only carried out in Season One. All other treatments were applied only in Seasons One and Two.

Figure 53: Correlation between leaf layer number approximately three weeks after FB and BSN incidence (%) across all three seasons. \( Y = 4.6x - 4.21 \), \( R^2 = 0.65, p=0.0085 \).

Figure 54: Percentage of assimilated C\(^{14}\) distributed to the bunch from particular areas of the cane at different stages in berry development. Values from Hunter and Visser (1988a). Bunch leaves were not treated with C\(^{14}\).

Figure 55: Veraison development for vines with and without laterals removed, and the application of the growth regulators GA\(_3\), NPA or IAA to bunches.

Figure 56: BSN incidence from 100% veraison to harvest in Season Two for canopy manipulated vines of laterals retained (control) and laterals removed and plant growth regulator dipped bunches of NPA, GA\(_3\), IAA and control.

Figure 57: Stylised diagram of training system used for potted vines.

Figure 58: Diagram of how conditions changed and related to each other in the CE rooms using the low humidity room as an example. Day/night VPD conditions for the high humidity room were 0.57/0.27.

Figure 59: Time line indicating treatment stages, physiological stages, and dates of measurement and assessments carried out on potted vines during the 2004/05 season.

Figure 60: Mean leaf area for canes with laterals retained or removed, and placed in the CE rooms pre-flowering (Stage One), post-flowering (Stage Two) and pre-veraison (Stage Three).

Figure 61: Mean number of growing points per cane for canes with laterals retained or removed, and placed in the CE rooms pre-flowering (Stage One), post-flowering (Stage Two) and pre-veraison (Stage Three).

Figure 62: Twenty-four hour cycle of sap flow through the peduncle of bunches from low (40%) and high (80%) RH treatments with similar leaf areas, and the temperature change in the CE rooms during the early stages of Stage Two (immediately post-flowering). VPD changes follow an identical pattern to that of temperature change. Green arrow indicates lights on, black arrow indicates lights off.

Figure 63: Twenty-four hour cycle of sap flow through the peduncle of bunches from low (40%) and high (80%) RH treatments with similar leaf areas and with laterals retained, and the temperature change in the CE rooms during the late stages of Stage Two (approx. three weeks post-flowering). VPD changes follow an identical pattern to that of temperature change. Green arrow indicates lights on, black arrow indicates lights off.

Figure 64: Twenty-four hour cycle of sap flow through the peduncle of bunches from low (40%) and high (80%) RH treatments and with laterals removed, and the temperature change in the CE rooms during the late stages of Stage Two (approx. three weeks post-flowering). VPD changes follow an identical pattern to that of temperature change. Green arrow indicates lights on, black arrow indicates lights off.

Figure 65: Twenty-four hour cycle of sap flow through the peduncle of bunches from low (40%) and high (80%) RH treatments with similar leaf areas and laterals retained, and the temperature change in the CE rooms during Stage Three (approx. four weeks post-flowering to one week pre-veraison). VPD changes follow an identical pattern to that of temperature change. Green arrow indicates lights on, black arrow indicates lights off.

Figure 66: Twenty-four hour cycle of sap flow through the peduncle on vines in the low RH (40%) treatment for canes with laterals and canes with laterals removed, and the temperature
change in the CE rooms during the later stages of Stage Two (approx. three weeks post-flowering). VPD changes follow an identical pattern to that of temperature change. Leaf area of cane with laterals was 1200 cm², leaf area of cane without laterals was 1500 cm². Green arrow indicates lights on, black arrow indicates lights off.

Figure 67: Twenty-four hour cycle of sap flow through the peduncle on vines in the high RH (80%) treatments for canes with laterals and canes with laterals removed, and the temperature change in the CE rooms during the later stages of Stage Two (approx. three weeks post-flowering). VPD changes follow an identical pattern to that of temperature change. Leaf area of cane with laterals was 1200 cm², leaf area of cane without laterals was 1700 cm². Green arrow indicates lights on, black arrow indicates lights off.

Figure 68: Forty-eight hour cycle of sap flow through the peduncle of a bunch that was girdled at 11:00 (red arrow) and the temperature change in the CE rooms during Stage Three (approx. four weeks post-flowering to one week pre-veraison). VPD changes follow an identical pattern to that of temperature change. Green arrow indicates lights on, black arrow indicates lights off.

Figure 69: Twenty-four hour cycle of sap flow through canes with laterals from low (40%) and high (80%) RH treatments with similar leaf areas and the temperature change in the CE rooms during Stage Two (approx. three weeks post-flowering). VPD changes follow an identical pattern to that of temperature change. Green arrow indicates lights on, black arrow indicates lights off.

Figure 70: Twenty-four hour cycle of leaf area adjusted sap flow through canes with laterals removed from low (40%) and high (80%) RH treatments and the temperature change in the CE rooms during Stage Two (approx three weeks post-flowering). VPD changes follow an identical pattern to that of temperature change. Green arrow indicates lights on, black arrow indicates lights off.

Figure 71: Twenty-four hour cycle of sap flow through canes from low (40%) and high (80%) RH treatments with similar leaf areas and the temperature change in the CE rooms during Stage Three (approx. four weeks post-flowering to one week pre-veraison). VPD changes follow an identical pattern to that of temperature change. Green arrow indicates lights on, black arrow indicates lights off.

Figure 72: An example of a twenty-four hour cycle of sap flow through canes with laterals intact and canes with laterals removed, and the temperature change in the CE rooms. VPD changes follow an identical pattern to that of temperature change. Green arrow indicates lights on, black arrow indicates lights off.

Figure 73: Twenty-four hour cycle of water use of a vine measured by container weight change, sap flow through a cane on that vine, soil temperature and the temperature change in the CE rooms. VPD changes follow an identical pattern to that of temperature change. Green arrow indicates lights on, black arrow indicates lights off.

Figure 74: Relative humidity and lateral manipulation interaction effect on BSN incidence at the first, second and third assessments for vines placed in CE rooms during Stage Two (post-flowering).

Figure 75: Maximum and minimum daily temperature (°C), rain fall and irrigation (mm) applied in A) Season One and B) Season Two at Motex, Taradale, New Zealand. No irrigation was applied in Season Two. F – approximate time of flowering, V – approximate time of veraison.

Figure 76: Correlation between leaf layer number two months after FB and BSN incidence (%) across all three seasons. Y=3.2x + 0.64, R² = 0.75, p<0.0001.

Figure 77: Correlation between percentage gaps approximately three weeks after FB and BSN incidence (%) across all three seasons. Y=-0.46x + 15.5, R² = -0.59, p=0.1856.

Figure 78: Relationship to determine leaf area from leaf diameter, y = 0.6339x² + 2.177x – 10.644.
List of Tables

Table A: Mean BSN incidence (%) for bunches from vines placed in the CE rooms at either one of three stages. Stage One – pre-flowering, Stage Two – post-flowering, Stage Three – pre-veraison. Means for vines not placed in the CE rooms also included (control).

Table 1: Cation concentration of standards used in cation analyses using AA.

Table 2: Mean leaf layer number (LLN) within measured canopies for Seasons One and Two.

Table 3: Mean leaf layer number (LLN) of root pruned and control vines for Seasons One, Two and Three. The LLN at the first measurement in Season Two was estimated in order to provide comparable values.

Table 4: Mean percentage gaps (PG) within measured canopies for Seasons One and Two.

Table 5: Mean percentage gaps (PG) of root pruned and control vines for Seasons One, Two and Three. The first measurement in Season Two was estimated in order to provide comparable values.

Table 6: Percent interior leaves within measured canopies for Seasons One and Two.

Table 7: Percent interior leaves (PIL) of root pruned and control vines for Seasons One, Two and Three. The first measurement in Season Two was estimated in order to provide comparable values.

Table 8: Mean effective cane number (ECN), pruning weights (PW) and effective cane weight (ECW) for treatments in Season One (2002/2003) and Season Two (2003/2004).

Table 9: Mean growing point number (GPN), growing point weight (GPW), and mean growing points per cane (GP/C) for Season Two (2003/2004).

Table 10: Mean date of 50% veraison development rate of field treatments in Season One. Values obtained from SAS fitted sigmoid curves.

Table 11: Mean date of 50% veraison and mean veraison development rate of all field treatments in Season Two. Values obtained from SAS fitted sigmoid curves.

Table 12: Mean date of 50% veraison and mean veraison development rate of field treatments in Season Three. Values obtained from SAS fitted sigmoid curves.

Table 13: Mean date of 50% veraison and mean veraison development rate of root pruning treatments and their controls in Season Three. Values obtained from SAS fitted sigmoid curves.

Table 14: Means of post-flowering and pre-veraison berry magnesium concentrations (mg l⁻¹) in Season Three.

Table 15: Means of post-flowering and pre-veraison calcium concentrations (mg l⁻¹) in berries in Season Three.

Table 16: Means of post-flowering and pre-veraison berry potassium concentrations (mg l⁻¹) in Season Three.

Table 17: Means of post-flowering and pre-veraison magnesium concentrations (mg l⁻¹) in the rachis in Season Three.

Table 18: Means of post-flowering and pre-veraison calcium concentrations in the rachis in Season Three.
List of Tables

Table 19: Means of post-flowering and pre-veraison potassium concentrations (mg l⁻¹) in the rachis in Season Three. 103

Table 20: Season Three Brix, TA, maturity ratio, pH, and malic and tartaric acid concentrations of the six main treatments in Seasons One and Two. Berries sampled on 6 April, 2005, prior to commercial harvest. 105

Table 21: Means of gas exchange measurements carried out on field grown vines in Season Two. Measurement times are 1) full bloom, 2) two weeks post-FB, 3) pre-veraison. Means shown are least squares means due to unbalanced data sets. 123

Table 22: Harvest measurements carried out on field grown vines in Seasons One and Two. 125

Table 23: Mean seed number per berry and mean seed weight for 'Cabernet Sauvignon' vines in Season Two (2003/2004). 130

Table 24: Unadjusted BSN scores (%) for the two assessments in Season One. 132

Table 25: BSN incidence (%) for all treatments in Season Two. 136

Table 26: BSN incidence (%) for Season Three from 100% veraison to one week before commercial harvest. 141

Table 27: BSN incidence (%) from vines root pruned in Season Three and Season Three control vines assessed from 100% veraison to one week before commercial harvest. 144

Table 28: Mean leaf layer number, percent interior leaves and percentage gaps of control and vines where laterals had been removed. 184

Table 29: Mean total cane number, total vine pruning weight, total growing point number, cane weight, growing point weight and growing point number per cane for control, and vines with laterals removed in Season Two. 185

Table 30: Mean time of 50% veraison and mean veraison development rate for bunches treated with plant growth regulators and vines that have retained their laterals or vines that have had their laterals removed. 187

Table 31: Harvest juice quality measurements carried out on juice from vines with canopy manipulation and with the application of plant growth regulators to bunches. 189

Table 32: Harvest measurements on bunches from canopy manipulated vines and from bunches treated with plant growth regulators. 190

Table 33: Mean seed number per berry and mean seed weight for 'Cabernet Sauvignon' vines in Season Two (2003/2004) for vines with canopy manipulation and plant growth regulator treatments applied to bunches. 191

Table 34: Mean leaf area per cane for canes with laterals retained or removed, and placed in the CE rooms at the three different stages 1) pre-flowering, 2) post-flowering and 3) pre-veraison. Assessments were carried out on 5 November (early Stage One), 5 December (early Stage Two) and 5 January (early Stage Three). 221

Table 35: Mean growing point number per cane for canes with laterals retained or removed, and placed in the CE rooms at the three different stages 1) pre-flowering, 2) post-flowering and 3) pre-veraison. Assessments were carried out on 5 November (early Stage One), 5 December (early Stage Two) and 5 January (early Stage Three). 225

Table 36: Means of gas exchange measurements carried out on potted vines in Season Three for vines placed in CE rooms at either high (80%) or low (40%) RH. Assessment times are during 1)
Stage Two – post-flowering and 2) Stage Three – pre-veraison. Values are means of vines with and without laterals.

Table 37: Means of gas exchange measurements carried out on potted vines in Season Three for canes with or without laterals removed. Assessment times are during 1) Stage Two – post-flowering, 2) Stage Three – pre-veraison. Values are means of vines with high and low RH treatments.

Table 38: Mean BSN incidence (%) for bunches placed in the high (80%) and low (40%) RH treatments. Values are means of vines with laterals either retained or removed.

Table 39: Mean BSN incidence (%) for bunches from canes with either laterals retained or removed. Values are means for vines in high and low RH treatments.

Table 40: Overall mean BSN incidence (%) for bunches in high (80%) and low (40%) RH CE rooms and from canes with either laterals retained or with laterals removed.

Table 41: Mean BSN incidence (%) for bunches from vines placed in the CE rooms at any one of three stages. Stage One – pre-flowering, Stage Two – post-flowering, Stage Three – pre-veraison. Means for vines not placed in the CE rooms also included (control).

Table 42: Approximate BSN incidence overall, only for Stage Two and only for vines assessed with sap flow sensors, night peduncle sap flow rate, day peduncle sap flow rate and difference between night and day peduncle sap flow rates, for vines in the CE conditions during Stage Two.
List of Plates

Plate 1: Rachis of BSN – affected (left) and healthy (right) bunches. Arrows indicate necrotised areas.

Plate 2: Left: healthy stomata opening in the epidermis. Right: necrotised (dead) stomata opening of a rachis, where the primary symptoms of BSN can develop (Theiler, 1975b).

Plate 3: A healthy bunch (left) and BSN affected bunch (right). Necrosis of rachis and shrivelling of berries can be seen in the lower three-quarters of the affected bunch.

Plate 4: BSN affected bunch. The distal end is exhibiting BSN symptoms with rachis necrosis and shrivelled berries. The demarcation between healthy and necrotised tissue is evident.

Plate 5: Necrosis and shrivelled berries on a rachis branch in the middle of a bunch. As the necrosis has not girdled the primary axis of the rachis, berries distal to the branch are not exhibiting visual symptoms of BSN.
List of Appendices


Appendix 2: Spray Schedule 277

Appendix 3: Weather data for Seasons One and Two. 278

Appendix 4: Correlations between point quadrat assessments and BSN. 279

Appendix 5: Harvest juice quality measurements carried out on juice from vines with canopy manipulation and plant growth regulator applications to the bunches. 281

Appendix 6: Modified Hoagland’s Nutrient Solution (1/2 strength) 282

Appendix 7: Inflorescence distribution 283

Appendix 8: Leaf diameter/leaf area relationship 283

Appendix 9: Gauge specifications 284

Appendix 10: Stem heat balance theory: Extracted directly from van Bavel (2000) therefore references to sections and figure numbering applies to the Flow32™ manual and not this thesis. 285

Appendix 11: Means of gas exchange measurements carried out on potted vines in Season Three in the high and low RH CE rooms and for canes with or without laterals removed. Assessment times are 1) Stage Two – post-flowering, and 2) Stage Three – pre veraison. 287

Appendix 12: Mean values for all treatment combinations of leaf area, increase in leaf area between assessments two and three and BSN incidence for the last assessment. 288

Appendix 13: Mean values for all treatment combinations of lateral number, increase in lateral number between assessments two and three and BSN incidence for the last assessment. 289

Appendix 14: Bunch measurements for bunches with sensors during Stage Two and Three. Assessments carried out on 17/12/04 and 20/01/05, respectively 290
List of Abbreviations

ABA  
abscisic acid

AD  
apical dominance

A_{max}  
light saturated photosynthetic rate

ANOVA  
analysis of variance

BSN  
bunch stem necrosis

BTOA  
benzothiazole-2-oxyacetic acid

Ca  
calcium

CaCl_2  
calcium chloride

Ca(NO_3)_2  
calcium nitrate

CE  
controlled environment

CN  
cane number

CsCl  
cesium chloride

dT  
temperature increase of the sap

EBSN  
early bunch stem necrosis

ECN  
effective cane number

ECW  
effective cane weight

FB  
full bloom

GAs  
gibberellic acids

GAs_n  
gibberellic acid, n denotes the acid number

Gs  
stomatal conductance

GPN  
growing point number

GP/C  
growing points per cane

GPW  
growing point weight

HCl  
hydrochloric acid

HNO_3  
nitric acid

IAA  
β-indole-3-acetic acid

IBA  
3-indolebutyric acid

IN  
inflorescence necrosis

K  
potassium

Ksh  
thermal conductance constant for a particular gauge

LCP  
light compensation point

LLN  
leaf layer number

LSD  
Fisher’s protected least significant difference

LSE  
light saturation estimate

LSMeans  
Least significant means

Mg  
magnesium

MgCl_2  
magnesium chloride

Mg(NO_3)_2  
magnesium nitrate
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgSO₄</td>
<td>magnesium sulphate</td>
</tr>
<tr>
<td>NAA</td>
<td>1-naphthalene acetic acid</td>
</tr>
<tr>
<td>NPA</td>
<td>naphthyl-phthalamic acid</td>
</tr>
<tr>
<td>PD</td>
<td>primigenic dominance</td>
</tr>
<tr>
<td>PIL</td>
<td>percent interior leaves</td>
</tr>
<tr>
<td>PG</td>
<td>percentage gaps</td>
</tr>
<tr>
<td>Pn</td>
<td>net photosynthetic rate</td>
</tr>
<tr>
<td>PPF</td>
<td>photosynthetic photon flux</td>
</tr>
<tr>
<td>PW</td>
<td>pruning weight</td>
</tr>
<tr>
<td>QE</td>
<td>quantum efficiency</td>
</tr>
<tr>
<td>Qf</td>
<td>heat convection carried by sap</td>
</tr>
<tr>
<td>RBD</td>
<td>random block design</td>
</tr>
<tr>
<td>RH</td>
<td>relative humidity</td>
</tr>
<tr>
<td>SAS</td>
<td>SAS system for statistical analysis</td>
</tr>
<tr>
<td>SrCl₂</td>
<td>strontium chloride</td>
</tr>
<tr>
<td>SrCs</td>
<td>strontium and cesium</td>
</tr>
<tr>
<td>Sr(NO₃)₂</td>
<td>strontium nitrate</td>
</tr>
<tr>
<td>TA</td>
<td>titratable acidity</td>
</tr>
<tr>
<td>TIBA</td>
<td>2,3,5-triiodobenzoic acid</td>
</tr>
<tr>
<td>Tr</td>
<td>transpiration rate</td>
</tr>
<tr>
<td>VPD</td>
<td>vapour pressure deficit</td>
</tr>
</tbody>
</table>
Chapter One – Literature review

1 Literature Review

1.1 History
The grape has a very long history with fossil records indicating that the ancestors of the grapevine were present during the Jurassic period, 181 million years ago (Dry and Coombe, 2004). Seeds found in south-central Europe indicate that grapes have been a food source for man since at least the Bronze Age (Winkler, 1965). The earliest humans to enjoy wine are thought to be the Assyrians and later the Pharaohs of Egypt five or six thousand years ago (Winkler, 1965; Jackson and Schuster, 2001).

Little is known about the early movement of winemaking knowledge from Egypt to Asia and the Mediterranean but it is known that the Phoenicians exchanged wine for other goods around 1000 B.C. (Winkler, 1965; Jackson and Schuster, 2001). This wine originated in Asia-Minor but because the Phoenicians were a seafaring nation, vineyards were also planted in the Mediterranean and North Africa regions. Later, the spread of the Roman Empire helped move winemaking towards the centre of Europe, with the vine entering Germany no later than the second century A.D. (Winkler, 1965). However, the first vineyards were planted in the south of France by the Greeks around 500 B.C. (Jackson and Schuster, 2001).

With the colonisation of the New World, winemaking spread further. The first non-native vines were planted in America in 1524 by Cortez (Winkler, 1965) and in South Africa in 1652 by Jan van Riebeeck (Jackson and Schuster, 2001). Vines were transported to Australia with the settlers on the First Fleet in 1788 (Dry and Coombe, 2004) and James Busby was instrumental in the planting of vines in New Zealand in the early nineteenth century (Jackson and Schuster, 2001).

1.2 Taxonomy
Grapevines belong to the family Vitaceae that consists of up to 14 genera and about 700 species, with Vitis being the most commercially important genera to commercial viticulture (Dry and Coombe, 2004). Vitis is distinguished from most of the other
genera by the flower that has fused petals at the distal end that form a cap, and during flowering this detaches at the basal end to reveal the reproductive parts of the flower (Dry and Coombe, 2004).

Although the species of the genus *Vitis* occur naturally in very different geographical areas they are related closely enough to allow for interbreeding. *Vitis vinifera*, which is the main species of cultivated wine grape, originated in Eurasia (Weaver, 1976; Dry and Coombe, 2004). Another species native to Eurasia is *V. amurensis* that was used in hybridisation to introduce cold hardiness and early maturity (Dry and Coombe, 2004). *V. labrusca*, originated in eastern America, and due to its tolerance of many pests and diseases also native to America, is used in many of the American hybrids. Other American native species are *V. aestivalis, V. riparia, V. rupestris, V. cinera, V. mustangensis, V. champinii* and *V. vulpines*, many of which have a resistance to phylloxera and other pests and diseases and are therefore used in the development of rootstocks for *V. vinifera* (Weaver, 1976; Jackson and Schuster, 2001; Dry and Coombe, 2004). Most of the hybrids obtained from crosses with American species are mainly used for grape juice, table grapes, preserves and food additives, and some wine produced in America. It is now illegal to plant these hybrids in France due to the fact that they are claimed to produce wines of very poor quality (Dry and Coombe, 2004).

### 1.3 Physiology

Reproductive and vegetative anatomy of grape vines is well documented (Weaver, 1976; Kennedy, 2002; Dry and Coombe, 2004) and are reported here only briefly in order to give a general overview of normal development and to define terms used throughout the rest of this thesis.

#### 1.3.1 Inflorescence

Inflorescence primordial initiation occurs in the season prior to the season in which the flowers and berries are formed (Winkler, 1965; Pratt, 1971; Weaver, 1976; Jackson and Schuster, 2001). It begins around the time of flowering and continues throughout that season until veraison (Dry and Coombe, 2004). Therefore environmental conditions
around this time can affect the next season’s flowering and crop load. Warm sunny weather during the time of inflorescence initiation is required for a large crop in the following season (Jackson and Schuster, 2001).

Soon after bud break, once the current season’s canes have reached the node number at which the inflorescence is situated, the form of the inflorescence can be seen with the naked eye (Winkler, 1965). From bud swell through bud break until flowering, inflorescence primordia growth resumes from the previous season. The peduncle and rachis of the inflorescence develop, lengthening and expanding in width, and flower formation occurs (Dry and Coombe, 2004).

Although Jackson and Coombe (1995) state that the term peduncle includes “the central axis (rachis), the stems of the laterals, and the pedicels” of the inflorescence and later the bunch, Soule (1985) defines the peduncle as “a stalk of a flower cluster (inflorescence)” and the rachis as “the extension of the peduncle, to which the pedicels are attached if present” and the pedicel as a “stalk supporting a single flower”. Winkler (1965) also states very clearly that “the main axis of the cluster is called the rachis. Branches arise from the rachis at irregular intervals and divide to form the pedicels which bear the individual flowers ........... The region of the rachis extending from the shoot to its first branch is called the peduncle, or stem”. Therefore, throughout this thesis the terms used to describe the different parts of the inflorescence and bunch will be the three separate terms as described by Winkler (1965) (Figure 1). Some studies have used the term “bunch stem” and it is unclear, therefore whether the authors mean the peduncle, rachis or both. Consequently, when discussing particular literature, the term that is used by the authors will be used.
1.3.2 Flower

Flower initiation on the inflorescence can occur at any time from the summer before to the spring of flowering, depending on environmental factors (Pratt, 1971). This means that the primordia of individual flowers can have developed by the end of the summer prior to actual flowering in mature buds. However, the buds in the upper parts of the cane that are less mature may not develop individual flower primordia until the following spring, and are therefore small and less typical in shape for that cultivar (Winkler, 1965).

The petals of a grape flower are green and fused at the top to form a cap called the corolla or calyptra (Weaver, 1976; Jackson and Schuster, 2001; Dry and Coombe, 2004). At flowering the calyptra separates from the rest of the flower at the base near the pedicel and falls off (Weaver, 1976). This is called “cap” fall and is determined to be the time of flowering (Jackson and Schuster, 2001; Dry and Coombe, 2004). This event is also termed anthesis.
1.3.3 Pollination

Most *V. vinifera* cultivars have perfect (hermaphrodite) flowers (Pratt, 1971). In the wild many are still dioecious, with separate male and female plants, but when domesticated, perfect flowers appear to have been selected to avoid having unproductive males in a vineyard (Dry and Coombe, 2004). The flowers are pollinated by the wind and can also self pollinate (Jackson and Schuster, 2001). If weather conditions around flowering are wet, caps may not fall totally from the flower, and although pollination can still occur, fruit set may be adversely affected (Weaver, 1976).

1.3.4 Berry growth and veraison

Many authors have used different methods to describe grape berry growth. These include classification due to colour and chemical change, cell metabolism, and diameter, length, volume or weight measurements (Pratt, 1971). Eichhorn and Lorenz developed a comprehensive scheme that included all growth stages of the vine (referenced in Dry and Coombe, 2004). It is known as the E-L system with 47 numbers detailing the growth stages from winter bud to leaf fall. The E-L number for the following stages are: flowering – 23, berry set – 27, veraison – 35, harvest – 38 (Dry and Coombe, 2004). Veraison in French means the commencement of berry colour change and has become a term used to describe the event at the end of the lag stage in berry growth when, among other things, berries soften, sugars increase, malic acid decreases, colour changes and berry growth accelerates again (Coombe and Hale, 1973; Dry and Coombe, 2004).

By using berry growth measurements it can be shown that berry growth follows a double sigmoid curve from anthesis to maturity with (I) a period of rapid growth, (II) a period of slow growth (termed the lag stage) and (III) a final period of rapid growth (Harris *et al.*, 1968; Pratt, 1971; Weaver, 1976; Jackson and Schuster, 2001). However, other authors have either condensed this into two phases of growth, or expanded it into four stages. This depends on whether they separate out the lag stage or not in their definition, or add an extra stage at the beginning when there is little berry growth, and berry drop and nucleus growth occur (E-L 23 – 27) (Pratt, 1971).
Figure 2: Central Diagram: Appearance of berries at 10-day intervals revealing the two successive sigmoidal growth curves of a grape berry, designated 'berry formation' and 'berry ripening'. Three generalised x-axes are shown – days after flowering, approximate juice "Brix values during ripening, and developmental growth stages using modified E-L system. The key growth stages and the approximate timing of the accumulation of major solutes are shown. At bottom: Scale drawings of anatomical features in the longitudinal sections of developing grape seeds at days 4, 14, 28, 42 and 98 days after flowering. Figure and caption from Dry and Coombe, (2004).

For the sake of simplicity in this thesis, three stages are considered making up the two phases of berry growth. Many authors continue to include the lag stage in their discussions even when considering there to be two phases of growth. Phase One is
therefore from anthesis to veraison (E-L 23 – 33) when cell division and cell expansion both account for the growth in the berry (Harris et al., 1968; Weaver, 1976). Cell division has generally stopped by approximately 40 days after bloom, with it first ceasing in the placenta and inner pericarp 7 – 11 days after full bloom (FB), in the outer pericarp 19 – 20 days after FB and finally in the hypodermis and epidermis 32 – 38 days after FB (Pratt, 1971). This growth phase is called berry formation (Dry and Coombe, 2004) and includes Stages I and II. The seeds attain their full size in this phase but the endosperm and embryo are incompletely developed (Pratt, 1971; Dry and Coombe, 2004). The end of this phase is characterised by Stage II, the lag stage in which it is thought that parts of the embryo are differentiated (Pratt, 1971) and little extra growth in the berry occurs (Figure 2). The lag stage can sometimes be brief or prolonged depending on the environmental conditions and whether it is a seeded or non seeded cultivar (Weaver, 1976). It has been suggested that this lag stage is due to a reduction in the sink strength of berry flesh for assimilates (Zhang et al., 2003). This may be due to the reduction in gibberellins in the berry (Bhullar and Dhillon, 1974). At this stage the seeds become much stronger sinks than the flesh of the berry (Zhang et al., 2003).

Phase Two begins at veraison and continues through to maturity (E-L 34 – 38). Berry growth is due to cell expansion with the importation of sugars through the phloem. This phase is called berry ripening (Dry and Coombe, 2004).

1.3.5 Ripening

From veraison onwards the berry content changes as part of the normal ripening process. The concentration of soluble solids stays relatively constant prior to veraison and then increases sharply (Hrazdina et al., 1984; Morrison and Iodi, 1990; Dry and Coombe, 2004) as both the glucose and fructose concentrations increase and tartaric and malic acid concentrations decline rapidly until harvest (Downton and Loveys, 1978; Cawthon and Morris, 1982; Crippen and Morrison, 1986). The reduction in acids results in the titratable acidity also declining after veraison (Morrison and Iodi, 1990). Tartaric acid has a high initial concentration after anthesis and steadily declines throughout the season, whereas malic acid has a low initial concentration after anthesis, increases sharply until veraison, and then declines rapidly (Hrazdina et al., 1984;
Gutierrez-Granda and Morrison, 1992; Dry and Coombe, 2004). The pH, which is generally constant prior to veraison, increases sharply from post-veraison until harvest (Morrison and Iodi, 1990; Gutierrez-Granda and Morrison, 1992; Dry and Coombe, 2004).

Xylem connections between the berry and the vine become discontinuous near veraison (Figure 2), but are thought to not disappear completely (Creasy and Lombard, 1993). Studies have shown that, although initially water flows predominantly in the peripheral xylem system, once the discontinuity occurs, water flow in the xylem occurs in the axial system which is less conductive (During et al., 1987). However, this is thought not to occur in the cultivar ‘Shiraz’ (Rodgers et al., 2000). This changes the berry water relations, with berries pre-veraison being more susceptible to water stress than post-veraison berries (Creasy and Lombard, 1993). The change in water flow in the xylem is thought to be due to the appearance of breaks in the xylem tracheid wall membranes in the peripheral bundles (During et al., 1987; Findlay et al., 1987). It has been hypothesised that this rupture is due to the rapid expansion of the berry in the final growth stage (During et al., 1987). However, Creasy et al., (1993) found that dye uptake was affected around the time of softening, which occurred before the rapid increase in growth. The hypothesis of xylem rupture has also been questioned lately with a new hypothesis of a loss of hydrostatic gradient in the berry apoplastic causing the decrease in xylem flow (Bondada et al., 2005).

A rise in sugar accumulation has also been found to coincide with the decline in dye uptake, which is the suggested timing of xylem dysfunction (Creasy et al., 1993). The reduction in xylem flow due to the dysfunction is thought to cause the resulting increase in phloem translocation in normal ripening fruit (During et al., 1987). This increase in phloem translocation may therefore result in the increase in sugar accumulation and also the change in cation accumulation in the berry (During et al., 1987).
1.3.5.1 Cation accumulation

Potassium (K) concentration is initially high in the berry after anthesis but declines throughout Stage I, remains constant during the lag stage and increases during Stage III after veraison (Hrazdina et al., 1984; Crippen and Morrison, 1986; Morrison and Iodi, 1990; Gutierrez-Granda and Morrison, 1992). The potassium content per berry continues to increase through the final stage of ripening indicating that potassium movement into the berry is still possible (Esteban et al., 1999). Calcium (Ca) concentration increases up to veraison, but steadily declines throughout Stage III with the decline levelling off towards harvest (Hrazdina et al., 1984; Morrison and Iodi, 1990; Creasy et al., 1993; Esteban et al., 1999). This decline in calcium concentration is mainly due to the increase in berry size and the cessation of calcium being imported into the berry, as calcium content per berry increases up until veraison then remains constant throughout the ripening period (Hrazdina et al., 1984; Esteban et al., 1999). Magnesium (Mg) concentration declines until veraison and then remains relatively constant throughout Stage III (Hrazdina et al., 1984), with berry magnesium content continuing to increase throughout this stage (Esteban et al., 1999).

The rupture of xylem vessels in the peripheral vascular system of the grape is thought to be the reason that calcium influx is reduced during ripening. The increase in potassium concentration after veraison is associated with the increased phloem translocation as, although potassium moves in the xylem and phloem, it is present in very high concentrations in the phloem sap (Mengel and Kirkby, 1987).

The potassium and calcium concentrations in the peduncle follow a similar pattern to that of concentrations in the berry, although potassium concentration decreases again close to maturity and calcium increases slightly. Magnesium concentration increases up until veraison, decreases rapidly and then increases again towards maturity (Stellwaag-Kittler, 1975). Cocucci et al. (1988) found that reduced berry number had no affect on potassium concentration in the rachis of the bunch, but calcium concentration does decrease with a reduction in berry number.
1.3.5.2 Plant growth substances

Auxin-like substances have been found at FB in the berry. Studies have found that they increase slowly until the end of Stage II and then decrease sharply (Alleweldt and Hifny, 1972; Bhullar and Dhillon, 1974; Zhang et al., 2003). Gibberellic acid (GA₃) has not been found in the berry at FB but has been found to increase after FB to reach a peak before Stage II, then decrease until there was no gibberellic activity after Stage II (Bhullar and Dhillon, 1974). Abscisic acid (ABA) has been found to be present before flowering but then to progressively decline (Lilov and Angelova, 1977) and then to be present in low concentrations until gibberellin and auxin concentrations begin to drop. After this ABA has been found to increase slowly until Stage II (Bhullar and Dhillon, 1974; Zhang et al., 2003). During Stage II, ABA concentration has been found to increase sharply to a peak after veraison, then decline again throughout Stage III until harvest (Coombe and Hale, 1973; Bhullar and Dhillon, 1974; Downton and Loveys, 1978).

1.4 BSN

Bunch stem necrosis (BSN) is a physiological disorder that occurs in grapes around the world that can be detrimental to wine and table grape quality (Ureta et al., 1981). In some cases crop losses of more than 60% have been reported (Rumbos, 1989). Due to its international occurrence it is known by many names. These include Stiellähme in Germany (translation being stalk necrosis, also used), le dessèchement de la rafle in France (translation being the drying (or withering) of the peduncle) and palo negro in Chile. Other English names include waterberry used in the United States of America and shanking used in New Zealand (Jackson and Coombe, 1995). However, some references state that waterberry and palo negro are similar to but not the same disorders as BSN (Pearson and Goheen, 1988). Throughout this thesis the term BSN will be used to describe the disorder under investigation.

BSN is a different disorder to that of early bunch stem necrosis (EBSN), also known as inflorescence necrosis (IN). EBSN affects the pedicels, rachis and peduncles any time from the early appearance of the inflorescence up until flowering and possibly shortly after, and not after veraison as in the case of BSN (Jackson and Coombe, 1988).
Symptoms of BSN can occur any time after the beginning of the lag stage when the concentration of most plant growth regulators are low and sugar accumulation begins to increase. This is also the time when the osmotic value of the berries is higher than that of the rachis (Stellwaag-Kittler, 1975, 1983). Symptoms occur on the peduncle, rachis and berries and continue to develop progressively from véraison until harvest (Theiler, 1975b; Stellwaag-Kittler, 1983).

*Botrytis cinerea* infection can produce symptoms that can also be confused with BSN, but again there are many differences between the disorders (Delas *et al.*, 1976). *Botrytis* is caused by a fungus and often the conidia are visible (Delas *et al.*, 1976; Pearson and Goheen, 1988). The margins of the necrotic tissue caused by *Botrytis* are not as well defined as in BSN and can also occur at any stage in the development of the berry (Delas *et al.*, 1976; Pearson and Goheen, 1988). However, often the presence of BSN will then predispose bunches to botrytis infection and therefore often both can be present (Bolay *et al.*, 1966; Delas *et al.*, 1976).

BSN incidence is dependent on grape cultivar, rootstock (Stellwaag-Kittler, 1975; Theiler, 1976; Scienza, 1982; Boselli *et al.*, 1983) and vineyard (Boselli *et al.*, 1986), with some cultivars and vineyards demonstrating consistently higher incidences of BSN (Stellwaag-Kittler, 1975; Jahnl, 1983). The incidence of BSN can also be higher in some seasons compared to others (Holzapfel and Coombe, 1995).

The first known reports of a disorder matching the symptoms of BSN in Europe was in 1937 (Osterwalder, 1937) and in Australia in 1953 (Coombe and Allan, 1953). The first record of BSN in Californian vineyards was by Bioletti in 1923 (cited in Weaver, 1976; Morrison and Iodi, 1990). Interestingly, Weaver (1976) states that there are in fact two conditions that the name waterberry covers. The first, which Bioletti (cited in Weaver, 1976) described, was stated to be caused by over-cropping and therefore under-nourishment of the berries and only occurred at the tips of the clusters. The symptoms included berries with lack of sugar, colour, flavour and shipping quality but Weaver (1976) did not mention any necrosis. The second condition had the same symptoms as the first, but did include necrosis of the pedicel and could occur in all parts of the bunch.
and was found not to be caused by over-cropping (Kasimatis, 1957). This second disorder was also found to be directly associated with the blockage of xylem vessels in the pedicel by tyloses (gummy or resinous secretions (Soule, 1985)). The extent of this blockage determined the degree of deterioration in berry development, and it was not until the blockage was almost complete that necrosis of the pedicel occurred (Kasimatis, 1957). It is therefore difficult to determine if either or both of these two conditions are in fact the same condition as that termed BSN but it is very likely that the second condition is the same as BSN.

1.4.1 Symptoms

Initial symptoms of BSN include the appearance of dark brown sunken necrotic spots on the pedicel, rachis or peduncle (Plate 1) (Delas et al., 1976; Theiler, 1976, 1986a). They can be of varying shape and size and if they remain as small spots, are harmless (Stellwaag-Kittler, 1983). These initial symptoms are called primary symptoms (Theiler, 1976). However, if the necrosis spreads to girdle the entire stem (Plate 1), then secondary symptoms occur (Delas et al., 1976; Theiler, 1976; Haub, 1986).

The first cells to become necrotic are the stomata, epidermis and hypodermis of the grape peduncles (Plate 2) (Delas et al., 1976; Theiler, 1976; Brendel et al., 1983). At the cellular level it appears that the middle lamella is initially affected (Jahnl, 1975). The necrosis then spreads to the cortical tissue and finally to the phloem cells in more severe cases (Theiler, 1976; Brendel et al., 1983).

Berries adjacent to the affected rachis show visual symptoms and are typically dull and opaque in appearance, soft in texture and juice analyses shows that they lack sugar and flavour due to disruption to the phloem cells (Delas et al., 1976; Theiler, 1976, 1986a). If the necrosis spreads to girdle the entire part of the affected area, the rachis may then desiccate and may either abscise or remain on the bunch in a shrunken or completely dry condition (Delas et al., 1976; Jackson and Schuster, 2001) (Plates 3, 4 and 5). The tips of the rachis and wings (shoulders) are areas where this condition most frequently develops (Stellwaag-Kittler, 1983) and the pattern of necrosis development corresponds
with the pattern of polyphenol distribution in the cortical parenchyma (Stellwaag-Kittler, 1975).

Plate 1: Rachis of BSN – affected (left) and healthy (right) bunches. Arrows indicate necrotised areas.

Plate 2: Left: healthy stomata opening in the epidermis. Right: necrotised (dead) stomata opening of a rachis, where the primary symptoms of BSN can develop (Theiler, 1975b).
Plate 3: A healthy bunch (left) and BSN affected bunch (right). Necrosis of rachis and shrivelling of berries can be seen in the lower three-quarters of the affected bunch.
Plate 4: BSN affected bunch. The distal end is exhibiting BSN symptoms with rachis necrosis and shrivelled berries. The demarcation between healthy and necrotised tissue is evident.

Plate 5: Necrosis and shrivelled berries on a rachis branch in the middle of a bunch. As the necrosis has not girdled the primary axis of the rachis, berries distal to the branch are not exhibiting visual symptoms of BSN.
Before symptoms develop, BSN affected berries cannot be distinguished from healthy berries by either their size or chemical composition. BSN affected berries, however, expand at a much slower rate after veraison (Morrison and Iodi, 1990). The rapid increase in the concentration of soluble solids in the first two weeks after veraison in healthy berries is not seen in BSN affected berries, but there is a slow increase later (Morrison and Iodi, 1990). However, BSN affected berries never reach the Brix concentrations of healthy berries (Osterwalder, 1943; Ureta et al., 1981; Morrison and Iodi, 1990). This trend is also seen in berry pH (Morrison and Iodi, 1990) and in the phenolic compounds of proanthocyanidins and anthocyanins (Ureta et al., 1981). The decrease in titratable acidity is also delayed in BSN affected berries and concentrations never drop to that of healthy berries (Morrison and Iodi, 1990). Tartaric acid concentrations do not drop in BSN affected berries as in healthy berries (Ureta et al., 1981) due to the reduced expansion of the berry and therefore a reduced dilution effect (Morrison and Iodi, 1990). However, there is a further accumulation of tartaric acid in BSN affected berries not seen in healthy berries (Morrison and Iodi, 1990). The concentration of potassium in the juice of BSN affected berries, does not increase as it should (Morrison and Iodi, 1990). Calcium concentration also increases greatly in BSN affected berries (Morrison and Iodi, 1990).

Generally potassium and calcium concentrations in rachis samples increase throughout the season. Some studies have found little difference between susceptible and non-susceptible cultivars (Cocucci et al., 1988) while others have found the resistant cultivar to have a higher potassium concentration in the rachis (Scienza, 1982). However, the continued accumulation of calcium and reduced accumulation of potassium in BSN affected berries is due to the normal xylem dysfunction not occurring in BSN affected bunches (Osterwalder, 1943; Morrison and Iodi, 1990). The slower, post-veraison growth rate may be related to the lack of dysfunction. It has been hypothesised that there must be some physiological change in BSN affected berries before softening, in order for the xylem to remain continuous (Creasy et al., 1993).

Concentrations of certain plant growth regulators have also been studied to determine what their concentrations are in healthy and affected bunches. Ruiz and Moyano (1994)
found that, in the peduncle and rachis of BSN affected bunches, putrescine concentration was higher than in healthy bunches. In that study, putrescine concentration had an increasing gradient from relatively healthy tissue towards the necrotised area of the bunch. Although putrescine synthesis can occur from the decarboxylation of arginine, high concentrations of nitrogen are not considered to be the sole reason behind the high concentrations of putrescine (Ruiz and Moyano, 1994) as potassium deficiency has been demonstrated to increase putrescine concentrations in plants (Davies, 1995a).

The plant growth regulator ABA has also been implicated in BSN incidence. It is often higher in the peduncle of BSN affected berries although there appears to be no correlation between the incidence of BSN and the concentration of ABA in the peduncle (Holzapfel and Coombe, 1997). ABA concentrations have also been found to be higher in the seeds of affected berries. ABA concentrations decreased in value from affected berries, to unaffected berries on affected bunches, to unaffected bunches on affected vines, with healthy bunches on healthy vines having the lowest ABA value (Broquedis and Bouard, 1981). However, studies have shown that removing the berries after fruit set resulted in there being no symptoms of BSN (Scienza and Fregoni, 1978; Theiler and Coombe, 1985) and yet ABA concentrations in the rachis of bunches without berries reached higher values before veraison than bunches with berries (Scienza and Fregoni, 1978).

In the above studies, as the concentrations of cations and plant growth regulators were assessed after the visual symptoms of BSN had begun to appear, it is likely that they are a consequence of the physiological disruption caused by BSN development, rather than the cause of the disorder.

1.4.2 Causes

1.4.2.1 Environmental

The severity of BSN cannot only fluctuate dramatically among vineyards, but also between seasons (Holzapfel and Coombe, 1995). It is not caused by any pathogen
(Stellwaag-Kittler, 1975; Haub, 1986) and, due to the apparent influence the environment has on the severity of BSN (Stellwaag-Kittler, 1975), it is now generally accepted that BSN is a result of the grapevine reacting to its surrounding environmental conditions. These conditions include the climate, site, soil management including fertilisation, and pruning, as well as other factors such as cultivar and rootstock (Theiler, 1975a). However, exactly what combination of these factors consistently lead to BSN is still not proven.

Rain events at certain times have been associated with high incidences of BSN (Boselli et al., 1983). In France and Austria a high frequency of rain events around veraison was found to be highly correlated with the incidence of BSN (Brechbuhler, 1987; Redl, 1987; Baldacchino-Reynaud, 2000). However in Australia, Germany and Italy this was not the case (Hartmair, 1975; Boselli et al., 1986, 1987; Holzapfel and Coombe, 1995). Theiler and Muller (1986) found a high amount of rain over the flowering period increased the incidence of BSN. In Greece, high incidences of BSN were associated only with high rainfall if it followed a long period of dry weather (Rumbos, 1989).

During rain events, temperatures usually drop and light levels are lower, both of which have been implicated in the incidence of BSN (Koblet et al., 1997). Studies in Germany and France have found a negative correlation between the average midday temperature at flowering (Theiler, 1983, 1986b; Theiler and Muller, 1986), or at the first growth phase of the berries, and BSN incidence (Boselli et al., 1987; Baldacchino-Reynaud, 2000) but this was not found to be true in Australia (Holzapfel and Coombe, 1995). In Australia it was found that it was the 20 days before flowering and the week over which veraison occurred where the temperature had the highest inverse relationship with BSN incidence (Holzapfel and Coombe, 1995). However, in Austria no correlation of average midday temperature around flowering with BSN incidence was found but there was a relationship between average daily temperature and maximum daily temperature from flowering to a berry diameter of 2 – 3 mm and BSN incidence. In contrast to other studies this relationship was positive (Redl, 1987).
Perez and Gaete (1986) found that vines grown under shade conditions had significantly more BSN incidence than those grown in full sunlight. Theiler and Muller (1986) also found a significant inverse relationship between mean duration of sunshine during flowering and BSN incidence.

Rain events also result in an increase in air humidity. Nicolli et al. (1977) concluded that high relative humidity affected BSN incidence more than either maximum or minimum temperature. Jordan (1985) found a two-fold increase in the incidence of BSN due to a two-fold increase in relative humidity for vines grown in controlled environment rooms. Leaf damage due to hail storms has also been found to increase BSN incidence (Koblet et al., 1997).

These various studies, although contradictory, do indicate that environmental conditions around certain physiological events in the berries development, particularly around flowering, can influence the incidence of this disorder.

1.4.2.2 Mineral nutrition in vine and berry

It has been hypothesised that BSN is a deficiency in calcium and/or magnesium (Hartmair and Grill, 1965; Haub, 1986; Baldacchino-Reynaud, 2000) and it has therefore been considered to be a similar disorder to other calcium-related disorders such as bitter pit and watercore in apples, and blossom end rot in tomatoes and peppers (Boselli and Fregoni, 1986; Haub, 1986). Because high concentrations of potassium tend to compete negatively against the uptake of calcium and magnesium (Mengel and Kirkby, 1987) it was hypothesised that high values of potassium would therefore cause calcium and magnesium deficiencies.

An imbalance between potassium, magnesium and/or calcium in the rachis and leaf tissue has therefore often been reported as the cause of BSN (Brechbuhler, 1975; Donna, 1985; Cocucci et al., 1988). Nahdi et al. (1993) found that BSN was related to an imbalance in the K/Mg ratio and that an optimum ratio in the petiole at veraison was four. Yet other studies have found no correlation between leaf nutrition and BSN.
incidence (Boselli et al., 1986), or only that it was able to be used for a prognosis of BSN at certain locations (Boselli et al., 1987).

Hartmair (1975) found a positive correlation between the ratio of K/(Ca+Mg) in the peduncle and BSN, and stated that an increase in this ratio, due to a decrease in calcium or magnesium, or an increase in potassium, resulted in an increase in BSN incidence. Other studies, have also found that tissue from susceptible cultivars showed a higher K/Ca ratio than that in tissue from less susceptible cultivars (Cocucci et al., 1988). Also, healthy appearing rachis tissue from diseased bunches showed a higher K/Ca ratio than healthy tissue from unaffected bunches of the same cultivar (Feucht et al., 1975). Lauber and Koblet (1967) found that at the first signs of BSN, the bunch stems had higher K/Mg, K/Ca and K/(Ca+Mg) ratios than healthy stems, and in healthy tissue that had been treated with foliage sprays and had no signs of BSN symptoms, the ratios were much smaller.

Feucht et al. (1975) also found the ratio of K/Ca to be high in tissue taken from rachis that were determined by an electron microscope to be developing BSN, even though visual symptoms had not appeared. The ratio was highest in the parenchyma cells of the cortex, but not as high in the xylem and on the surface of the rachis. The ratio became higher as the disorder progressed.

However, Christensen and Boggero (1985) found that due to a reduction in potassium, and consistent concentrations of calcium and magnesium, that the ratio of K/(Ca+Mg) was lower in the rachis of BSN affected bunches. Fregoni and Scienza (1975) found that a resistant cultivar, ‘Barbera’, consistently had a higher K/(Ca+Mg) ratio in the bunch stem than the susceptible cultivar, ‘Bonarda’. Shin et al. (1984) found that in the two cultivars they were investigating, all three ratios of K/Ca, K/Mg and K/(Ca+Mg) were higher in healthy vines compared to vines with BSN. Yet other studies have found no correlation between nutrient content of the peduncle and rachis and the occurrence of BSN (Claus, 1965; Redl, 1983).
**Potassium.** Jordan (1985) reported that vines with BSN demonstrated a higher potassium concentration in the leaf tissue than vines without BSN. However, it was the rate at which potassium accumulated that was better correlated with BSN incidence; a high rate resulted in high BSN incidence (Jordan, 1984). However, Spring *et al.* (1999) investigated potassium concentration in the leaf tissue of two cultivars of varying BSN incidence, and the cultivar with higher BSN incidence did not consistently demonstrate higher leaf potassium concentration across all rootstocks studied compared to the other cultivar.

Christensen and Boggero (1985) did not find a correlation with the concentration of potassium in the petiole and BSN incidence. Shin *et al.* (1984) found that in one cultivar the potassium concentration in the petiole was higher in vines with BSN, and yet in another cultivar the potassium concentration was lower in vines with BSN. However, when looking at the potassium concentration in the rachis, Shin *et al.* (1984) found that in both cultivars the potassium concentration was lower in BSN affected bunches. Other studies (Scienza, 1982; Christensen and Boggero, 1985; Ruiz and Moyano, 1994) have also found that potassium concentrations were lower in the rachis of BSN affected bunches, whereas Redl (1983) found the opposite with potassium concentration being higher. Scienza and Fregoni (1978) also found that in the rachis of bunches without berries and that did not demonstrate BSN, that potassium concentration was lower, and therefore potassium concentration was higher in the BSN affected bunches. Some research has found that potassium concentration is lower in BSN affected berries (Osterwalder, 1943; Morrison and Iodi, 1990), but Scienza (1982) found that a cultivar resistant to BSN had lower potassium concentration in the berry juice compared to a susceptible cultivar.

The results from these various studies therefore demonstrate that there is no consistent relationship between potassium concentration in the leaves, petioles, rachis or berries and the incidence of BSN across all cultivars and rootstocks. Conclusions have ranged from stating that there is a positive relationship to stating a negative relationship between potassium concentration and the incidence of BSN.
Calcium. Early in the study of BSN it was thought that the incidence of BSN was due to a deficiency of calcium in cells (Hartmair and Grill, 1965; Alleweldt and Hifny, 1972) as BSN symptoms are similar to that of other calcium deficiency symptoms (Schaller, 1983). High calcium concentrations have also been found in leaves from cultivars that are less susceptible to BSN compared to more susceptible cultivars (Spring et al., 1999). Wood from affected vines also had lower calcium concentrations than that from healthy vines (Hartmair and Grill, 1965). However, Christensen and Boggero (1985) did not find a correlation between petiole calcium concentration and BSN incidence.

At veraison, unaffected berries and resistant cultivars have slightly higher concentrations of calcium than BSN affected berries and susceptible cultivars (Scienza, 1982; Morrison and Iodi, 1990). Berry respiration rate of less susceptible cultivars can also be higher, which is hypothesised as the reason the calcium concentration was higher (Boselli and Volpe, 1990). Foliar fertilisers that contain magnesium and reduce BSN incidence have also been found to increase the calcium content of the rachis (Redl and Weindlmayr, 1985). On bunches where the berries had been removed and BSN symptoms did not occur, the calcium concentration in the peduncle was also higher than on bunches with berries and BSN symptoms (Scienza and Fregoni, 1978). Calcium concentration was also found to be higher in berries containing more seeds (Boselli et al., 1995). Haub (1986) states that “diseased stalks” have 20% less calcium on average than healthy ones.

However, in an extensive survey over many cultivars, Redl (1983) did not find a correlation between rachis calcium concentration and BSN incidence, but they did find higher calcium concentrations in the peduncles of affected bunches. Christensen and Boggero (1985) also found no relationship between rachis calcium concentration and BSN incidence. Boselli et al. (1995) found that calcium concentration was higher in a cultivar that was more susceptible to BSN incidence (‘Croatina’) when compared to a cultivar that was less susceptible (‘Barbera’) and yet Scienza (1982) found the exact opposite. Therefore, like the results for potassium, those for calcium concentration are
inconsistent, and therefore no conclusive relationship between calcium concentration and BSN incidence can be found.

**Magnesium.** Some studies have shown that BSN affected bunches tend to have lower concentrations of magnesium in the rachis and the vine also exhibit lower magnesium values in the leaf blade (Redl, 1983; Spring et al., 1999). Spring et al. (1999) showed that a cultivar that consistently demonstrated high BSN incidence (‘Chasselas’) had lower leaf magnesium values than the cultivar ‘Gamay à Pully’ that consistently had low BSN incidence. However, Brechbuhler and Meyer (1988) found that although applications of nitrogen to the soil also increased magnesium concentrations in the leaves, the incidence of BSN also increased. Other studies have found no correlation between magnesium concentration in the leaves and leaf petioles and BSN incidence (Jordan, 1984; Christensen and Boggero, 1985).

Haub (1986) reported 40% lower magnesium concentration in BSN affected bunch stems compared to healthy ones and Scienza (1982) found that a susceptible cultivar also had lower magnesium concentrations in the rachis compared to a resistant cultivar. The uptake of magnesium from a solution also appears to be cultivar dependant, with the more susceptible cultivars having a lower capacity to take up magnesium (Schimansky, 1983). Applications of foliar fertilisers that decrease BSN have also been found to increase rachis magnesium content. On bunches where berries had been removed and no BSN symptoms occurred, magnesium concentrations in the peduncle were higher than in peduncles with berries and exhibiting BSN symptoms (Scienza and Fregoni, 1978). Christensen and Boggero (1985) found no correlation between magnesium concentration in the rachis and BSN incidence.

Therefore, unlike potassium and calcium concentrations, the majority of studies do demonstrate a relationship between magnesium concentration in the rachis and peduncle and the incidence of BSN. However, it appears likely that leaf and petiole magnesium concentrations are not related to BSN incidence and are therefore not a good indicator for determining the cause of this disorder.
**Nitrogen.** Spring *et al.* (1999) found when comparing two cultivars with differing BSN incidence, that the cultivar with the lower incidence (‘Gamay à Pully’) also demonstrated higher nitrogen concentrations in the leaves than the cultivar with the higher incidence (‘Chasselas’). However, Jordan (1985) reported that vines with a high incidence of BSN had higher nitrate values in leaf tissue than vines without BSN, yet in the season before, Jordan (1984) found no such evidence. Brechbuhler and Meyer (1988) also found that an increase in leaf nitrogen concentrations due to nitrogen application to the soil, increased BSN incidence, and Christensen and Boggero (1985) found high incidence areas to have higher petiole nitrogen than the low incidence areas.

Ammonium concentrations in the rachis have been shown to be higher in BSN affected bunches (Redl, 1983; Christensen and Boggero, 1985; Ruiz and Moyano, 1994). Foliar applied substances that decrease the incidence of BSN have also been found to decrease the berry nitrogen content. However, high nitrogen concentrations in the rachis and berry are not always found to be associated with BSN (Ruiz and Moyano, 1993; Capps and Wolf, 2000). Holzapfel and Coombe (1997) found a negative correlation between released-NH$_4$ and BSN but no relationship between free-NH$_4$ and BSN. Christensen and Boggero (1985) did not find a correlation between nitrate and BSN incidence. Due to the conflicting results between studies it was suggested that high ammonium values in BSN affected rachis may in fact be the result of some dysfunction of nitrogen metabolism associated with BSN (Ruiz and Moyano, 1993) or a secondary effect related to the senescence of the peduncle tissue (Keller and Koblet, 1995b). Therefore, as with potassium and calcium concentrations, there appears to be no clear, consistent relationship between nitrogen concentrations in the leaves, rachis and berries and BSN incidence.

Increasing the organic matter in the soil has also been found to reduce the incidence of BSN (Frisullo and Faretra, 2003) as has growing a ground cover between rows rather than leaving the soil bare (Koblet and Lauber, 1968; Perret and Koblet, 1973; Hinkel, 1992).
1.4.2.3 Plant growth regulators

The plant growth regulator ABA has been shown to be an important hormone in the process of senescence in plants. This, and the fact that high ABA concentrations have been found in the peduncle and berries of BSN affected bunches, have lead to the hypothesis that BSN may be caused by an imbalance in hormone metabolism during the ripening of the berries (Baldacchino et al., 1987a).

1.4.2.4 Cultivars and rootstocks

Some cultivars of grape are more susceptible to BSN than others. Susceptible cultivars include ‘Cabernet Sauvignon’, ‘Riesling’, ‘Gewürztraminer’, ‘Chasselas’, ‘Carignan’, ‘Grenache’, and the table cultivars ‘Muscat de Hambourg’ and ‘Cardinal’. Less susceptible cultivars include ‘Merlot’, ‘Pinot’ cultivars and ‘Sylvaner’ (Clement, 1977, 1978a). The rootstock onto which vines have been grown can also influence the incidence of BSN. High vigour rootstocks or those with a tendency towards magnesium deficiency, such as SO4, 161-49C and 45-53M tend to demonstrate more BSN incidence (Delas et al., 1976; Clement, 1978a; Lupton, 1985). This correlation is consistent with the studies into magnesium and the incidence of BSN.

1.4.2.5 Canopy development

Vine canopy development and the training systems used to grow vines have been shown to influence the incidence of BSN. Early in the investigations into BSN incidence it was reported that BSN was more prevalent in vigorous vines that had been over pruned, over watered, over fertilised with nitrogen or potassium or that had heavy crop loads (Bolay et al., 1965; Delas et al., 1976; Cline, 1987). Later, Moreno and Pavez (2000) found a positive correlation between high leaf layer number (LLN), low canopy gaps and excessive growth during the veraison to harvest period and the incidence of BSN. Theiler (1975b) also found that vigorous vine growth increased BSN incidence while weak growth reduced it.

Training systems that prune to a high trunk height have resulted in high BSN incidence (Hifny, 1971; Redl, 1984a) as have those training systems that encourage the vigour of
individual canes (Hifny, 1971; Redl, 1984a). Brendel and Hofmann (1983) found that by decreasing bud number on canes BSN incidence increased in cultivars ‘Riesling’, ‘Thurling’ and ‘Ehrenfelser’. However, they also found that increasing bud number on canes increased BSN incidence for the cultivars ‘Kanzler’, ‘Optima’ and ‘Schönburger’.

Reports on BSN published in the 1970s suggested the use of training systems to reduce vine vigour and therefore BSN incidence (Clement, 1978b). Holzapfel and Coombe (1995) found that vines that were spur-pruned developed considerably more BSN than vines that were minimally pruned. Redl (1988) found that a training system, traditionally used in Austria, which resulted in an improved leaf exposure, decreased the incidence of BSN on ‘Green Veltliner’. This training system also resulted in larger leaves, increased leaf area and longer shoots, although it may not necessarily have resulted in a more vigorous canopy.

However, Capps and Wolf (2000) did not find a significant difference in canopy development between their treatments on ‘Cabernet Sauvignon’ (as measured by LLN and canopy gaps), although their treatments did demonstrate a significant difference in BSN incidence. Also, in a vineyard with greater canopy development, BSN incidence was lower (Capps and Wolf, 2000). Some studies have found BSN to be more prevalent on vines with less vigorous shoots (Scienza and Fregoni, 1978; Song et al., 2003). Shin et al. (1984) also found similar results for the two cultivars ‘Golden Queen’ and ‘Himrod Seedless’. Becker (1990) found a vigorous cultivar, ‘FR946-60’, which is a cross between European and American vines, to be resistant to BSN.

The number of bunches per vine has been found to be inversely correlated with BSN incidence (Nicolli et al., 1977) especially when training systems were compared (Holzapfel and Coombe, 1995). However, this correlation did not hold up when the year susceptibility was compared with the crop loads in those years (Holzapfel and Coombe, 1995).
Some studies have also found that defoliation around the bunches, a common practice in New Zealand around veraison time to control *Botrytis*, actually increases the incidence of BSN (Koblet *et al.*, 1969).

Therefore, although high vigour has been positively correlated with a high incidence of BSN, the results are not always consistent. However, the description of “vigour” is not always precise and the time that the vigour of the vines is measured is not always stated or consistent across all studies.

### 1.4.2.6 Xylem development

During and Lang (1993) found that in BSN susceptible cultivars the xylem development was suppressed just distal to each node of the peduncle creating a restriction in the xylem system. The cross sectional area was reduced and only small primary vessels were present in this area. Cultivars that were less susceptible did not demonstrate such a limitation. The restriction demonstrated a high hydraulic resistance that in turn would affect the transport of nutrients through the xylem. From this work they developed a screening procedure for new cultivars to determine if they may be susceptible or not to BSN (Lang *et al.*, 1994). During and Lang (1993) also found that the extent of xylem development close to the node was generally less in the distal and lateral branches, which are areas more prone to BSN (compared to the proximal branches of a bunch).

In trials investigating how flowers and berries influence peduncle health, it was found that removal of flowers resulted in the death of the peduncle, but removal of set berries did not have this effect. Peduncles with berries removed stayed green and turgid until the end of the growing season, and symptoms of BSN did not develop (Theiler and Coombe, 1985). The final cross section of de-berried peduncles was less than that of attached berry peduncles. The peduncle obtains 75% of its final cross-sectional area by the middle of flowering (Theiler and Coombe, 1985) and therefore it appears the presence of the berry influences xylem development.
Theiler and Coombe (1985) found that by applying GA₃ to the peduncle during fruit set, not only did the peduncle remain healthy with no symptoms of BSN but that the area of the metaxylem was also significantly increased. Another plant growth regulator, α-naphthalene acetic acid (NAA), when applied to deflowered bunches had the same effect as GA₃ in that peduncles remained healthy, but when applied to intact bunches it did not decrease the incidence of BSN as GA₃ did. It also did not increase the area of the metaxylem (Theiler and Coombe, 1985). It may therefore be a plant growth regulator, or a combination of plant growth regulators, produced in the berry that influences xylem development and also the incidence of BSN.

1.4.3 Application of substances

1.4.3.1 Potassium

Sandy soils fertilised with potassium demonstrated higher values of BSN in France (Baldacchino-Reynaud, 2000) yet Cooper et al. (1987) found no effect on BSN incidence by applying potassium to soils in Chile. Jordan (1985) also found no effect of high potassium in a nutrient solution on BSN incidence. In Italy, the practice of ploughing in organic matter increased the exchangeable potassium in the soil but reduced the incidence of BSN (Frisullo and Faretra, 2003).

Applying potassium directly to the bunch by spraying potassium chloride (KCl) does significantly increase berry potassium concentration, although there is no effect when potassium nitrate (KNO₃) is used instead (Failla et al., 1996). Application of substances containing potassium sulphate (K₂SO₃) have been found to increase the incidence of BSN (Rumbos, 1989). As studies into potassium concentration and BSN incidence do not consistently demonstrate a relationship between potassium and BSN incidence, it is not surprising that studies into the application of potassium and this disorder are also not consistent.

1.4.3.2 Calcium

Some studies have shown that application of calcium, either as a soil fertiliser or as a foliar spray, can reduce the incidence of BSN in some cultivars (Stellwaag-Kittler and
Haub, 1964; Cline, 1987). Hartmair and Grill found that the application of lime reduced the incidence of BSN (Hartmair and Grill, 1965). Applying calcium chloride (CaCl\(_2\)) either by itself, or in conjunction with magnesium chloride (MgCl\(_2\)) can also reduce BSN incidence (Lauber and Koblet, 1967). Application of calcium to the bunch by spraying CaCl\(_2\) significantly increases berry calcium concentration (Failla et al., 1996). However, the effectiveness varies from year to year and in some cases and some cultivars, has no control over the disorder (Jordan, 1985; Cline, 1987; Capps and Wolf, 2000). Christensen and Boggero (1985) also referenced work that found that calcium nitrate (Ca(NO\(_3\))\(_2\)) sprays increased the incidence of BSN. They also found that dipping bunches in various solutions containing calcium did not effect the incidence of BSN. Again, as with potassium, the inconsistencies within these studies are therefore similar to the inconsistencies within studies on tissue calcium.

1.4.3.3 Magnesium

Many studies have found that foliar application of substances containing magnesium can reduce the incidence of BSN (Lauber and Koblet, 1967; Koblet et al., 1969; Brechbuhler, 1975; Schaller, 1977; Haub, 1983; Cline, 1987; Brechbuhler, 1991 and many more). Application of magnesium can occur through many different products, but magnesium sulphate (MgSO\(_4\)) and magnesium nitrate (Mg(NO\(_3\))\(_2\)) have been found to be very effective (Beetz and Bauer, 1983; Fabre et al., 1983; Rumbos, 1989). Jordan (1985) reported that spraying bunches with magnesium five times from berry set to veraison achieved good control of BSN. Good control was also achieved with just two applications near veraison (Theiler, 1979; Beetz and Bauer, 1983; Haub, 1986), although Theiler (1979) recommended an additional spray after bloom in the case of persistent severe occurrences. Better control of BSN was obtained when magnesium sprays were directed at the bunches, rather than the entire vine (Theiler, 1980; Haub, 1986) as foliar sprays have been found to be ineffective.

However, Christensen and Boggero (1985) referenced work that found Mg(NO\(_3\))\(_2\) sprays increased the incidence of BSN. They also found that dipping bunches in various solutions containing magnesium had no effect on the incidence of BSN. The presence of calcium or potassium in some solutions has been found to impede the
uptake of magnesium but this was counteracted by increasing the amount of magnesium in the solution or by spraying the bunches more often (Schimansky, 1983).

Overall however, these studies are in agreement with those carried out on tissue magnesium and the incidence of BSN, where low rachis and berry magnesium concentrations were correlated with a high incidence of the disorder.

1.4.3.4 Nitrogen

Results concerning the application of nitrogen on the incidence of BSN are as conflicting as the results obtained from applications of other mineral nutrients. Studies have shown that the application of nitrogen or high nitrogen in the soil solution around bloom time increases BSN incidence (Gysi, 1983; Christensen and Boggero, 1985; Brechbuhler and Meyer, 1988; Perret et al., 1994; Keller et al., 2001). The application of di-ammonium phosphate ((NH₄)₂HPO₄) directly to the bunches has also been found to increase BSN incidence (Christensen and Boggero, 1985). However, Capps and Wolf (2000) found that the application of nitrogen fertiliser, including around bloom time, increased veraison rachis nitrogen concentration and decreased BSN incidence. They also found that in years with elevated tissue nitrogen, BSN incidence was low.

1.4.3.5 Plant growth regulators

The application of gibberellins (GAs) around flowering to bunches or directly to the rachis has been shown to reduce BSN incidence (Alleweldt and Hifny, 1972; Beetz and Bauer, 1983; Haub, 1983; Theiler and Coombe, 1985). Application before and during flowering resulted in small or “shot” berries, but application during fruit set only affected fruit development slightly (Theiler and Coombe, 1985). Hifny (1971) also found that GAs applied 43 days after flowering reduced BSN incidence. Therefore timing of gibberellin application for BSN control is important in order to reduce the possible negative side effects.
Theiler and Coombe (1985) found that the auxins NAA, β-indole acetic acid (IAA) and 3-indolebutyric acid (IBA) had no affect on BSN incidence. However, other studies found that IAA reduced BSN, but not to such an extent as GAs (Hifny, 1971; Alleweldt and Hifny, 1972). NAA reduced the amount of secondary symptoms, but not primary. Cooper et al. (1987) also found a reduction in BSN incidence due to NAA, although this was not statistically significant. In an experiment where flowers were removed and various plant growth regulators were applied to determine the effect on peduncle death, Theiler and Coombe (1985), found that the application of NAA and GA3 prevented peduncle death after de-flowering, and GA3 increased the peduncle thickening by increasing the cross sectional area of metaxylem. This thickening occurred no matter when the GA3 was applied. Alleweldt and Hifny (1972) found that the application of GA3 caused the development of numerous, non-lignified xylem cells. They also found that GA3 in combination with IAA, gave rise to robustly walled parenchyma cells, sclerenchyma cells and lignified metaxylem.

Holzapfel and Coombe (1998) also found that certain substances can cause the appearance of necrotic tissue similar to that found in BSN. Agmatine had the highest potency for inducing BSN. Ammonium sulphate ((NH4)2SO4), benzothiazole-2-oxyacetic acid (BTOA), ABA-M (a 50/50 mixture of ABA and t-ABA) and ABA all induced BSN symptoms to the same degree, though less than agmatine. All except (NH4)2SO4, caused large increases in the concentrations of ABA extracted from bunch stems and it was therefore thought that ABA may play an important role in inducing BSN. However, the correlation between extracted ABA and BSN incidence was not strong. Baldacchino et al. (1987c) also found that application of ABA around veraison at concentrations of 25 mM and above induced symptoms similar to that of BSN. The concentration of ABA required to induce BSN in a susceptible cultivar such as ‘Cabernet Sauvignon’ was lower than that required in a more tolerant cultivar such as ‘Merlot’ (Baldacchino et al., 1987b).

A strong positive correlation between BSN incidence and putrescine in bunch stems has been found by Ruiz and Moyano (1994). Ruiz et al. (2004) also found similar results in table grapes demonstrating the “soft berry” problem. Holzapfel and Coombe (1998)
hypothesised that the effect of agmatine in inducing BSN was not due to an increase in free NH₄ ion concentrations, but rather the catabolism of it to putrescine.

1.5 Summary

BSN is a physiological disorder that is detrimental to grape quality as berry ripening is retarded and berry quality compromised. Symptoms include dark necrotic spots on the peduncle, rachis or pedicel of the bunch post-veraison and berries become typically dull and opaque in appearance and soft in texture. Lesions may grow to girdle the entire affected area of a pedicel or rachis and if this occurs desiccation of berries distal to the necrotic tissue may follow with shrivelled berries either abscising or remaining on the bunch. Sugars and pH in berry juice remain low and acid concentrations remain high resulting in berries not attaining commercial maturity. There is further accumulation of calcium but the accumulation of potassium that usually accompanies berry ripening does not occur. It is thought that this is due to xylem dysfunction not occurring as is surmised to occur in healthy bunches, and therefore xylem and phloem flows remain at concentrations found pre-veraison. Plant growth substances putrescine and ABA are also higher in BSN affected berries compared to healthy berries.

Susceptibility to BSN is dependant on cultivar, rootstock, vineyard and season. Low temperature, rain and shade at varying times during berry development have all been associated with the disorder, although different publications are often conflicting. It has been hypothesised that BSN is a calcium and/or magnesium deficiency and high ratios of K/Ca, K/Mg or K/(Ca+Mg) have been implicated in BSN development. Also, high nitrogen concentrations are also thought to lead to BSN. However, again the literature on plant/bunch nutrition and BSN is conflicting. High vigour vines, or training systems that lead to high vigour shoots, are also thought to increase BSN, although BSN has also been associated with weak growth.

Applications of magnesium and/or calcium have been found to decrease BSN, although the success with soil and foliage applications have often been limited. Sprays directed at the bunch alone have proven to be most effective although again the literature is conflicting with varying results in different vineyards, in different seasons and on
different cultivars. Application of nitrogen has in some cases increased BSN, while in others it has decreased BSN incidence. The application of ABA directly to bunches has caused BSN-like symptoms while some auxin-like substances can decrease BSN incidence. The application of gibberellins have also reduced BSN incidence although at certain times in berry development this may be detrimental to both fruit set and berry development. It is thought that the decrease in BSN incidence due to gibberellin application is through increased xylem differentiation (Theiler and Coombe, 1985). More susceptible cultivars tend to have reduced xylem formation and therefore function, creating a restriction in the xylem for substances going into the developing bunch.

Much of the literature has concentrated on the nutrient balance within the vine and/or bunch and has endeavoured to find ways in which to correct this perceived nutrient imbalance. However, as much of the literature is conflicting and recommendations do not work for all situations and cultivars, it is hypothesised that there is an underlying cause to BSN which, depending on other circumstances may, or may not then affect the nutrient balance. Some of these underlying mechanisms have been implied in past literature, but further research has not been carried out to determine the validity of these hypotheses. Also, there are many conflicting studies in regard to the time during which environmental conditions may or may not influence the susceptibility of the bunch to BSN. Most studies into this area have used survey techniques to determine the validity of their claims, but other external factors may have also influenced the results obtained.

The main hypotheses investigated in this study are therefore that:

a) The vigour of the vine, and therefore possibly how this influences nutrient and/or plant growth regulator concentrations, may influence the incidence of BSN, and

b) that there is a critical time in which factors may predispose bunches to the disorder, although other factors may be required for the disorder to manifest itself.
The validation of hypotheses already put forward by international authors under New Zealand conditions, further investigations into the underlying mechanisms behind BSN incidence, and further investigation into the possible critical timing of influencing factors, therefore form the basis of the research presented in this thesis.
2 General Materials and Methods

2.1 Vine vigour

2.1.1 Point quadrat

Vine vigour was determined using the point quadrat method as detailed by Smart and Robinson (1991). Vine canopy area was determined by counting leaf and gap numbers at 57 points within a sample area of 2 m by 2 m. Survey points were spread evenly horizontally at 10 cm intervals and vertically at 1, 1.5 and 2 m intervals above the soil surface.

Leaf plucking, which removed approximately 50% of leaves around the fruiting zone, occurred in all seasons close to the time of veraison. After this time, point quadrat measures were only carried out on the top two intervals of 1.5 and 2 m, across a total of 38 points.

Leaf layer number (LLN), percentage gaps (PG) and percent interior leaves (PIL) were determined by the following formulae;

\[ LLN = \frac{\text{total leaves counted}}{\text{number of points}} \]

\[ PG = \frac{\text{number of gaps}}{\text{number of points}} \times 100\% \]

\[ PIL = \frac{\text{number of interior leaves}}{\text{total leaves counted}} \times 100\% \]

2.1.2 Dormant canopy measurements

Pruning weights (PW) were taken in the winters of Seasons One and Two from two vines per replication per treatment. Prior to winter pruning, effective cane number
(ECN) per vine was determined. ECN is the number of substantial canes on a vine as opposed to entire cane number (CN). Pruning weight was then divided by ECN to determine effective cane weight (ECW).

In Season Two, growing points (GP) were also counted on one vine per replication per treatment prior to winter pruning. The vigour of vines per growing point was determined by calculating growing point weight (GPW = PW/GP) as a measure of vegetative vigour, and mean growing points per cane (GP/C = GP/CN) as a measure of the amount of branching.

2.2 Veraison
Veraison progress was determined by non-destructively scoring individual bunches visually for percentage of colouring. This score was purely an estimate of how many berries on the bunch had turned a red colour. Data for each individual replicate were plotted and 50% veraison and rate of change around 50% veraison were estimated as detailed later and values were then statistically analysed using the GLM procedure in SAS system for Windows, release 8.02 (SAS Institute Inc., North Carolina).

2.3 Analyses of berry juice
The sample size taken to obtain berry juice in all instances was approximately 800 berries. Berries were air-bag pressed at 35 psi. The air-bag press was self-designed and engineered by Corbans Winery Ltd. Pressure was applied to berries for approximately 5 s and the free flow juice collected. This was repeated three times for each sample. At least 100 ml and no more than 250 ml of juice was collected for analysis. The press was cleaned thoroughly between each sample pressing.

Brix, titratable acidity (TA), pH, tartaric and malic acid concentrations and potassium, magnesium, calcium and ammonium concentrations were all determined in both Seasons One and Two. In Season Three, only Brix, TA, pH, tartaric and malic acid concentrations were determined to define maturity development.
Chapter Two – General Materials and Methods

2.3.1 Brix concentration

Brix concentration was determined using a hand-held refractometer (Atago PR1) that was calibrated at the start of each sampling day. It was also zeroed before the start of sampling using distilled water and again after every 10 samples.

A small amount of juice (approx. 1 ml) was placed on the prism surface using a disposable pipette and a reading was taken. This was repeated with a new sub-sample until three consecutive readings were obtained. The prism was cleaned between each sample.

2.3.2 Titratable acidity and pH

Titratable acidity and pH were determined using a Methrohm Autotitrator 702SM. A 20 ml sample was pipetted into a 100 ml beaker and placed under the sparge head and stirrer of the Methrohm Autotitrator. The head was lowered to ensure the probe, doser, stirrer and sparging head were all below the surface of the liquid. The stirrer speed was set to 4 and once titration was complete, TA (g l⁻¹) and pH values were recorded.

2.3.3 Acid and cation determination

Acid and cation concentrations were determined by capillary electrophoresis using a BioRad BioFocus 3000 TC. Corbans Winery Ltd laboratory staff carried out the procedures to determine tartaric and malic acid concentrations and potassium, magnesium, calcium and ammonium concentrations.

2.4 Harvest

The field trial harvest was not carried out at the same time as the commercial harvest, but approximately five weeks after 100% veraison. This was to ensure that all harvest assessments had been carried out before the commercial harvest commenced. In Season One, harvest occurred over a one week period that began on 29 March 2003 and finished on 4 April 2003. Assessments were carried out on individual bunches as each experimental block was harvested. However, at the end of five days all the bunches had
Chapter Two – General Materials and Methods

not been harvested so the remainder were harvested over a two day period and stored in a cool store until measures could be carried out. Bunch weight was recorded at the time of harvest and again when measures were carried out to ensure that no weight loss had occurred over the storage period. Because of the length of time the harvest had taken in Season One, and the increase in BSN incidence during that time, in Season Two all bunches were harvested over a two day period, 5/6 April 2004, and stored in a cool store until harvest measures could be carried out. As in Season One, bunches were weighed at the time of harvest and reweighed when harvest measures were carried out.

Entire bunches were harvested directly into individual paper bags in order to minimise the loss of any BSN affected berries from the bunches. Bunches were then weighed to the nearest gram and the length and width of the bunch was measured to the nearest centimetre, only measuring the berries, i.e., measurements did not take into account the rachis or peduncle. The diameter of the peduncle was measured to 0.01 mm using electronic callipers. BSN incidence was estimated using the same technique as described later for estimating BSN incidence through the final stage of berry growth.

Individual healthy and BSN affected berries were then counted on each bunch and the healthy berries were re-weighed and from this, mean healthy berry weight could be determined. Also, from the BSN affected berry number for each bunch, the exact percentage of BSN incidence could be determined and compared against the estimate taken before harvest.

2.5 Seed number and weight

After harvest a random sample of 50 berries was selected from all the healthy berries in each block of each treatment. The number of seeds per berry was counted for each of the randomly selected berries. After the seeds had been counted, the excess flesh was removed from the seeds and the total weight of all of the seeds for each block in each treatment was recorded. This weight was then divided by the total number of seeds for each block in each treatment to obtain an mean seed weight.
2.6 Bunch stem necrosis (BSN)
Symptoms of BSN begin to appear approximately one week after 100% veraison. Consequently, following completion of veraison, regular checks of all bunches were carried out to identify the progression of symptom development. Once symptoms began to be exhibited non destructive visual scoring of individual bunches on one vine per replication within each treatment was carried out.

Scoring was achieved by examining individual bunches and visually estimating what percentage of berries were exhibiting BSN symptoms. Symptoms consisted of shrivelled berries and necrosis on the rachis. Canes on which bunches were positioned were checked to ensure leaves were still present as bunches on totally defoliated canes can exhibit similar symptoms. Comparison of values using exact counts of BSN berries at harvest and estimation of BSN showed that the estimation was reasonably accurate.

In the ANOVAs carried out for BSN incidence the probability of a Type I error of the statistical test ($\alpha$) was chosen to be 15% due to high variability between vines within treatments. This probability is regarded as being biologically sound.

2.7 Data handling and statistical analyses

2.7.1 Data handling
Wherever possible, data were collected directly into a computer file to reduce the occurrence of input errors. This included measures using digital callipers and balances having a direct interface with a laptop computer and using the SimKey program (ID Innovations Incorporated, Hurst, TX, USA) to write data directly into a Microsoft excel worksheet (Microsoft Corporation, Redmond, WA, USA).

However, due to the extent of field work carried out, often this was not possible. In these instances data were recorded manually into a data collection book and manually transferred onto a Microsoft excel worksheet at a later date.
In both cases, datasets were checked for errors using the following methods:

1. Datasets were sorted by treatment and blocks in order to determine that all treatment x block combinations had been measured and that the data had been entered.

2. Datasets were then visually checked for any obscure values that may have been an input error.

3. Residual plots were plotted using SAS to check for outliers. Any outliers were then double checked to ensure the input of the data was correct and values were removed if necessary.

### 2.7.2 Experimental design

#### 2.7.2.1 Field trial

Field trial experiments were randomised complete block designs with the exception of the plant growth regulator application experiment. In most cases the experimental unit that was measured was an entire grape vine. The vine was situated in a bay of five vines and treatments were applied to the entire bay. The two end vines of each bay were used as guard vines. Of the remaining three vines, one or possibly two uniform vines were chosen to carry out measurements. In the case where two vines were chosen, the mean of the two vines was taken and statistical analyses were carried out on this mean. Of these two vines, if only one vine was measured, that vine was nominated to be the alpha vine on which all measurements were carried out. In some cases, measurements were carried out on individual bunches, but in this situation a weighted mean for the entire vine was obtained and statistical analyses were carried out on this value.

In the case of the plant growth regulator application experiment the experimental design was a split plot with blocks. Individual vines were used as the main plot and bunches were used as the split plot. Vines used for the main plot effect were two of the three remaining vines in each bay.
Chapter Two – General Materials and Methods

Soil analyses was carried out prior to the start of the field trial and this indicted a fertility gradient down the rows. Blocks were therefore arranged to take into account this changing soil fertility and encompassed a set number of rows in width and a set number of bays, down the length of the rows. Treatments were randomly allocated to each bay within each block. There were an additional three bays at each end of each row and these were classed as guard bays (Appendix 1). The fertility of the soil in the top 10 cm was considered to be high.

2.7.2.2 Controlled environment room experiments

Experiments carried out in the controlled environment (CE) rooms were blocked split – split – plot designs. In most cases the experimental unit was either individual canes or the bunch on each individual cane. The CE rooms were replicated in order to obtain blocking.

2.7.3 Statistical analyses

The SAS system for Windows, release 8.02, was used to statistically analyse all data. Univariate analyses were carried out on all data.

Data were tested for outliers, homogeneity of variance and a normal distribution. If the data was not homogenous and normally distributed then transformations were carried out on the raw data before statistical analyses were carried out.

Repeated measures analysis was carried out on data that were collected over time on the same experimental unit. Such analyses were carried out on data collected from both the field trial and the CE room experiments. Between-subject, within subject and interaction effects were examined using this analysis.
Chapter Two – General Materials and Methods

2.7.3.1 Test statistics

In most cases the ANOVA test statistic F was used to test the null hypothesis that all treatment means were the same. This was carried out in SAS using the general linear model (proc GLM). If this hypothesis was rejected due to a high F value then the alternative hypothesis that treatment means were different was accepted. The probability of a Type I error of the statistical test (α) was generally chosen to be 5%, however other test statistics where used when appropriate.

When data did not conform to the assumptions underlying the ANOVA procedure, the non-parametric method of Friedman’s test for two-way designs was used. This is a rank-based analogue of the F-test on ANOVA that provides a test statistic of Q in which to test the null hypothesis against. Q was used in the same way as F to either accept or reject the null hypothesis.

2.7.3.2 Treatment comparison testing

When treatment differences were found by using the test statistic F, treatment means were compared using one of the following comparisons:

- Fisher’s Least Significant Difference (LSD) is presented on some graphs to help indicate treatment differences. The LSD procedure controls the comparison-wise error rate and therefore can inflate the Type I error rate, declaring more treatment means to be different, and is therefore less conservative than other commonly used Multiple Comparison Procedures (MCPs) (Ott, 1993). It is due to this that the LSD method was chosen over other MCPs as the naturally high variation in grape vines can create the possibility of accepting the null hypothesis when it is false. Also, by applying Fisher’s LSD only after the test statistic has shown there to be treatment differences, the method becomes a protected LSD and the experiment-wise error rate is controlled at a level approximately equal to the α-level for the F test (Ott, 1993).
- Linear Contrasts (SAS proc GLM) were used for pre-planned comparisons between treatment groups or when comparison of only some pairs of treatments were wanted. Contrasts could only be carried out on balanced data or data with very few missing values. Orthogonal polynomial contrasts were also used on repeated measures data to test whether there were significant linear or curvilinear relationships among the mean responses of the treatments. Furthermore, once the type of relationship was determined, regression lines were fitted to the treatments to determine whether regression lines, and therefore treatments, were genuinely different from each other. This was carried out by determining whether parallel lines fitted the data as well as separate lines, and if so whether a common line would fit the data as well as the parallel lines.

- Least squares means (LSMeans) were used for paired comparisons where missing observations in the data set occurred. Misleading results may arise from comparisons from data containing missing values due to the means being weighted towards cells with fewer missing data. For consistency LSMeans were also used and reported in tables for balanced data when comparing two means and P values were required. Only P values of pre planned comparisons were used. LSMeans are computed using linear combinations of the effects fitted in the ANOVA model and therefore adjusts for the missing values.

When the non-parametric test statistic Q was used to test the null hypothesis, no comparison testing could be carried out due to there being no non-parametric equivalents. Therefore, in these cases, the trends of the means were discussed.
3 Vigour and Light Effects on BSN

3.1 Introduction

Previously published studies on BSN have indicated that the vigour of the vine, or more importantly, the vigour of individual shoots, may play a role in the incidence and severity of this disorder (Theiler, 1975b; Clement, 1978b; Redl, 1984a; Moreno and Pavez, 2000). Different training systems (Hifny, 1971; Redl, 1984a), pruning techniques (Redl, 1988; Holzapfel and Coombe, 1995) and changing bud number per cane (Brendel and Hofmann, 1983) have all been found to influence the incidence of BSN. However, there has been a wide range of results obtained in these studies and results may be regarded as being inconclusive.

Research has shown that root pruning is a good method of controlling the excessive vigour of many crops including apples (McArtney and Belton, 1992; Mika and Krzewinska, 1995) and grape vines (McArtney and Ferree, 1999a). It has been found that root pruning grape vines reduces shoot elongation and leaf area more effectively than doubling the number of canes per vine (McArtney and Ferree, 1999a). The closer the root pruning is to the trunk, the more effective it is in decreasing shoot growth. The timing of the root pruning is also important with pruning at different times throughout the growing season having differing effects on growth attributes and fruit quality (McArtney and Belton, 1992).

Generally, root pruning has the added benefit of producing higher quality fruit, with increased soluble solids (Schupp and Ferree, 1987; Stone, 2002), reduced TA (Stone, 2002), increased firmness (Schupp and Ferree, 1987) and reduced incidence of some diseases such as cork spot in apples (Schupp and Ferree, 1987) and nectarine pox in nectarines (Baugher and Miller, 1991). Fruit size tends to be reduced by root pruning (Schupp and Ferree, 1987; McArtney and Belton, 1992; Mika and Krzewinska, 1995) which in some crops, such as grapes grown for wine, is a desired quality aspect. However, in other crops such as apple this may not be so. Yield has on occasion been affected, depending on the crop and the cultivar. Mika and Krzewinska (1995) found no effect on the yield of ‘Empire’ apple trees for some years, but there was an increase in
yield for the cultivar ‘Spartan’. Schupp and Ferree (1987) found that root pruning during dormancy or at full bloom increased yield efficiency while Mika and Krzewinska (1995) found that generally it was the trees that were root pruned in the summer that experienced the greatest reduction in yield.

Some studies have found that leaf cation concentrations can also be affected by root pruning, with calcium content being increased and magnesium and nitrogen content being decreased (Mika and Krzewinska, 1995). However, other studies on root pruning have found no change in leaf calcium, magnesium and nitrogen but a decrease in potassium (Baugher and Miller, 1991). Although leaf nutrients were not affected in Baugher and Miller’s (1991) study, the nitrogen, phosphorus, potassium and magnesium concentrations in nectarine fruit were all reduced. Calcium concentration was increased in the fruit flesh, but no change occurred in the calcium concentration of the fruit peel compared to the control.

Several studies have found that environmental conditions around the time of flowering influenced the incidence of BSN (Theiler and Muller, 1986). Theiler and Coombe (1985) hypothesised that this was a critical time when environmental events could influence the incidence of BSN due to the development of the peduncles, and the known effects of flower and berry removal on peduncle development. However, there is no consistency in the literature as to whether a critical time exists before or after flowering or around veraison in relation to the development of this disorder.

In some studies, rain events are one environmental factor that have been shown to influence BSN incidence at certain times (Boselli et al., 1983; Brechbuhler, 1987; Redl, 1987) although how, is not known. Low water stress favours vegetative growth (Holzapfel and Coombe, 1995) and therefore rain events may increase vine vigour at critical times. Other authors have suggested that possibly the increase in the K/(Ca + Mg) ratio in the soil after a rain event is what actually influences BSN incidence (Hartmair, 1975). However, during rain events, PPF values are usually low due to cloud cover and any increased vegetative growth arising from lower stress levels will also reduce light around the fruiting zone and increase the proportion of shaded interior
leaves within the canopy. Within-vine shading can reduce the PPF to 5% of ambient or less within the interior of a grapevine canopy (Smart, 1974, 1987). Among other factors, shading affects juice quality, vine photosynthesis and vine mineral nutrition including leaf and peduncle mineral concentrations (Smart et al., 1988). Therefore it may be the reduction in PPF due to cloud cover, or increased vine vigour due to the improved vine water status, which is influencing the incidence of BSN.

Shade has been implicated in the incidence of a similar disorder, early bunch stem necrosis (EBSN) (Jackson and Coombe, 1988; Jackson, 1991; Gu et al., 1996). Keller and Koblet (1994) found that extensive shading of vines during bloom time dramatically increased the incidence of EBSN. As the two disorders are similar, it has been suggested that BSN may also be influenced by shade (Jackson and Coombe, 1988; Koblet et al., 1996). There is very little literature on how shade influences the incidence of BSN. However, in an experiment where ‘Sultanina’ (Thompson Seedless) grape vines were shaded from after flowering to harvest, Perez and Gaete (Perez and Gaete, 1986) did find that as the intensity of shade increased so did the incidence of BSN. Fifty percent shade cloth resulted in, on average, 42% of clusters being affected by BSN, while the control had only 14%.

In this study the impacts of vigour and shade on the incidence of BSN, and whether or not there exists a possible critical time for factors to predispose vines to BSN, were investigated. Root pruning has already been demonstrated to be an effective de-vigorator, and also to improve other quality aspects of grape production, but no information on how it may affect the incidence of BSN has been reported. In contrast, the number of buds per cordon were reduced in this study to stimulate individual cane growth (Smart, 1988; Smart and Robinson, 1991; Carbonneau, 1996), and shade cloth and a reflective mulch were used to manipulate the light environment within the canopy. As mineral nutrition of the vine is often associated with BSN, the effects of selected treatments on vine and bunch mineral nutrition were also investigated.

Most research into how environmental factors influence the incidence of BSN has solely concentrated on the current season’s environment. However, as already
Chapter Three - Field trial: vigour and light effects on BSN. Introduction.

mentioned in Chapter One, inflorescence primordial initiation occurs in the season prior to the season in which the flowers and berries are formed. Therefore, it is feasible that the environmental conditions around inflorescence initiation in the season prior to the current growing season may, in fact, influence the incidence of BSN. This study was designed therefore to also investigate any carry over effects that treatments may have had on the incidence of BSN.
Chapter Three - Field trial: vigour and light effects on BSN. Materials and Methods

3.2 Materials and Methods

3.2.1 Plant material

Seven-year-old field grown ‘Cabernet Sauvignon’ grape vines on SO4 root stock were used for this study. Vines were planted on a Montana vineyard at Moteo, Taradale, New Zealand. The vineyard was situated on an ashy sandy loam soil called Poporangi. There was a cemented pan over stones more than 60 cm down and this was ripped in both directions prior to planting.

Spacing between vines was at 2 m within-rows, and 2.4 m between-rows. Vines were arranged within-rows in bays that consisted of five vines. Each row was 30 bays long. Vines were cane pruned in the winter months and trained to a vertical shoot position (VSP) with two cordons being laid down either side of the main trunk approximately 0.8 m above the soil. There was no permanent irrigation system but in dry years a travelling irrigator could be used. Common commercial practices were carried out to maintain the vines (for spray schedule see Appendix 2). The application of foliar fertiliser throughout the growing season, undertaken on the rest of the vineyard, was not applied to the vines used in the study.

3.2.2 Treatments

Treatments applied in this study were a combination of one, two or no (control) main treatments. These treatments were chosen to modify vine vigour in various ways and to alter the environmental conditions under which the vines were growing. The main treatments consisted of root pruning, heading back of the canes, application of shade cloth and the application of a reflective mulch called Extenda™ (Extenda New Zealand Ltd, Auckland).

Root pruning was undertaken to reduce the vegetative vigour of the vines and was carried out on vines in the winter months prior to bud break in the first growing season (2002/2003). Vines were root pruned approximately 25 cm from the trunk on either side to a depth of 60 cm. A large spinning modified wood saw blade mounted on the
back of a tractor was used to cut through the soil and roots. In the winter months prior to Season Three a second group of vines, in rows adjacent to the rows used for root pruning in Season One, were root pruned 25 cm from the trunk on either side to a depth of 45 cm. In this season, a rigid blade mounted on the back of a tractor was used to tear through the soil and roots.

Heading back was undertaken to encourage vigorous growth of the canes during the growing season. Prior to bud break the cordons that had been laid down during winter pruning were reduced in length by 50%. Bud count per cordon was reduced from ten to approximately six.

Fifty percent shade cloth was applied to reduce the light environment at two defined development stages. These stages had been previously implicated in the literature as possible critical times in which the vines might react to certain environmental conditions. Each stage was applied to two different groups of vines. The shade cloth was draped over a wire 2 m from the ground to enclose the vines on both sides in both instances.

1. Shade cloth was applied three weeks prior to full bloom (FB) for a period of three weeks (Shade pre-FB).

2. Shade cloth was applied after FB for a period of three weeks (Shade post-FB).

Shade cloth was not applied around the time of 50% flowering in order to reduce any detrimental effects that shade may have had on berry set. Full bloom was determined to be when at least 90% capfall had occurred.

Extenday™ cloth with a width of 0.5 m was used in order to increase the light in the middle and lower zones of the canopy. It was laid down on either side of the vines at bud break and was removed after leaf fall. The increase in the light reflected back into the canopy was six-fold than from the soil/ward groundcover alone when the cloth was first laid in the rows.
These main treatments were combined and applied to vines to produce 13 treatment combinations.

1. Control – no treatments applied.
2. Root pruning alone.
3. Root pruning with Extenda™.
4. Root pruning with shade pre-FB.
5. Root pruning with shade post-FB.
6. Root pruning with heading back.
7. Heading back alone.
8. Heading back with Extenda™.
9. Heading back with shade pre-FB.
10. Heading back with shade post-FB.
11. Extenda™ alone.
12. Shade pre-FB alone.

Treatment combinations were chosen to study the impact of individual vigour and light manipulation effects on BSN incidence, as well as combinations of these vigour and light effects that may occur in the field. Root pruning and heading back were combined to determine if the invigorating effects of heading back would counteract the de­vigorating effects of root pruning. Light manipulation treatments were not combined as these combinations were not deemed as important as other combinations as these would not help to determine the critical time of BSN predisposition. These combinations and a full factorial statistical design were also not used due to the need to reduce treatment numbers and limit the overall trial to a manageable size.

All individual treatments, except as detailed for root pruning, were applied in both Season One (2002/2003) and Two (2003/2004).
3.2.3 Statistical analyses

The 13 treatment combinations were applied as a randomised block design that consisted of eight blocks (Appendix 1). Experimental unit and vine nomination for assessments were as detailed in Chapter Two.

Univariate analyses were carried out on the data as described in Chapter Two.

3.2.4 Assessments

3.2.4.1 Vine vigour

Point quadrat. Leaf layer number (LLN), percentage gaps (PG) and percent interior leaves (PIL) were measured using the point quadrat method as described in Chapter Two. Measurements began close to flowering and were repeated four times approximately one month apart in both Seasons One and Two. In Season Three only two measurements were taken; the first close to flowering, and the second approximately six weeks later when it was estimated that maximum measurable vigour had occurred as indicated by Season One and Two measurements. Of the thirteen treatments, point quadrat measurements were only carried out on nine in Seasons One and Two, as treatments that involved interactions with shade were not measured (mainly because of labour and time constraints). The treatments that were measured were therefore: control, root pruning, heading back, Extenda™, shade pre-FB and shade post-FB as the main treatments, and root pruning with heading back, root pruning with Extenda™, and heading back with Extenda™ as the interaction treatments. In Season Three, measurements were carried out on the control and root pruning treatments, along with the new root pruning treatment and the second group of control vines.

Dormant canopy measurements. As described in Chapter Two, pruning weights (PW), effective cane number (ECN) per vine, cane number (CN) per vine, and effective cane weight (ECW) were taken in the winters following Seasons One and Two. Following Season Two, growing points (GP) were also counted.
3.2.4.2 **Veraison scores**

Veraison scores were carried out on vines as described in Chapter Two. In Seasons One and Two grape bunches were checked weekly for initial signs of veraison from four weeks after 100% FB. Once veraison began scores were taken three times weekly in Season One and twice weekly in Season Two. This assessment was carried out on 5 bunches per vine replicate. Scorings continued until all treatments had reached at least 95% veraison development. Data for each treatment x block combination were plotted using SAS and a best fit sigmoidal curve was plotted. An ANOVA, carried out in SAS using the GLM procedure, was then used to determine statistical differences in the day of 50% veraison and the rate of change around 50% veraison.

In Season Three scores were only carried out three times over the veraison period. Scores were one week apart from each other and began approximately two weeks after initial signs of veraison were noticed. All of the bunches on each vine replicate were scored for colouring of berries. Scores were carried out on all 13 treatments in the vigour trial in Seasons One, Two and Three and the additional root pruned and control vines in Season Three.

3.2.4.3 **Cation analyses**

Petiole samples for cation analyses were taken from vines pre- and post-veraison in Season One. Samples taken pre-veraison were pooled across all eight replicates whereas, at post-veraison all eight replications were sampled separately.

Bunch samples for cation analyses were taken pre-veraison in both Season One and Two, and at both post-FB and pre-veraison in Season Three. In Season One, two replicates selected at random were taken for all treatments. In Season Two treatments of control, heading back and root pruning were sampled randomly across four of the eight replicates. In Season Three, samples were taken from the control and root pruned vines from Season One, and the additional control and root pruned vines in Season Three where all eight replicates were sampled in each instance.
Chapter Three - Field trial: vigour and light effects on BSN. Materials and Methods

Digestion of dried samples. All samples were oven dried at 65°C to constant weight. Bunch samples were split into berry and rachis and samples were then ground using a ‘Cyclotec Mill’. A dried sample of 0.100 g ± 0.005 g was weighed into digestion tubes that were pre-marked at 25 ml. For every 29 samples, two herbage standards and a blank were included. A total of 32 test tubes fitted into each ‘block’.

In a fume cupboard 4 ml conc. nitric acid (HNO₃) was added to each sample and a small glass funnel was placed on top of each tube to help condense any vapour back into the sample. Samples were then digested at 150°C for 16 hours in a controlled temperature aluminium block.

After digestion, aluminium foil was wrapped around each block to aid evaporation and funnels were removed. The temperature was increased to 200°C in small increments, and samples were left to evaporate until dryness.

After samples had evaporated, the sample tubes were removed from the block. While still warm, 5 ml of 2M hydrochloric acid (HCl) was added and samples were mixed using a vortex mixer. Samples were left to digest for 1-2 h using the vortex mixer intermittently to mix samples and ensure all material was digested. Next 1 ml of 25,000 ppm strontium and cesium (SrCs) solution was added to each test tube. Samples were then made up to 25 ml using deionised water and further mixed using the vortex mixer.

Samples, diluted where necessary, were then read using an Atomic Absorption (AA) Spectrophotometer (GBC Avanti Sigma).

Standards and solutions

HCl – 0.4M HCl was made up using 172 ml conc. HCl l⁻¹ of deionised water.

SrCs – 25,000 ppm SrCs solution was made up using 15.84 g CsCl and either 30.19 g Sr(NO₃)₂ or 38.0 g SrCl₂·6H₂O dissolved in 500 ml of deionised water.

Standards - Standards were made up for AA at the concentrations outlined in Table 1.
Chapter Three - Field trial: vigour and light effects on BSN. Materials and Methods

Table 1: Cation concentration of standards used in cation analyses using AA.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Mg (mg l⁻¹)</th>
<th>Ca (mg l⁻¹)</th>
<th>K (mg l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>one</td>
<td>2</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>two</td>
<td>4</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>three</td>
<td>6</td>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td>four</td>
<td>8</td>
<td>40</td>
<td>16</td>
</tr>
<tr>
<td>five</td>
<td>10</td>
<td>50</td>
<td>20</td>
</tr>
</tbody>
</table>

A bulk solution was made up using 10 ml of 1,000 mg l⁻¹ Mg, 5 ml of 10,000 mg l⁻¹ Ca and 20 ml of 1,000 mg l⁻¹ K and made up to 100 ml with 0.4M HCl. To each of five 250 ml flasks 5, 10, 15, 20 or 25 ml of this bulk solution was added to make standards one, two, three, four and five, respectively. 10 ml of 25,000 mg l⁻¹ SrCs solution was added to each flask and then made up to 250 ml with 0.4M HCl.

Analysis of berry juice. Analysis of berry juice was carried out as described in Chapter Two. In Season One all treatments were sampled weekly from 100% veraison for six weeks. Due to sample numbers, treatments were not replicated on a regular basis. One sample, taken across all eight replicates for each treatment (i.e., pooled) was taken each week, except in the case of one main treatment. This treatment was sampled so that all eight replicates were analysed separately and each week a different main treatment was treated in this way.

In Season Two, to allow statistical analysis, the number of treatments sampled was reduced but three replications (i.e., blocks) were sampled. The treatments that were included were control, heading back, heading back with Extenday™, Extenday™, shade post-FB, root pruning and, on alternative weeks, shade pre-FB or root pruning with shade post-FB. As in Season One, samples were taken weekly starting at 100% veraison and continued for six weeks.

In Season Three, only main treatments of control, root pruning, heading back, Extenday™, shade pre-FB and shade post-FB were sampled. Samples were replicated across four blocks, and sampling occurred only once, one week prior to harvest.
3.2.4.4 Gas exchange measurements

Gas exchange measurements were carried out on vines using a CIRAS-1 portable photosynthesis system (PP systems, United Kingdom) in both Seasons One and Two. In Season One, light response curves were determined in the field on all treatments. The PPF at which gas exchange measurements were carried out were 0, 50, 100, 200, 400, 800 and 1600 μmolm⁻² s⁻¹. Due to time constraints only four replicates per treatment were measured. Measurements were carried out only once, two weeks after full bloom.

The measured CO₂ exchange rates were used to plot light response curves using ‘Photosyn assistant’ software (Dundee Scientific, Dundee). Maximum photosynthesis (Amax), quantum efficiency (QE), light compensation point (LCP) and light saturation estimate (LSE) were determined from each plot and statistically analysed using SAS software.

In Season Two, due to time constraints and the lack of significant differences among treatments in Season One, measurements were carried out at only one PPF value. As maximum photosynthetic rate was the only measurement of interest in Season Two, a PPF level of 1600 μmolm⁻² s⁻¹ was used as no further increase in the photosynthesis rate after this was determined from the previous season’s light response curves. The first measurement was carried out at 100% FB. All treatments except those involving shade post-FB were measured. Measurements were replicated three times and each replicate was duplicated. The second measurement was carried out two weeks later but due to weather deterioration only the main treatments of root pruning, heading back, Extenda™ and control were measured. All treatments were replicated three times and each replicate was duplicated. The third measurement, four weeks later, was immediately pre-veraison and eight replications per treatment were measured, with each replicate being duplicated. Measurements were only carried out on the main treatments of root pruning, heading back, Extenda™, shade pre-FB, shade post-FB and the control. No other measurements were able to be performed during the season due to inappropriate weather conditions.
In both seasons, measured leaves were chosen from the same area of the canopy and were determined to be the last fully expanded leaf on a cane. Transpiration rate (Tr), photosynthesis rate (Pn), stomatal conductance (Gs) and internal CO$_2$ concentration at 1600 μmol m$^{-2}$ s$^{-1}$ were statistically analysed using a RBD model and the SAS software in both seasons.

### 3.2.4.5 Harvest measurements

In Seasons One and Two measurements were carried out on five bunches randomly selected from the alpha vine in each bay, in all eight blocks, from all treatment combinations at harvest time. Harvest measurements were carried out as described in Chapter Two.

### 3.2.4.6 Seed number and weight

In Season Two the seed number and weight per berry was determined as described in Chapter Two.

### 3.2.4.7 BSN incidence

In Season One, the method of scoring as described in Chapter Two, was carried out at two-weekly intervals until harvest (but not at harvest). In Seasons Two and Three, the above method was carried out weekly until harvest, and including harvest time in Season Two. In Seasons One and Two, scores were also carried out on the five bunches per vine that were harvested.

BSN scores were adjusted for days after 50% veraison and for the stage of grape maturity. The number of days after 50% veraison that the BSN scores were adjusted to, was chosen so that most of the treatments were as close to the final time of assessment as possible. This was 36 days after 50% veraison in Season One, 40 days in Season Two and 43 days in Season Three. Actual rather than interpolated scores were used.
Maturity of berries was determined, for the purpose of this thesis, by the ratio of Brix/TA. This is not a ratio that is used commercially but is a basic indicator of maturity taking into account two of the major influences that are used to determine harvest time. The ratio value that was chosen to define maturity was selected so that most of the treatments fell within actual measured concentrations, i.e., there was minimal prediction. The ratio value that was chosen was also influenced by the desire to have a value that was similar across all seasons. However, further adjustment of BSN scores to identical maturity values was carried out in order to compare seasons.

A general overview of physiological stages, treatment application dates and assessment dates for all three seasons is shown in Figure 3.
Figure 3: Time line indicating physiological stages, dates when treatments were applied, and dates of measurements and assessments carried out on field grown ‘Cabernet Sauvignon’ vines during Seasons One (2002/2003), Two (2003/2004) and Three (2004/2005). Note that not all factors were measured in all seasons.
3.3 Results

3.3.1 Vine vigour

3.3.1.1 Point quadrat

Leaf layer number (LLN)

Seasons One and Two. The weather patterns (Appendix 3) during each growing season were very different and consequently, vine growth differed markedly as each season progressed (Figure 4). Vine vigour, as measured by LLN, prior to flowering was similar in both Seasons One and Two. However, one month later it was apparent that the vigour in Season Two was twice that of Season One. Vine growth continued into February in Season Two, as evidenced by the continued vine trimming that was required to control growth, whereas it did not in Season One (data not shown).

![Figure 4: Mean leaf layer number (LLN) of vines for main treatments for Seasons One and Two. F = 50% flowering, V = 50% veraison, H = harvest date.](image)

In the first two seasons and for all measurement times, vines in the root pruning treatment had significantly lower LLN than the control vines \((P \leq 0.0001 - 0.0485)\), and all of the other main treatments \((P \leq 0.0001 - 0.0765)\) (Table 2). Treatments that included interactions with root pruning (such as root pruning with the use of Extenday\textsuperscript{TM} and root pruning with heading back) also had significantly lower LLN...
Table 2: Mean leaf layer number (LLN) within measured canopies for Seasons One and Two.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Season</th>
<th>First measurement</th>
<th>Second measurement</th>
<th>Third measurement</th>
<th>Fourth measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>S1</td>
<td>S2</td>
<td>S1</td>
<td>S2</td>
</tr>
<tr>
<td>Control</td>
<td>S1</td>
<td>1.6 a</td>
<td>1.5 a</td>
<td>2.9 b</td>
<td>4.5 ab</td>
</tr>
<tr>
<td>Root pruned</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root pruned/heading back</td>
<td>S1</td>
<td>1.0 b</td>
<td></td>
<td>1.7 c</td>
<td>3.1 d</td>
</tr>
<tr>
<td>Root pruned/Extenday™</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heading back</td>
<td>S1</td>
<td>1.5 a</td>
<td>1.2 b</td>
<td>2.9 b</td>
<td>3.7 c</td>
</tr>
<tr>
<td>Heading back/Extenday™</td>
<td>S1</td>
<td>1.0 n</td>
<td></td>
<td>1.3 n</td>
<td>2.9 o</td>
</tr>
<tr>
<td>Extenday™</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shade pre-FB</td>
<td>S1</td>
<td>1.9 a</td>
<td></td>
<td>3.2 ab</td>
<td>4.4 ab</td>
</tr>
<tr>
<td>Shade post-FB</td>
<td>S1</td>
<td>1.7 a</td>
<td></td>
<td>1.3 ab</td>
<td>2.8 b</td>
</tr>
</tbody>
</table>

Means in the same column with a different letter are significantly different from each other at $P=0.08$ (LSMeans. SAS). *abc - main treatments, mno - comparisons of interaction treatments that included root pruning with respective main treatments, xyz - comparisons of interaction treatments that included heading back with respective main treatments.

**Due to rounding shade pre- and post-FB appear to have identical LLN values. However, shade post-FB was slightly lower than shade pre-FB and hence the significant difference with Extenday™.
values than the control throughout the entire second season, and for the last measurement of the first season ($P<0.0001$). In the first season, LLN measurements of the interaction treatment of root pruning with Extenday™ were only taken on the last measurement. Neither of these treatments were assessed on the last measurement of the second season. There was no interaction effect among treatments, with root pruning being the predominant treatment in both seasons (Table 2).

The initial LLN measurement in Season One was carried out at a comparatively later date than the initial measurement in Season Two. Therefore, even though LLN values for vines that had been headed back at the first measurement were higher in Season One, in Season Two vines were obviously more vigorous when compared at a similar time in each season. In Season One there was no significant difference in LLN in the heading back treatment compared with the control (Table 2). However in Season Two, LLN for the heading back treatment was moderately lower than the control at the first and second measurements ($P \leq 0.0365 - 0.0719$) but not by the third measurement. Hence, in both seasons, although 50% of the buds had been removed, vines in the heading back treatments filled the same canopy area as the control vines.

For the last measurement of LLN in the first season, the increase in LLN due to the application of Extenday™ was significant ($P=0.0092$) when compared with the control treatment (Table 2). The second and third measurements of LLN in Season One, and all measurements in Season Two also demonstrated a trend for vines with the Extenday™ treatment to have a higher LLN, although differences were not significant. It was noted during each season however, that more canopy tended to be removed from the vines in the Extenday™ treatment at the time of mechanical trimming (data not recorded), and this practice may therefore have obscured any significant increase in LLN that is likely to have occurred due to the use of Extenday™. Interaction treatments that included Extenday™ tended to have higher LLN when compared to values obtained from vines in treatments without the use of Extenday™. This was generally not significant except for the last measurement in Season One for the interaction with heading back ($P=0.0374$) (data not shown). There was no significant difference in LLN between the
control and the two shade treatments in both seasons for all measurements. There was also no significant difference in LLN between the two shade treatments themselves.

**Season Three.** LLN values for the controls demonstrate that vigour in Season Three was higher than that in Season One, but somewhat less than in Season Two (Figure 5). Root pruning that was carried out only in the third season did not reduce vigour in that initial year to the same LLN values achieved in Season One but the reduction in vigour was still marked (Figure 5). This may have been due to the more vigorous growing season, or to the change in pruning technique, or to both factors combined.

![Figure 5: Leaf layer number (LLN) assessments carried out in Seasons One, Two and Three. Control B and root pruned B are treatments that were applied in Season Three. All other treatments were applied in Season One. LLN values for each of the three years have been superimposed on a common date scale. F = approximate flowering date, V = approximate 50% veraison date.](image)

LLN was still significantly lower ($P \leq 0.0001 - 0.025$) than either control in the third season for vines that had been root pruned at the start of Season One (Table 3). Additional vines that had been root pruned at the start of the third season also had a significantly lower LLN to the control values ($P < 0.0001$) at both measurements. They also had significantly lower LLN compared to the first seasons root pruned vines at the
first measurement \((P=0.0023)\), but not the second. By the second measurement (13 February 2005) LLN between the two root pruned treatments was the same.

**Table 3:** Mean leaf layer number (LLN) of root pruned and control vines for Seasons One, Two and Three. The LLN at the first measurement in Season Two was estimated in order to provide comparable values.

<table>
<thead>
<tr>
<th></th>
<th>Season One</th>
<th>Season Two</th>
<th>Season Three</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3/01/03</td>
<td>18/02/03</td>
<td>31/12/03 (est)</td>
</tr>
<tr>
<td>Control (Season One)</td>
<td>1.6a</td>
<td>2.6a</td>
<td>3.1</td>
</tr>
<tr>
<td>Control (Season Three)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root pruned (Season One)</td>
<td>1.0b</td>
<td>1.4b</td>
<td>2.2</td>
</tr>
<tr>
<td>Root pruned (Season Three)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means in a column with a different letter are significantly different from each other at \(P=0.05\) (LSMeans, SAS).

**Percentage gaps (PG).**

Due to the statistical nature of the PG data in Season Two, LSMeans could only be carried out on the first measurement in the second season. Therefore, all other measurements in Season Two were statistically analysed using Friedman’s test and consequently only trends are discussed for these data. PG data for all measurements in Season One did meet all assumptions and therefore statistical analyses using LSMeans could be carried out.

**Seasons One and Two.** Differences in weather patterns among years did not have such a dramatic effect on PG as it did on LLN (Figure 6). PG dropped dramatically between the first and second measurements in both seasons. However, on average PG never reached 0% in Season One for any treatment, whereas it fell to 0 - 3% by the second measurement, and under 1% by the third measurement for all treatments except root pruning in Season Two. PG values for all treatments in both seasons had become reasonably constant by the second measurement.
Root pruning significantly increased PG at the initial measurement for both seasons when compared to both the control vines \((P \leq 0.0285 - 0.0361)\) and the Extenday™ treatment \((P \leq 0.0018 - 0.0327)\). However, when compared with all the other main treatments at the initial measurement, root pruning vines significantly increased PG only in Season One \((P \leq 0.0028 - 0.0238)\) although there was a trend for root pruning to also increase PG in Season Two. This significant increase in PG for root pruned vines persisted for the entire first season \((P \leq 0.0001 - 0.0014)\) compared with all other main treatments (Table 4). At all other measurements in Season Two, the Friedman’s test showed that there was a significant difference among treatments with root pruned treatments tending to have higher PG values at all measurements compared to all other treatments (Table 4).

Interaction treatments with root pruning demonstrated a similar pattern as the main treatment of root pruning alone. All of these interaction treatments had either significantly higher PG \((P \leq 0.0001 - 0.0552)\) or the trend was for higher PG than the control. Root pruned vines with heading back were not significantly different from root pruned vines alone at any measurement in either season (Table 4). These vines had significantly higher PG \((P < 0.0001)\), or the trend was for higher PG than heading back alone, showing that the main effect of root pruning was dominant (Table 4).
Table 4: Mean percentage gaps (PG) within measured canopies for Seasons One and Two.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>First measurement</th>
<th>Second measurement</th>
<th>Third measurement</th>
<th>Fourth measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Season</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>S1</td>
<td>S2</td>
<td>S1</td>
<td>S2</td>
</tr>
<tr>
<td></td>
<td>26 b*</td>
<td>37 b</td>
<td>9 b</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root pruned</td>
<td>S1</td>
<td>S2</td>
<td>S1</td>
<td>S2</td>
</tr>
<tr>
<td></td>
<td>46 a</td>
<td>48a</td>
<td>30 a</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>m</td>
<td>m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root pruned/heading back</td>
<td>S1</td>
<td>S2</td>
<td>S1</td>
<td>S2</td>
</tr>
<tr>
<td></td>
<td>47 m</td>
<td>30 m</td>
<td>9.4</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>m</td>
<td>m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root pruned/Extenday™</td>
<td>S1</td>
<td>S2</td>
<td>S1</td>
<td>S2</td>
</tr>
<tr>
<td></td>
<td>52 m</td>
<td>4.1</td>
<td>2.2</td>
<td>26 n</td>
</tr>
<tr>
<td>Heading back</td>
<td>S1</td>
<td>S2</td>
<td>S1</td>
<td>S2</td>
</tr>
<tr>
<td></td>
<td>27 b</td>
<td>41 ab</td>
<td>4 b</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heading back/Extenday™</td>
<td>S1</td>
<td>S2</td>
<td>S1</td>
<td>S2</td>
</tr>
<tr>
<td></td>
<td>46 x</td>
<td>2.0</td>
<td>0.9</td>
<td>6 x</td>
</tr>
<tr>
<td></td>
<td>y</td>
<td>x</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extenday™</td>
<td>S1</td>
<td>S2</td>
<td>S1</td>
<td>S2</td>
</tr>
<tr>
<td></td>
<td>16 b</td>
<td>37 b</td>
<td>5 b</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shade pre-FB</td>
<td>S1</td>
<td>S2</td>
<td>S1</td>
<td>S2</td>
</tr>
<tr>
<td></td>
<td>21 b</td>
<td>40 ab</td>
<td>5 b</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shade post-FB</td>
<td>S1</td>
<td>S2</td>
<td>S1</td>
<td>S2</td>
</tr>
<tr>
<td></td>
<td>25 b</td>
<td>42 ab</td>
<td>6 b</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>x</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means in the same column with a different letter are significantly different from each other at $P=0.05$ (LSMeans, SAS). * abc – main treatments, mno – comparisons of interaction treatments that included root pruning with respective main treatments, xyz – comparisons of interaction treatments that included heading back with respective main treatments.
Vines with the interaction treatment of root pruning with \textit{Extenday\textsuperscript{TM}} were only assessed on the last measurement of Season One and were significantly different from vines that were only root pruned ($P=0.0861$). Also, the trend in Season Two was for the vines with root pruning combined with \textit{Extenday\textsuperscript{TM}} to have lower PG than root pruned vines alone. The root pruning with \textit{Extenday\textsuperscript{TM}} treatment tended to have higher PG in both seasons than \textit{Extenday\textsuperscript{TM}} alone (Table 4). This was significant ($P \leq 0.0042 - 0.0052$) in Season One and at the initial measurement in Season Two.

Heading back did not significantly affect the PG within the canopy for either season when compared with the control. It did, however, tend to have a higher PG value for the first two measurements in Season Two (Table 4). There was no interaction effect for treatments with heading back and \textit{Extenday\textsuperscript{TM}} in either season.

In both seasons, when heading back was applied to vines with \textit{Extenday\textsuperscript{TM}}, there tended to be either no difference or vines had higher PG than with \textit{Extenday\textsuperscript{TM}} alone. This was significant ($P=0.0632$) at the initial measurement of the second season (Table 4). \textit{Extenday\textsuperscript{TM}} alone did not significantly affect PG compared to the control in both seasons, although at times there was a trend for the application of \textit{Extenday\textsuperscript{TM}} to decrease PG (Table 4).

PG values from shade post-FB or shade pre-FB vines were not significantly different from the control or from one another.

\textbf{Season Three.} PG measurements for the controls in Season Three show that the ability of the control vines to fill the canopy space was very similar to Season One (Figure 7), and therefore canopy development was very similar to Season One. This is different from the result obtained using LLN, although both measurements still indicate a lower vigour in Season One compared to Season Two.
Figure 7: Percentage gaps (PG) for Seasons One, Two and Three. Control B and root pruned B are treatments that were applied in Season Three. All other treatments were applied in Season One. PG values for each of the three years have been superimposed on a common date scale. F = approximate flowering date, V = approximate 50% veraison date.

The vines that were root pruned in Season One, and the vines that were root pruned in Season Three each filled the canopy faster in Season Three than the Season One root pruned vines had done in Season One. Both, however, were slower than in Season Two.

Unlike Seasons One and Two, vines that had been root pruned in Season One did not have significantly higher PG at the first measurement in Season Three compared to the control (Table 5). Vines that were root pruned in Season Three did, however, have significantly higher PG than both the control ($P \leq 0.0016 - 0.0030$) and the first season’s root pruned vines ($P = 0.0279$). By the second measurement the vigour of the control vines resulted in there now being a significant difference ($P \leq 0.0018 - 0.0362$) between the controls and the first season’s root pruned vines (Table 5). Furthermore, at the second measurement there was no difference between the two root pruning treatments.
Table 5: Mean percentage gaps (PG) of root pruned and control vines for Seasons One, Two and Three. The first measurement in Season Two was estimated in order to provide comparable values.

<table>
<thead>
<tr>
<th></th>
<th>Season One</th>
<th>Season Two</th>
<th>Season Three</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Season One)</td>
<td>26a</td>
<td>7a</td>
<td>15</td>
</tr>
<tr>
<td>Control (Season Two)</td>
<td></td>
<td></td>
<td>0.9</td>
</tr>
<tr>
<td>Root pruned (Season One)</td>
<td>46b</td>
<td>32b</td>
<td>24</td>
</tr>
<tr>
<td>Root pruned (Season Three)</td>
<td></td>
<td></td>
<td>8.3</td>
</tr>
</tbody>
</table>

Means in a column with a different letter are significantly different from each other at P=0.05 (LSMeans, SAS).

Percent interior leaves (PIL)
Seasons One and Two. Conclusions drawn from PIL measurements about overall vine growth for the first two seasons are similar to those drawn from LLN measurements. For example, it is clear from PIL measurements that vine growth in Season Two was more vigorous than Season One (Figure 8), although the magnitude of the differences when using PIL measurements was smaller than when using LLN.

![Figure 8: Mean percent interior leaves (PIL) within vine canopy area for main treatments for Seasons One and Two. F = 50% flowering, V = 50% veraison, H = harvest date.](image)
Chapter Three - Field trial: vigour and light effects on BSN. Results

Root pruning the vines reduced PIL at all measurements in both seasons compared to the control. Although this difference in Season One was not significant at the first assessment (Table 6), by the time of the second assessment it was significant ($P<0.0003$) and continued to be so throughout the rest of the season ($P\leq0.0006 - 0.0114$). In the second season the reduction in PIL with root pruning was significant at all assessments ($P\leq0.0001 - 0.0032$).

Treatments with an interaction with root pruning, such as heading back and Extenday™, were generally not significantly different from root pruning alone (Table 6). The exception was in Season Two at the third assessment where the interaction treatment of root pruning with Extenday™ was significantly different ($P=0.0367$) to root pruning alone. There was a significant decrease in PIL when these interaction treatments were compared to the other main treatments alone of heading back ($P\leq0.0048 - 0.0001$) or Extenday™ ($P\leq0.0036 - 0.0001$) in both seasons (Table 6). The only exceptions were that at the first assessment in Season Two when root pruning with heading back was not significantly different from heading back alone, and at the second assessment in Season Two when root pruning with Extenday™ was not significantly different to Extenday™ alone. Heading back did not significantly change the PIL of vines at any assessment in Season One compared to the control (Table 6). In Season Two heading back reduced PIL values compared to the control treatment only at the second assessment ($P=0.0323$). The interaction treatment of heading back with Extenday™ also did not significantly affect PIL compared to the control.

Other than the significant differences already discussed with root pruning and the final assessment in Season One, where it was significantly different from the control ($P=0.0138$), Extenday™ did not significantly effect PIL (Table 6). Neither of the shade treatments had a significant effect on PIL (Table 6).
### Table 6: Percent interior leaves within measured canopies for Seasons One and Two.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>First measurement</th>
<th>Second measurement</th>
<th>Third measurement</th>
<th>Fourth measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Season</td>
<td>S1 ab</td>
<td>S2 a</td>
<td>S1 ab</td>
<td>S2 a</td>
</tr>
<tr>
<td>Control</td>
<td>18 ab</td>
<td>25 a</td>
<td>35 a</td>
<td>54 a</td>
</tr>
<tr>
<td>Root pruned</td>
<td>12 b</td>
<td>15 b</td>
<td>20 b</td>
<td>43 b</td>
</tr>
<tr>
<td>Root pruned/heading back</td>
<td>17 n</td>
<td>18 n</td>
<td>39 n</td>
<td>46 n</td>
</tr>
<tr>
<td>Root pruned/Extenday™</td>
<td>18 n</td>
<td>46 mn</td>
<td>49 n</td>
<td>19 n</td>
</tr>
<tr>
<td>Heading back</td>
<td>21 ab</td>
<td>21 a</td>
<td>33 a</td>
<td>47 b</td>
</tr>
<tr>
<td>Heading back/Extenday™</td>
<td>22 x</td>
<td>55 x</td>
<td>64 x</td>
<td>38 xy</td>
</tr>
<tr>
<td>Extenday™</td>
<td>18 ab</td>
<td>27 a</td>
<td>41 a</td>
<td>57 a</td>
</tr>
<tr>
<td>Shade pre-FB</td>
<td>23 a</td>
<td>21 a</td>
<td>31 a</td>
<td>53 a</td>
</tr>
<tr>
<td>Shade post-FB</td>
<td>20 ab</td>
<td>20 a</td>
<td>32 a</td>
<td>50 a</td>
</tr>
</tbody>
</table>

Means in a column with a different letter are significantly different from each other $P=0.05$ (LSMeans, SAS). *abc - main treatments, mno - comparisons of interaction treatments that included root pruning with respective main treatments, xyz - comparisons of interaction treatments that included heading back with respective main treatments.
Chapter Three - Field trial: vigour and light effects on BSN. Results

Season Three. In Season Three, PIL values were initially higher than those recorded in the vigorous year of Season Two (Figure 9). However, as shown by the gradient of the trend lines, limited growth occurred after the first assessment in Season Three, whereas growth continued in both Seasons One and Two. Vines in Season Three had more vigorous growth before flowering, and less after flowering than had occurred in the other two seasons (Figure 9).

Figure 9: Percent Interior Leaves (PIL) for Seasons One, Two and Three. Control B and root pruned B are treatments that were applied in Season Three. All other treatments were applied in Season One. PIL values for each of the three years have been superimposed on a common date scale. F = approximate flowering date, V = approximate 50% veraison date.

PIL values were not significantly different between the controls in Season Three (Table 7). PIL in vines that were root pruned in the first season were significantly lower ($P=0.0104$) than the second group of control vines, but not different from the first group at the first assessment in Season Three (Table 7). PIL in these vines was however significantly lower than both groups by the second measurement ($P\leq0.0001 - 0.0003$). Due to there being no significant difference between the control vines, first season root pruned vines were statistically compared against the controls as one group for the first assessment and they were found to be significantly different ($P=0.0250$) to the control group (mean $=42$) as a whole at this time. In Seasons One and Two the increase in PIL from the first assessment to the second assessment for the vines root pruned in Season One was small. In Season Three there was no increase (Table 7).
Vines that were root pruned in the third season had significantly lower PIL ($P \leq 0.0001$ – 0.0029) than both groups of controls at both assessments (Table 7). These vines also had a significantly lower PIL than the first season root pruned vines at the first measurement ($P=0.0669$) but the difference was not significant by the second measurement (Table 7).

## Table 7: Percent interior leaves (PIL) of root pruned and control vines for Seasons One, Two and Three. The first measurement in Season Two was estimated in order to provide comparable values.

<table>
<thead>
<tr>
<th></th>
<th>Season One</th>
<th>Season Two</th>
<th>Season Three</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3/01/03</td>
<td>18/02/03</td>
<td>31/12/03 (est)</td>
</tr>
<tr>
<td>Control (Season One)</td>
<td>18a</td>
<td>36a</td>
<td>50</td>
</tr>
<tr>
<td>Control (Season Three)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root pruned (Season One)</td>
<td>12a</td>
<td>18b</td>
<td>30</td>
</tr>
<tr>
<td>Root pruned (Season Three)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means in a column with a different letter are significantly different from each other at $P=0.05$ (LSMeans, SAS).
3.3.1.2 Dormant canopy measurements

Effective cane number (ECN). Treatments had a significant influence on ECN in both seasons (Table 8). The root pruning treatment significantly reduced ECN on vines compared to the control (Figure 10). This was by approximately 14% in both seasons (Table 8). However, though significant in Season One ($P=0.0289$) there was no significant difference in the second season. Apart from the interaction treatment of root pruning with heading back ($P=0.0004 - 0.0007$) no other interaction treatment with root pruning was significantly different from root pruning alone in both seasons (Table 8).

Statistical analyses on the effect of heading back on ECN was the same for both Seasons One and Two. The heading back treatment significantly ($P\leq0.0001 - 0.004$) reduced final ECN on vines by approximately a third compared to the control treatment values (Figure 10). When combined, as expected all treatments where heading back of canes had been applied, vines had a significantly lower ECN than the treatments where there was no heading back ($P<0.0001$) (Table 8). Individually, the interaction treatments of heading back with shade post-FB, shade pre-FB, Extenday\textsuperscript{TM} or root pruning all had a significantly lower ECN than the control, but were not significantly different from heading back alone (Figure 10). The main effect of interaction treatments that included heading back was primarily due to the heading back of the canes.

The use of Extenday\textsuperscript{TM} did not significantly affect ECN in either season compared to the control (Table 8 and Figure 10).
Shading, whether it was pre- or post-FB, did not affect ECN in Season One (Figure 10). However, in Season Two shading significantly decreased ECN compared to the control ($P=0.0178 - 0.0351$). Shade pre-FB significantly ($P=0.0246$) reduced ECN overall when compared with treatments without shading in Season One (Table 8). Shade post-FB also moderately reduced ($P=0.0820$) ECN overall when compared to the other main treatments in Season Two. The two shade treatments were not significantly different from each other in either season (Table 8).
Table 8: Mean effective cane number (ECN), pruning weights (PW) and effective cane weight (ECW) for treatments in Season One (2002/2003) and Season Two (2003/2004).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Effective cane number (ECN)</th>
<th>Pruning weight (PW) (kg)</th>
<th>Effective cane weight (ECW) (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>02/03</td>
<td>03/04</td>
<td>02/03</td>
</tr>
<tr>
<td>Control</td>
<td>14.6 a</td>
<td>21.3 a</td>
<td>2.3 ab</td>
</tr>
<tr>
<td>Root pruned</td>
<td>12.8 b</td>
<td>18.4 ab</td>
<td>1.1 c</td>
</tr>
<tr>
<td>Root pruned/Extenday™</td>
<td>13.1 n</td>
<td>19.8 mn</td>
<td>1.5 o</td>
</tr>
<tr>
<td>Root pruned/shade pre-FB</td>
<td>11.7 n</td>
<td>17.8 n</td>
<td>1.2 n</td>
</tr>
<tr>
<td>Root pruned/shade post-FB</td>
<td>11.9 n</td>
<td>18.9 mn</td>
<td>1.2 n</td>
</tr>
<tr>
<td>Root pruned/heading Back</td>
<td>9.9 o</td>
<td>13.6 o</td>
<td>0.9 n</td>
</tr>
<tr>
<td>Heading back</td>
<td>9.8 c</td>
<td>15.8 c</td>
<td>2.0 b</td>
</tr>
<tr>
<td>Heading back/Extenday™</td>
<td>10.1 y</td>
<td>15.8 y</td>
<td>2.4 y</td>
</tr>
<tr>
<td>Heading back/shade pre-FB</td>
<td>9.1 y</td>
<td>16.3 y</td>
<td>1.9 x</td>
</tr>
<tr>
<td>Heading back/shade post-FB</td>
<td>10.2 y</td>
<td>14.3 yz</td>
<td>2.1 xy</td>
</tr>
<tr>
<td>Extenday™</td>
<td>14.1 ab</td>
<td>20.4 a</td>
<td>2.5 a</td>
</tr>
<tr>
<td>Shade pre-FB</td>
<td>13.2 ab</td>
<td>17.6 bc</td>
<td>2.2 ab</td>
</tr>
<tr>
<td>Shade post-FB</td>
<td>14.1 ab</td>
<td>17.3 bc</td>
<td>2.1 ab</td>
</tr>
</tbody>
</table>

Means within a column with a different letter are significantly different from each other at P=0.05 (LSMeans, SAS). * abc – main treatments, mno – comparisons of interaction treatments that included root pruning with respective main treatments, xyz – comparisons of interaction treatments that included heading back with respective main treatments.
Pruning weight (PW). Root pruning significantly \((P<0.0001)\) reduced PW by approximately a half in both seasons when compared to the control (Figure 11 and Table 8). Root pruning was also significantly different from all other treatments not containing root pruning \((P<0.0001)\). Interaction treatments containing root pruning were not significantly different from root pruning alone, except for the treatment of root pruning with Extenday™ (Table 8). Here, PW was higher than for root pruning alone, though PW was still lower than the control, and the difference was highly significant \((P<0.0001)\).

Heading back of the vines resulted in a slight decrease in PW from the control values (Figure 11 and Table 8), although the difference was not significant in the first season and was only moderately significant in the second \((P=0.0562)\) (Table 8). When all treatments where heading back was applied were taken into account and compared to treatments without heading back, the decrease in PW became significant (Table 8) (Season One \(P=0.0683\), Season Two \(P=0.0002\)). Interaction treatments that contained heading back and either shade post- or shade pre-FB were not significantly different from heading back alone in both seasons (Table 8). However, the interaction treatments with Extenday™ \((P=0.0123 - 0.0144)\) and root pruning \((P<0.0001)\) were significantly different from heading back alone in both seasons.

The application of Extenday™ significantly affected PW in a similar way in both seasons (Figure 10). As a main treatment, Extenday™ did not significantly increase PW from the control values although there was a trend for these values to be higher (Table 8). However, when Extenday™ was applied in conjunction with the other main treatments of root pruning and heading back, values were significantly higher than values obtained for these main treatments alone \((P<0.0012 - 0.0144)\) (Table 8). Therefore, when all treatments containing Extenday™ were compared against treatments without Extenday™, the increase in PW was highly significant \((P=0.0002 - 0.0004)\).
Figure 11: Pruning Weights (PW) for Seasons One and Two. Season One LSD = 0.324, Season Two LSD = 0.509.

Neither shade treatment affected PW in Season One when compared to the control (Figure 11). However, both shade pre- and shade post-FB, significantly reduced PW compared to the control ($P=0.0071 - 0.0269$) in Season Two (Table 8). However, when all treatments with shading were compared to those treatments without shading in Season Two, neither shade pre- nor shade post-FB significantly affected PW (Table 8).

**Effective cane weight (ECW).** Root pruning the vines significantly reduced ECW by between a half and a third of the control values ($P<0.0001$) (Table 8 and Figure 12). Only the interaction treatment of root pruning with Extendal™ was significantly different from root pruning alone ($P=0.0099 - 0.0198$) in both seasons (Figure 12). In Season Two the interaction of root pruning with heading back was also significantly different from root pruning alone ($P=0.0055$). For the interaction treatments with root
pruning, the other main treatments acted in an additive way with root pruning, which resulted in ECW being higher than that of root pruning alone. However, ECW values for the interaction treatments were still significantly lower than that for the control vines ($P \leq 0.0001 - 0.0468$).

Heading back of the vines tended to increase ECW although this was only moderately significant in the first season ($P = 0.0440$) and not significantly different from the control values in the second season (Table 8 and Figure 12). However, when all treatments containing heading back of vines were compared against treatments without heading back, the increase in ECW was highly significant ($P < 0.0001$) in both seasons. Interaction treatments with heading back were not significantly different from heading back alone (Table 8) although they were all significantly higher than the values from the control vines ($P \leq 0.0009 - 0.0691$).

The application of Extenda$^{TM}$ tended to increase ECW, although not significantly compared to the control (Figure 12). However, the interaction with heading back was significantly different ($P = 0.0009 - 0.0014$) from the control (Table 8), which resulted in the application of Extenda$^{TM}$ with heading back having the highest ECW in both seasons (Figure 12). Also, when all treatments containing Extenda$^{TM}$ were compared against treatments without Extenda$^{TM}$, there was a significant effect ($P \leq 0.0053 - 0.0062$) from the use of Extenda$^{TM}$ in both seasons.

In both seasons neither shade pre- nor shade post-FB significantly affected ECW compared to the control (Table 8). They were also not significantly different from each other (Figure 12). When applied as an interaction with other main treatments, they did not significantly affect ECW when compared to the other main treatment alone.
Figure 12: Mean Effective Cane Pruning Weights (ECW) for Seasons One and Two. Season One LSD = 0.231, Season Two LSD = 0.202.

Season Two values for ECN, PW and ECW were all higher than those in Season One. This increase between seasons corresponds with the increase in LLN between Seasons One and Two.

**Growing point number (GPN).** Root pruning alone significantly ($P<0.0001$) decreased GPN per vine when compared with all other main treatments (Table 9). Heading back also reduced GPN though not significantly (Table 9). This resulted in the interaction treatment of root pruning and heading back having one of the lowest numbers of growing points per vine. This interaction was not only significantly less than the control ($P<0.0001$), but was also significantly less than heading back ($P=0.0016$) and root pruning ($P=0.0568$) alone (Table 9).
Chapter Three · Field trial: vigour and light effects on BSN. Results

The main treatment of Extenday™ tended to increase the number of growing points per vine and this was moderately significant ($P=0.0719$) when compared to the control alone (Table 9). However, when applied as an interaction with other main treatment effects, the interaction resulted in significantly more growing points when compared with the other main treatment effects alone ($P<0.0001$).

Except in the treatment of root pruning with shade post-FB, the two shade treatments did not significantly alter GPN (Table 9). The treatment of root pruning with shade post-FB significantly reduced the number of growing points per vine ($P=0.0008$).

**Growing point weight (GPW).** Root pruning ($P=0.0012$) significantly reduced GPW compared to the control (Table 9). Interaction treatments of root pruning with heading back ($P=0.0227$) and root pruning with shade post-FB ($P=0.0294$) had a significantly higher GPW compared to root pruning alone. Other interaction treatments were not significantly different from root pruning alone (Table 9).

Main treatments heading back, Extenday™, and shade pre-FB did not significantly influence GPW (Table 9) compared to the control. However, there was a slight reduction in GPW for Extenday™ and shade pre-FB treatments, and a slight increase in GPW with the heading back treatment. When all treatments with heading back were compared to treatments without heading back, there was a significant ($P=0.0005$) increase in GPW (Table 9).

Shade post-FB significantly decreased GPW from the control ($P=0.0338$) (Table 9). Though not significant, shade post-FB decreased GPW when combined in an interaction with heading back, compared to heading back alone. However, as mentioned above, when combined with root pruning, shade post-FB significantly increased GPW from root pruning alone. This resulted in GPW not being significantly different from the control for the root pruning with shade post-FB interaction treatment (Table 9).
Table 9: Mean growing point number (GPN), growing point weight (GPW), and mean growing points per cane (GP/C) for Season Two (2003/2004).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean growing points per vine (GPN)</th>
<th>Mean growing point weight (GPW) (g)</th>
<th>Mean growing points per cane (GP/C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>65.9 ab*</td>
<td>67 a</td>
<td>3.0 a</td>
</tr>
<tr>
<td>Root prune</td>
<td>50.6 c</td>
<td>45 bc</td>
<td>2.7 a</td>
</tr>
<tr>
<td>Root pruned/Extenda™</td>
<td>67.0 o</td>
<td>42 n</td>
<td>3.3 n</td>
</tr>
<tr>
<td>Root pruned/shade pre-FB</td>
<td>52.8 m</td>
<td>50 n</td>
<td>2.8 n</td>
</tr>
<tr>
<td>Root pruned/shade post-FB</td>
<td>36.8 p</td>
<td>69 m</td>
<td>1.9 o</td>
</tr>
<tr>
<td>Root pruned/heading back</td>
<td>41.9 p</td>
<td>59 m</td>
<td>3.1 mn</td>
</tr>
<tr>
<td>Heading back</td>
<td>56.3 bc</td>
<td>71 a</td>
<td>3.7 a</td>
</tr>
<tr>
<td>Heading back/Extenda™</td>
<td>77.8 x</td>
<td>62 xy</td>
<td>5.4 x</td>
</tr>
<tr>
<td>Heading back/shade pre-FB</td>
<td>65.4 y</td>
<td>63 xy</td>
<td>4.2 y</td>
</tr>
<tr>
<td>Heading back/shade post-FB</td>
<td>63.5 y</td>
<td>66 xy</td>
<td>4.6 y</td>
</tr>
<tr>
<td>Extenda™/shade pre-FB</td>
<td>77.0 a</td>
<td>59 ab</td>
<td>3.4 a</td>
</tr>
<tr>
<td>Shade pre-FB</td>
<td>66.4 ab</td>
<td>55 abc</td>
<td>3.7 a</td>
</tr>
<tr>
<td>Shade post-FB</td>
<td>72.3 a</td>
<td>47 bc</td>
<td>4.2 a</td>
</tr>
</tbody>
</table>

Means within a column with a different letter are significantly different from each other at $P=0.05$ (LSMeans, SAS). * abc - main treatments, mno - comparisons of root pruning with main treatments, xyz - comparisons of heading back with main treatments.
There were no significant differences in GPW between the two shade treatments (Table 9).

**Growing points per cane (GP/C).** There was no significant difference in GP/C between the control and the treatments root pruning, heading back, Extenday™, shade pre-FB or shade post-FB (Table 9). However, the interaction treatment of root pruning with shade post-FB resulted in the GP/C being significantly lower than that of root pruning alone ($P=0.0155$). This was the only interaction with root pruning to be significantly different from root pruning alone although there was a trend overall for GP/C to be reduced by root pruning (Table 9). When all treatments with root pruning were compared to those without root pruning there was a significant decrease in GP/C ($P<0.0001$).

The interaction treatment of heading back and Extenday™ had the highest GP/C, which was significantly different from the control ($P=0.0003$) and heading back alone ($P=0.0095$) (Table 9). All other interaction treatments with heading back, except root pruning with heading back, had significantly higher GP/C ($P=0.0054 - 0.0265$) than the control (Table 9). Therefore, when all treatments with heading back were compared to those without heading back, the increase in GP/C was highly significant ($P<0.0001$).

This also occurred with the use of Extenday™ (Table 9). Due to the trend of Extenday™ to increase GP/C, overall Extenday™ did significantly increase GP/C ($P=0.0037$).

### 3.3.2 Veraison

#### 3.3.2.1 Season One

In Season One 50% veraison was spread over a 2 week period (Figure 13, Table 10) and the entire length of veraison development was approximately 30 days (Figure 13). There was a significant difference among treatments for the day that 50% veraison occurred ($P<0.0001$) and the rate of development at this time ($P=0.0239$).
Bunches on root pruned vines had a significantly later 50% veraison date than bunches on headed back ($P=0.0145$), $\text{Extenda}^\text{TM}$ ($P<0.0001$) and shade post-FB ($P=0.00114$) treated vines. Vines that were only root pruned were also significantly later than the treatment combination of root pruned with $\text{Extenda}^\text{TM}$ ($P=0.0010$) and root pruned with shade post-FB ($P=0.0346$). Overall, when root pruned vines were compared against vines not root pruned there was a significant delay due to root pruning ($P<0.0001$).

Vines that were headed back were significantly later in reaching 50% veraison than $\text{Extenda}^\text{TM}$ treated vines ($P=0.0078$). Overall, heading back significantly affected the date of 50% veraison ($P=0.0153$).

$\text{Extenda}^\text{TM}$ significantly decreased the time to 50% veraison compared to the control, shade pre-FB ($P<0.0001$) and shade post-FB ($P=0.0100$). Overall, $\text{Extenda}^\text{TM}$ treated vines were significantly different to other vines without $\text{Extenda}^\text{TM}$ ($P<0.0001$).

![Figure 13: Veraison development for Season One for treatments applied to Cabernet Sauvignon vines in the field.](image)

The date that bunches reached 50% veraison was significantly different between shade pre- and shade post-FB ($P=0.0614$). Shade post-FB significantly affected the date of 50% veraison ($P<0.0001$)
Root pruning only significantly increased the rate of veraison development when combined with Extenday™. This combination was significantly faster compared to Extenday™ alone ($P=0.0112$) and to root pruning alone ($P=0.0140$). Root pruning with heading back had a significantly slower rate to heading back alone ($P=0.0272$) and the control ($P=0.0560$). Overall root pruning significantly delayed the rate of veraison development ($P<0.001$).

Table 10: Mean date of 50% veraison development rate of field treatments in Season One. Values obtained from SAS fitted sigmoid curves.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Date of 50% veraison (February 2003)</th>
<th>Veraison development rate (% day$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21 ab*</td>
<td>5.6 a mn y xa</td>
</tr>
<tr>
<td>Root pruned</td>
<td>23 a</td>
<td>4.1 a m y n</td>
</tr>
<tr>
<td>Root pruned/Extenday™</td>
<td>16 o</td>
<td>6.8 m n</td>
</tr>
<tr>
<td>Root pruned/shade pre-FB</td>
<td>24 m</td>
<td>4.1 no n</td>
</tr>
<tr>
<td>Root pruned/shade post-FB</td>
<td>18 no</td>
<td>3.8 no n</td>
</tr>
<tr>
<td>Root pruned/heading back</td>
<td>21 mn</td>
<td>3.6 o n</td>
</tr>
<tr>
<td>Heading back</td>
<td>18 bc</td>
<td>5.9 a m</td>
</tr>
<tr>
<td>Heading back/Extenday™</td>
<td>15 xz</td>
<td>4.8 x n</td>
</tr>
<tr>
<td>Heading back/shade pre-FB</td>
<td>20 y</td>
<td>3.3 y n</td>
</tr>
<tr>
<td>Heading back/shade post-FB</td>
<td>18 xy</td>
<td>5.6 x n</td>
</tr>
<tr>
<td>Extenday™</td>
<td>12 d</td>
<td>4.0 a n</td>
</tr>
<tr>
<td>Shade pre-FB</td>
<td>21 ab</td>
<td>5.8 a m</td>
</tr>
<tr>
<td>Shade post-FB</td>
<td>17 c</td>
<td>5.3 a n</td>
</tr>
</tbody>
</table>

Means in the same column with a different letter are significantly different from each other at $P=0.05$ (LSMeans, SAS). * abc – main treatments, mno – comparisons of interaction treatments that included root pruning with respective main treatments, xyz – comparisons of interaction treatments that included heading back with respective main treatments.
Chapter Three - Field trial: vigour and light effects on BSN. Results

Heading back alone significantly increased the rate compared to the combination of heading back with shade pre-FB ($P=0.0151$). Overall, heading back significantly affected the rate of veraison development ($P=0.0045$).

Overall both shade pre- and shade post-FB significantly affected the rate of veraison development ($P<0.0001$ and $P=0.0370$, respectively) although as individual treatments, compared to the control, they did not.

3.3.2.2 Season Two

Whereas in Season One 50% veraison was spread over a two week period, in Season Two 50% veraison was spread over only one week (Figure 14, Table 11). However, the time from the start of veraison development to 100% veraison was still approximately 30 days (Figure 14). As with Season One, analyses showed that there was a significant treatment difference for the date that 50% veraison was reached.

![Figure 14: Veraison development for Season Two for treatments applied to ‘Cabernet Sauvignon’ vines in the field.](image)
Table 11: Mean date of 50% veraison and mean veraison development rate of all field treatments in Season Two. Values obtained from SAS fitted sigmoid curves.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Date of 50% veraison (February 2004)</th>
<th>Veraison development rate (% day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21 a*</td>
<td>1.9</td>
</tr>
<tr>
<td>Root pruned</td>
<td>17 c</td>
<td>2.6</td>
</tr>
<tr>
<td>Root pruned/Extenday™</td>
<td>18 n</td>
<td>2.5</td>
</tr>
<tr>
<td>Root pruned/shade pre-FB</td>
<td>19 n</td>
<td>2.6</td>
</tr>
<tr>
<td>Root pruned/shade post-FB</td>
<td>13 p</td>
<td>2.1</td>
</tr>
<tr>
<td>Root pruned/heading back</td>
<td>17 o</td>
<td>2.1</td>
</tr>
<tr>
<td>Heading back</td>
<td>21 a*</td>
<td>2.5</td>
</tr>
<tr>
<td>Heading back/Extenday™</td>
<td>20 xy</td>
<td>2.1</td>
</tr>
<tr>
<td>Heading back/shade pre-FB</td>
<td>20 xy</td>
<td>2.4</td>
</tr>
<tr>
<td>Heading back/shade post-FB</td>
<td>20 xy</td>
<td>1.8</td>
</tr>
<tr>
<td>Extenda™</td>
<td>17 c</td>
<td>2.5</td>
</tr>
<tr>
<td>Shade pre-FB</td>
<td>21 a*</td>
<td>3.1</td>
</tr>
<tr>
<td>Shade post-FB</td>
<td>19 b</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Means in the same column with a different letter are significantly different from each other at $P=0.05$ (LSMeans, SAS). * abc – main treatments, mno – comparisons of interaction treatments that included root pruning with respective main treatments, xyz – comparisons of interaction treatments that included heading back with respective main treatments.

Root pruning had a significant effect on the day of 50% veraison compared to the control, heading back and both shade pre-FB and post-FB ($P<0.0001$). Unlike Season One, in Season Two root pruning brought forward 50% veraison.
Root pruning with Extendal™, root pruning with shade pre-FB and root pruning with shade post-FB were all significantly different from root pruning alone (\(P=0.0045, 0.0008\) and \(<0.0001\), respectively) for the date of 50% veraison. Root pruning with heading back was significantly earlier than heading back alone (\(P<0.0001\)), and root pruning with shade post-FB was significantly earlier than shade post-FB alone (\(P<0.0001\)). However, root pruning with Extendal™ was significantly later than Extendal™ alone (\(P=0.0018\)).

Root pruning over all made a significant difference on the date of 50% veraison (\(P<0.0001\)).

Heading back significantly delayed the date of 50% veraison compared to Extendal™, shade pre- and shade post-FB (\(P<0.0001\)). Heading back alone also significantly delayed the date of 50% veraison compared to heading back with Extendal™, heading back with shade pre-FB and heading back with shade post-FB (\(P=0.0554, 0.0016\) and \(0.0388\), respectively). Heading back with Extendal™ was also significantly delayed compared to Extendal™ alone (\(P<0.0001\)), heading back with shade pre-FB was significantly delayed compared to shade pre-FB (\(P=0.0592\)) and heading back with shade post-FB was significantly delayed compared to shade post-FB alone (\(P=0.0142\)). Therefore heading back made a significant difference overall to the date of 50% veraison (\(P<0.0001\)).

Extendal™ significantly brought forward the date of 50% veraison compared to the control, shade pre- and shade post-FB (\(P<0.0001\)). Overall, Extendal™ had a significant effect on the date of 50% veraison (\(P<0.0001\)).

Shade pre-FB significantly brought forward the date of 50% veraison compared to the control. Overall, shade pre-FB significantly affected 50% veraison (\(P<0.0001\)). Shade post-FB did significantly bring forward the date of 50% veraison (\(P=0.0005\)) but overall did not significantly affect it.
The rate of veraison development was slower in Season Two when compared to Season One for all treatments (Table 10 and Table 11). The difference in development rate among all the treatments was much less in Season Two when compared to Season One. The difference between maximum and minimum veraison rate was only 0.9% day$^{-1}$, whereas in Season One the difference was 3.5% day$^{-1}$. There was no significant difference in veraison development rate among treatments.

### 3.3.2.3 Season Three

In Season Three the difference among treatments for 50% veraison was less than a week (Figure 15, Table 12). Due to a reduction in the number of measurements over the entire veraison development stage, it is difficult to determine how many weeks veraison development was spread over and statistical analyses could not be carried out. However, vines that had root pruning as part of their treatment tended to reach 50% veraison earlier than the control and other main treatments. The veraison development rate also tended to be slightly faster for root pruned vines, including the treatment combinations that included root pruning (Table 12).

Unlike Seasons One and Two, no other treatment brought forward 50% veraison or sped up veraison development compared to the control. In fact, there may have been a slight delay in 50% veraison, and the rate of veraison development slowed, by the use of all the treatments apart from root pruning (Table 12).
Figure 15: Veraison development for Season Three for treatments applied to ‘Cabernet Sauvignon’ vines in the field.

Table 12: Mean date of 50% veraison and mean veraison development rate of field treatments in Season Three. Values obtained from SAS fitted sigmoid curves.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Date of 50% veraison (February 2005)</th>
<th>Veraison development rate (% day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18</td>
<td>3.0</td>
</tr>
<tr>
<td>Root pruned</td>
<td>17</td>
<td>3.4</td>
</tr>
<tr>
<td>Root pruned/Extenday™</td>
<td>18</td>
<td>2.5</td>
</tr>
<tr>
<td>Root pruned/shade pre-FB</td>
<td>18</td>
<td>2.8</td>
</tr>
<tr>
<td>Root pruned/shade post-FB</td>
<td>15</td>
<td>3.8</td>
</tr>
<tr>
<td>Root pruned/heading back</td>
<td>16</td>
<td>2.7</td>
</tr>
<tr>
<td>Heading back</td>
<td>20</td>
<td>2.0</td>
</tr>
<tr>
<td>Heading back/Extenday™</td>
<td>21</td>
<td>1.9</td>
</tr>
<tr>
<td>Heading back/shade pre-FB</td>
<td>19</td>
<td>3.3</td>
</tr>
<tr>
<td>Heading back/shade post-FB</td>
<td>19</td>
<td>2.6</td>
</tr>
<tr>
<td>Extenday™</td>
<td>20</td>
<td>2.2</td>
</tr>
<tr>
<td>Shade pre-FB</td>
<td>19</td>
<td>2.6</td>
</tr>
<tr>
<td>Shade post-FB</td>
<td>19</td>
<td>2.8</td>
</tr>
</tbody>
</table>
Root pruning in Season Three. There was very little difference in 50% veraison among root pruned treatments and their controls (Figure 16, Table 13). The two controls and the vines root pruned in Season Three, all reached 50% veraison around the same time, whereas the vines that were root pruned in Season One reached 50% veraison slightly earlier (Table 13).

Figure 16: Veraison development in Season Three for Season One and Season Three root pruned ‘Cabernet Sauvignon’ vines with their controls.

Vines that were root pruned in Season One also had a slightly faster veraison development rate compared to the two controls and the vines root pruned in Season Three (Table 13).
Table 13: Mean date of 50% veraison and mean veraison development rate of root pruning treatments and their controls in Season Three. Values obtained from SAS fitted sigmoid curves.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Date of 50% veraison (February 2005)</th>
<th>Veraison development rate (% day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Season One)</td>
<td>20</td>
<td>2.3</td>
</tr>
<tr>
<td>Control (Season Three)</td>
<td>20</td>
<td>2.6</td>
</tr>
<tr>
<td>Root pruned (Season One)</td>
<td>17</td>
<td>3.4</td>
</tr>
<tr>
<td>Root pruned (Season Three)</td>
<td>20</td>
<td>2.4</td>
</tr>
</tbody>
</table>

3.3.3 Petiole, rachis and berry cation analyses

3.3.3.1 Leaf petiole cation concentrations

Statistical analyses could not be carried out on pre-veraison petiole samples in Season One due to samples being pooled rather than replicated.

Magnesium concentration. Differences among treatments were very small for pre-veraison petiole samples. There was, however, a large increase in magnesium concentration in the petioles from pre-veraison to post-veraison (Figure 17).

Root pruning reduced the magnesium concentration in petioles post-veraison, though not by a statistically significant amount (Figure 17). Though not significantly different from the control when compared on the basis of individual treatments, when all root pruned treatments were compared to treatments without root pruning, there was a significant affect for magnesium ($P=0.0408$) concentration. However, both heading back with Extenday™ and shade post-FB also appeared to reduce magnesium concentrations in petioles, although not by statistically significant amounts. There also appeared to be a difference between the two shade treatments where shade pre-FB consistently had higher magnesium concentrations in the petioles than shade post-FB (Figure 17).
Figure 17: Mean petiole magnesium concentration (mg l⁻¹) for each treatment in Season One. LSD, for comparison among treatment means at post-veraison sampling time, was derived using SAS 8.2.

**Calcium concentration.** There were no significant differences among treatments for leaf petiole calcium concentration post-veraison (Figure 18), even when interaction treatments were taken into account. Similar to the results for petiole magnesium concentration, there was a trend for shade post-FB to reduce calcium concentrations in leaf petioles and for shade pre-FB to increase calcium concentrations. Calcium concentration also markedly increased from pre- to post-veraison.
Figure 18: Mean petiole calcium concentration (mg l⁻¹) for each treatment in Season One. LSD, for comparison among treatment means at post-veraison sampling time, derived using SAS 8.2.

Potassium concentration. The only consistent trend among treatments for the pre-veraison sample was shade pre-FB tended to increase potassium concentration. However, that trend was not apparent at post-veraison. There were no significant differences among treatments at the post-veraison sample time (Figure 19). Unlike magnesium and calcium, differences in potassium concentrations between pre- and post-veraison were smaller, but post-veraison concentrations were still higher than pre-veraison.
3.3.3.2 Berry cation concentrations

**Magnesium concentration.** There was no significant difference among treatments for magnesium concentrations in the berries that were sampled pre-veraison in Season One (Figure 20).

This was also the case for berry magnesium concentrations determined after flowering in Season Three for berries from vines that had been root pruned in Season One, root pruned in Season Three and those from control vines (Table 14). Though not significantly different, root pruning in Season Three appeared to reduce berry magnesium concentrations compared to the control, post-flowering.
Figure 20: Mean berry magnesium concentration (mg l\(^{-1}\)) from dried samples taken pre-veraison in Season One. LSD, for comparison among treatment means, was derived using SAS 8.2.

However, there was a significant difference \((P=0.0285)\) between these treatments when the berries were sampled pre-veraison (Table 14). Vines that were root pruned in Season Three had a significantly higher berry magnesium concentration than either the control \((P=0.0222)\) or vines that had been root pruned in Season One \((P=0.0157)\).

Magnesium concentrations in the berries also declined between post-flowering and pre-veraison (Table 14). This decline was highly significant for the control \((P<0.0001, F\text{ value}=141)\), berries from vines root pruned Season One \((P<0.0001, F\text{ value}=96)\) and vines root pruned Season Three \((P<0.0001, F\text{ value}=71)\). By comparing the F values it can be seen that the decline in magnesium concentrations between the two sample times was most significant in the control, and least significant in vines which were root pruned in Season Three.
Chapter Three - Field trial: vigour and light effects on BSN. Results

Though Season One values for pre-veraison berry magnesium concentration are slightly lower than in Season Three, they are generally similar (ref. Figure 20 and Table 14).

### Table 14: Means of post-flowering and pre-veraison berry magnesium concentrations (mg l⁻¹) in Season Three.

<table>
<thead>
<tr>
<th></th>
<th>Post-flowering</th>
<th>Pre-veraison</th>
<th>LSD (p≤0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.60a</td>
<td>4.10b</td>
<td>0.47</td>
</tr>
<tr>
<td>Root pruned Season One</td>
<td>6.40a</td>
<td>4.08b</td>
<td>0.53</td>
</tr>
<tr>
<td>Root pruned Season Three</td>
<td>6.23a</td>
<td>4.46c</td>
<td>0.49</td>
</tr>
<tr>
<td>LSD (p≤0.05)</td>
<td>0.62 (ns)</td>
<td>0.30</td>
<td></td>
</tr>
</tbody>
</table>

Means within a column and within a row with a different letter are significantly different from each other at P=0.05 (LSMeans, SAS).

**Calcium concentration.** There was a significant difference among treatments for berry calcium concentration in Season One (P=0.0609). Overall root pruning appeared to reduce berry calcium concentrations (Figure 21). Except for the interaction treatment of heading back with Extenday™, it appeared that Extenday™ increased berry calcium concentration. The shade treatments appeared to increase berry calcium concentration when applied alone or with heading back, but when shade was applied with root pruning it appeared that the berry calcium concentrations were reduced.

There were no significant differences in berry calcium concentration among the control, root pruned Season One or root pruned Season Three treatments for berries sampled either post-flowering or pre-veraison in Season Three (Table 15). This is in contrast to the results from Season One where root pruning reduced the calcium concentration.

There was however, a significant decrease in berry calcium concentration between the two sample times for the control (P<0.0001, F value = 83), vines root pruned in Season One (P<0.0001, F value = 44.25) and vines root pruned in Season Three (P=0.0015, F value = 25). Pre-veraison values were similar between Season One and Three (ref. Figure 21 and Table 15).
Chapter Three - Field trial: vigour and light effects on BSN. Results

Figure 21: Mean berry calcium concentration (mg l⁻¹) from dried samples taken pre-veraison in Season One. LSD, for comparison among treatment means, derived using SAS 8.2.

As in berry magnesium concentration, the difference between sample dates was the most significant for the control and the least significant for the root pruned Season Three treatment.

Table 15: Means of post-flowering and pre-veraison calcium concentrations (mg l⁻¹) in berries in Season Three.

<table>
<thead>
<tr>
<th></th>
<th>Post-flowering</th>
<th>Pre-veraison</th>
<th>LSD (p≤0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>33.6a</td>
<td>19.1b</td>
<td>3.6</td>
</tr>
<tr>
<td>Root pruned Season One</td>
<td>32.8a</td>
<td>18.3b</td>
<td>4.9</td>
</tr>
<tr>
<td>Root pruned Season Three</td>
<td>32.8a</td>
<td>19.8b</td>
<td>6.1</td>
</tr>
<tr>
<td>LSD (p≤0.05)</td>
<td>5.9(ns)</td>
<td>1.9(ns)</td>
<td></td>
</tr>
</tbody>
</table>

Means within a column and with a row with the same letter are not significantly different from each other at \( P=0.05 \) (LSMeans, SAS).
Potassium concentration. In Season One there was no significant difference among treatments for berry potassium concentration. There were also no consistent trends evident within the main treatments and the interactions (Figure 22).

![Figure 22: Mean berry potassium concentration (mg l⁻¹) from dried samples taken pre-veraison in Season One. LSD, for comparison among treatment means, derived using SAS 8.2.](image)

There were also no significant differences in berry potassium concentrations in Season Three, for either of the two sample dates (Table 16). Pre-veraison berry potassium concentrations tended to be lower overall in Season Three than Season One (ref. Figure 22 and Table 16). There was no significant difference in berry potassium concentrations from post-flowering to pre-veraison in Season Three (Table 16).
Table 16: Means of post-flowering and pre-veraison berry potassium concentrations (mg l⁻¹) in Season Three

<table>
<thead>
<tr>
<th></th>
<th>Post-flowering</th>
<th>Pre-veraison</th>
<th>LSD (p≤0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>70.4</td>
<td>67.7</td>
<td>3.4 (ns)</td>
</tr>
<tr>
<td>Root pruned Season One</td>
<td>68.6</td>
<td>70.3</td>
<td>5.1 (ns)</td>
</tr>
<tr>
<td>Root pruned Season Three</td>
<td>69.8</td>
<td>72.4</td>
<td>11.8 (ns)</td>
</tr>
<tr>
<td><strong>LSD (p≤0.05)</strong></td>
<td>5.0 (ns)</td>
<td>7.8 (ns)</td>
<td></td>
</tr>
</tbody>
</table>

3.3.3.3 Rachis cation concentration

Magnesium concentration. There were no significant differences among treatments for magnesium concentrations measured in the rachis in Season One (Figure 23).

![Figure 23: Mean rachis magnesium concentration (mg l⁻¹) from dried samples taken pre-veraison in Season One. LSD, for comparison among treatment, means derived using SAS 8.2.](image)
There was also no significant difference between rachis magnesium concentration determined post-flowering in Season Three when comparing the control vines with vines root pruned in Season One and vines root pruned in Season Three (Table 17).

However, magnesium concentration in the rachis was significantly different \((P=0.0036)\) by pre-veraison in Season Three (Table 17). As with berry magnesium concentration, root pruning in Season Three led to an increasing magnesium concentration in the rachis and this was significantly different from the control \((P=0.0050)\) and root pruning treatments in Season One \((P=0.0017)\).

Again, as with berry magnesium concentration, values generally declined in the rachis over the time from post-flowering to pre-veraison. This was significant for the control \((P=0.0025)\) but not for root pruning in Season One or Season Three (Table 17). Pre-veraison values for Season One and Season Three were similar (ref. Figure 23 and Table 17).

**Table 17:** Means of post-flowering and pre-veraison magnesium concentrations (mg l\(^{-1}\)) in the rachis in Season Three.

<table>
<thead>
<tr>
<th></th>
<th>Post-flowering</th>
<th>Pre-veraison</th>
<th>LSD ((p=0.05))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.2a</td>
<td>2.71b</td>
<td>0.26</td>
</tr>
<tr>
<td>Root pruned Season One</td>
<td>3.04ab</td>
<td>2.60b</td>
<td>0.52 ((ns))</td>
</tr>
<tr>
<td>Root pruned Season Three</td>
<td>3.04abc</td>
<td>3.39c</td>
<td>0.62 ((ns))</td>
</tr>
<tr>
<td>(LSD) ((p=0.05))</td>
<td>0.54 ((ns))</td>
<td>0.43</td>
<td></td>
</tr>
</tbody>
</table>

Means within a column and within a row with a different letter are significantly different from each other at \(P=0.05\) (LSMeans, SAS).

**Calcium concentration.** There was a significant difference among treatments for calcium concentration in the rachis \((P=0.0407)\), in Season One (Figure 24). As with calcium concentration in the berry, root pruning reduced calcium concentration in the rachis.
Figure 24: Mean rachis calcium concentration (mg l⁻¹) from dried samples taken pre-veraison in Season One. LSD, for comparison among treatment means, derived using SAS 8.2.

As with berry calcium concentration in Season Three, there was no significant difference among treatments at either sampling time in Season Three for the control, vines root pruned in Season One and vines root pruned in Season Three (Table 18).

There was also no significant difference in rachis calcium concentration between the two sample dates (Table 18). Pre-veraison values for Seasons One and Three were similar (ref. Figure 24 and Table 18).
Table 18: Means of post-flowering and pre-veraison calcium concentrations in the rachis in Season Three.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Post-flowering</th>
<th>Pre-veraison</th>
<th>LSD (p=0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.8</td>
<td>20.9</td>
<td>1.9 (ns)</td>
</tr>
<tr>
<td>Root prune Season One</td>
<td>20.4</td>
<td>21.3</td>
<td>3.1 (ns)</td>
</tr>
<tr>
<td>Root prune Season Three</td>
<td>21.4</td>
<td>19.9</td>
<td>2.9 (ns)</td>
</tr>
<tr>
<td>LSD (p=0.05)</td>
<td>3.0 (ns)</td>
<td>2.4 (ns)</td>
<td></td>
</tr>
</tbody>
</table>

**Potassium concentration.** There were no significant differences among treatments for rachis potassium concentrations in Season One (Figure 25).

![Figure 25: Mean rachis potassium concentration (mg L⁻¹) from dried samples taken pre-veraison Season One. LSD, for comparison among treatment means, derived using SAS 8.2.](image)

Neither was there a significant difference in rachis potassium concentrations post-flowering in Season Three (Table 19). By pre-veraison in Season Three there was a significant difference in rachis potassium concentrations (P=0.0588) where vines that
were root pruned in Season Three had significantly ($P=0.0194$) lower potassium than vines that were root pruned in Season One (Table 19).

Potassium concentrations also increased in the rachis from post-flowering to pre-veraison. This was highly significant in all the treatments (Table 19). The potassium concentrations in Season Three at this sample time appeared to be much higher than potassium concentrations in Season One around the same sample time (ref. Figure 25 and Table 19).

Table 19: Means of post-flowering and pre-veraison potassium concentrations (mg l$^{-1}$) in the rachis in Season Three.

<table>
<thead>
<tr>
<th></th>
<th>Post-flowering</th>
<th>Pre-veraison</th>
<th>$LSD(p=0.05)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>122a</td>
<td>157bc</td>
<td>13</td>
</tr>
<tr>
<td>Root prune Season One</td>
<td>117a</td>
<td>166b</td>
<td>16</td>
</tr>
<tr>
<td>Root prune Season Three</td>
<td>112a</td>
<td>142c</td>
<td>19</td>
</tr>
<tr>
<td>$LSD (p=0.05)$</td>
<td>17.5 (ns)</td>
<td>12.9</td>
<td></td>
</tr>
</tbody>
</table>

Means within a column and with a row with a different letter are significantly different from each other at $P=0.05$ (LSMeans, SAS).

3.3.4 Juice analyses

3.3.4.1 Brix

In Season One, treatment differences at each sample time (based on pooled samples) were unable to be determined statistically due to a lack of replication. However, regression analyses showed that linear trend lines fitted to the data were significantly different among treatments ($P<0.0001$). Brix values from treatments that included root pruning tended to increase more over the post-veraison sample period than those treatments without root pruning. Therefore, although these treatments tended to have lower values initially, they tended to finish with a higher Brix concentration at harvest (Figure 26). In Season One, the rate of increase in Brix values also tended to decline near to harvest. However, treatments that included root pruning did not have such a
decline in the rate of Brix increase as much as those treatments without root pruning (Figure 26).

Figure 26: Mean Brix concentrations of treatments for six weeks following 100% veraison to harvest in Season One.

In Season Two, significant treatment differences in Brix concentrations were found among treatments, two \((P=0.0213)\), three \((P=0.0248)\) and four \((P=0.0389)\) weeks after 100% veraison (Figure 27).

Although in Season One root pruning tended to have lower Brix values initially, in Season Two root pruning (carried out in Season One) tended to increase Brix values compared to the control and this was evident soon after 100% veraison \((P=0.0034\) second week, \(P=0.0163\) fourth week). Heading back, overall, tended to reduce Brix concentration compared to the control (this was however, only significant at three weeks after 100% veraison \((P=0.0567)\)).
Chapter Three - Field trial: vigour and light effects on BSN. Results

Figure 27: Mean Brix concentrations of treatments from one to five weeks after 100% veraison in Season Two.

All treatments, except shade pre-FB, showed a significant increase in Brix concentration from week 1 until week 4, after which Brix concentrations were not significantly different (Figure 27). Brix values for shade pre-FB did not significantly increase between weeks 1 and 3, but did so between weeks 3 and 5.

In Season Three, there was no difference in Brix values among the treatments sampled (Table 20). However, root pruning did tend to have higher Brix values than all other treatments except the control.

Table 20: Season Three Brix, TA, maturity ratio, pH, and malic and tartaric acid concentrations of the six main treatments in Seasons One and Two. Berries sampled on 6 April, 2005, prior to commercial harvest.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Brix</th>
<th>TA</th>
<th>Maturity ratio</th>
<th>pH</th>
<th>Malic acid concentration</th>
<th>Tartaric acid concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(%)</td>
<td>(g l⁻¹)</td>
<td>(Brix/TA)</td>
<td>(mg l⁻¹)</td>
<td></td>
<td>(mg l⁻¹)</td>
</tr>
<tr>
<td>Control</td>
<td>20.18</td>
<td>9.32 a</td>
<td>2.17 a</td>
<td>3.34 a</td>
<td>3.04 a</td>
<td>7.88 b</td>
</tr>
<tr>
<td>Root pruned</td>
<td>20.05</td>
<td>8.00 b</td>
<td>2.51 b</td>
<td>3.43 a</td>
<td>2.21 b</td>
<td>6.35 c</td>
</tr>
<tr>
<td>Heading back</td>
<td>19.48</td>
<td>9.15 a</td>
<td>2.13 a</td>
<td>3.33 a</td>
<td>3.07 a</td>
<td>7.40 b</td>
</tr>
<tr>
<td>Extenday™</td>
<td>19.65</td>
<td>9.67 a</td>
<td>2.03 a</td>
<td>3.41 a</td>
<td>3.22 a</td>
<td>8.89 a</td>
</tr>
<tr>
<td>Shade pre-FB</td>
<td>19.50</td>
<td>8.86 a</td>
<td>2.21 a</td>
<td>3.38 a</td>
<td>2.60 ab</td>
<td>7.18 b</td>
</tr>
<tr>
<td>Shade post-FB</td>
<td>19.15</td>
<td>9.23 a</td>
<td>2.09 a</td>
<td>3.40 a</td>
<td>3.25 a</td>
<td>7.16 b</td>
</tr>
<tr>
<td>LSD (p≤0.05)</td>
<td>1.28 (ns)</td>
<td>0.70</td>
<td>0.25</td>
<td>0.19 (ns)</td>
<td>0.69</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Means within a column with a different letter are significantly different from each other at P=0.05 (LSMeans, SAS).
3.3.4.2 Titratable Acidity (TA)

In Season One, regression analyses showed that linear trend lines fitted to the data were significantly different among treatments ($P<0.0001$). Treatments that included root pruning tended to have a lower TA in Season One, even at the stages immediately following veraison, compared to treatments without root pruning (Figure 28). This tended to be consistent across all sample dates.

TA tended to decline progressively over the sampling period with a marked decrease over the initial two weeks in most treatments, and a steady decline thereafter (Figure 28).

![Figure 28: Mean titratable acidity (g L⁻¹) values of treatments for six weeks following 100% veraison to harvest in Season One.](image)

Of the five sampling times in Season Two, TA was significantly affected by treatments at the first ($P=0.0746$), second ($P=0.0013$), third ($P=0.0285$) and fourth ($P=0.0019$) sampling dates (Figure 29). Root pruning significantly ($P=0.0109 - 0.0424$) decreased TA at all of these times, and although not significant, TA was also lower in the root pruning treatment five weeks after 100% veraison (Figure 29). This was the same trend as that determined in Season One.
Heading back also tended to reduce TA compared to the control, although not as much as root pruning (Figure 29). This reduction was significant at three ($P=0.0485$) and four ($P=0.0040$) weeks after 100% veraison.

The two shade treatments also tended to reduce TA, although not significantly except for shade pre-FB one week after 100% veraison ($P=0.0219$). This resulted in the interaction treatment of root pruning with shade post-FB having the lowest TA of all treatments at the two dates on which it was sampled ($P=0.0002 - 0.0031$). This tended to be significant when compared to all treatments except root pruning alone and heading back alone four weeks after 100% veraison (Figure 29).

![Figure 29: Mean titratable acidity (g l⁻¹) values of treatments from one to five weeks after 100% veraison in Season Two.](image)

All main treatments apart from heading back followed a similar pattern for TA decrease over time - there was a highly significant decrease each week except for between weeks three and four where, although there was still a decrease, it was not significant (Figure 29).

In Season Three root pruning still significantly reduced TA ($P=0.0011$) compared to the control and all other treatments ($P=0.0001 - 0.0190$) (Table 20).
3.3.4.3 Maturity ratio – Brix/TA

Regression analyses showed that linear trend lines fitted were significantly different among treatments \( (P<0.0001) \) in Season One. The maturity ratio showed that root pruning tended to increase the maturity of berries in Season One, even at the early stages when the Brix values may have been slightly lower than in the other treatments (Figure 30). The increase in maturity was most evident when the root pruned values were compared to that for the control. Overall, treatments with root pruning had higher maturity ratios than treatments without root pruning. The increase in maturity over the sampling period was generally linear (Figure 30).

![Mean maturity ratio of treatments for six weeks following 100% veraison to harvest in Season One.](image)

In Season Two, vines that had been root pruned in Season One tended to have more mature berries than the control vines over all five sampling times (Figure 31). This was, however, only significantly different from the control two weeks after 100% veraison \( (P=0.0267) \). Bunches from the interaction treatment of root pruning with shade post-FB were also significantly more mature than the control bunches and this was significant both times that bunches were sampled \( (P=0.0006 - 0.0007) \). Heading back also tended to increase maturity of berries when compared to the control (Figure 31), though not significantly except at four weeks after 100% veraison \( (P=0.0334) \). The control and heading back treatments followed a similar pattern over time for the maturity ratio as they did for TA (Figure 31). For all treatments, there was a highly significant increase in the maturity ratio each week.
Figure 31: Mean maturity ratio of treatments from one to five weeks after 100% veraison in Season Two.

There was a treatment difference ($P=0.0166$) for the maturity ratio in Season Three (Table 20) where bunches from root pruned vines were significantly more mature than bunches from either the control ($P=0.0129$) or all other treatments ($P\leq0.0012 – 0.0240$) as in the previous two seasons.

### 3.3.4.4 Calcium concentration

In Season One regression analyses showed that linear trend lines fitted to the data were significantly different among treatments ($P<0.0001$). Any trends among treatments regarding calcium concentration were difficult to determine as values tended to fluctuate within treatments throughout the sampling period (Figure 32). However, there was a trend for treatments that included Extenda™ to have higher calcium concentrations (Figure 32). Furthermore, treatments that included heading back appeared to have lower calcium concentrations than the control (Figure 32). Differences among treatments which included root pruning were not consistent but root pruning alone did tend to have lower calcium concentration than the control at most sample dates (Figure 32).
Figure 32: Mean calcium concentration (mg l⁻¹) of treatments for six weeks following 100% veraison to harvest in Season One.

There were no significant differences in calcium concentration of the berry juice among treatments throughout Season Two at each individual assessment date. However, in the first week following 100% veraison, berries from the control vines tended to have the highest calcium concentration but thereafter, in contrast to Season One, root pruning values were highest (Figure 33). The use of Extenda™ also tended to increase berry calcium concentration at most of the sampling dates (Figure 33), which was a similar result to that obtained in Season One.

Figure 33: Mean calcium concentration (mg l⁻¹) of treatments from one to five weeks after 100% veraison in Season Two.
3.3.4.5 Magnesium concentration

In Season One regression analyses showed that linear trend lines fitted to the data were significantly different among treatments \((P<0.0001)\). Treatments that included Extenday\(^\text{TM}\) appeared to indicate higher magnesium concentrations in the berry juice than treatments that did not include Extenday\(^\text{TM}\) and heading back alone tended to have higher magnesium values than the control (Figure 34).

![Figure 34: Magnesium concentration (mg l\(^{-1}\)) of treatments from 100% veraison to six weeks after in Season One.](image)

One, three and four weeks following 100% veraison in Season Two there was a significant difference \((P=0.0627, P=0.0193 \text{ and } P=0.0072, \text{ respectively})\) among treatments. Shade pre-FB significantly reduced magnesium concentration compared to the control \((P=0.0766 \text{ and } 0.0787)\) at the two dates that it was sampled. Shade post-FB also tended to reduce magnesium concentration although this difference was never significant (Figure 35).

Extenday\(^\text{TM}\) tended to lead to increased magnesium concentration (Figure 35) as was also evident in Season One. Root pruning and heading back also led to increased magnesium concentrations in Season Two (Figure 35). Heading back did not increase berry magnesium values to such an extent or as consistently as Extenday\(^\text{TM}\).
Chapter Three - Field trial: vigour and light effects on BSN. Results

All treatments, except shade pre-FB, had a moderate to highly significant increase in magnesium concentration over time. However, there did tend to be a decline in magnesium values near to harvest for all treatments (Figure 35). There was no significant change in berry magnesium concentration within the shade pre-FB treatment over time although it did increase from the first week following 100% veraison to harvest.

![Figure 35: Mean magnesium concentration (mg l⁻¹) of treatments from one to five weeks after 100% veraison in Season Two.](image)

3.3.4.6 Potassium concentration

In Season One, regression analyses showed that linear trend lines fitted to the data were significantly different among treatments ($P<0.0001$). Heading back used alone tended to result in an increase in berry potassium concentration, whereas root pruning alone tended to either have similar potassium concentration values or lower values than the control depending on the sampling time (Figure 36). All treatments had a dramatic decline in potassium concentration for the final sampling date by which time values among treatments were very similar (Figure 36).
**Figure 36: Potassium concentration (mg l⁻¹) of treatments for six weeks following 100% veraison to harvest in Season One.**

Potassium concentrations in Season Two were significantly affected by the treatments at all six sampling times between 100% veraison and harvest ($P<0.0001 - 0.0175$). Unlike in Season One, Heading back consistently reduced berry potassium concentration (Figure 37) and this was significant ($P=0.0008 - 0.0385$) at all times except two weeks after 100% veraison. Shade pre-FB also reduced berry potassium concentration significantly at all sample dates ($P\leq0.0066 - 0.0782$) and Extenday™ also tended to reduce potassium concentration although only significantly at one ($P=0.0674$) and four ($P<0.0001$) weeks following 100% veraison (Figure 37). Shade post-FB reduced berry potassium concentration at some sampling times, but did not do so consistently and values were, on occasion, higher than those for the control vines. This was also the case for root pruning (Figure 37) where results were similar to those obtained in Season One.
Although there were moderate differences among sampling dates for most treatments, there was often no significant difference between the first and last sample date. Potassium concentration tended to increase in most treatments over the period from one to four weeks following 100% veraison but then tended to decline nearer to harvest (Figure 37). However, this decline was not as dramatic as that determined in Season One.

### 3.3.4.7 Ammonium concentration

In Season One, regression analyses showed that linear trend lines fitted to the data were significantly different among treatments ($P<0.0001$). All treatments with root pruning tended to have lower berry ammonium concentrations than the other treatments (Figure 38). At some sampling dates, treatments with heading back also had lower ammonium concentrations than the control, although this was not consistent across all sampling dates (Figure 38).
In Season Two, berry ammonium concentrations were significantly different among treatments at three ($P=0.0112$) and four ($P=0.0190$) weeks following veraison. Root pruning reduced ammonium concentrations compared to the control at those sampling times ($P=0.0042$). Although not significant, root pruning also reduced ammonium concentrations at the other sampling times (Figure 39). This is the same result as that determined in Season One.

There were no consistent trends in berry ammonium concentration for the other treatments at any of the sampling dates.

Ammonium concentration did decline over the sampling period for all treatments (Figure 39) although this was not significantly different between the first and last sampling dates for the treatments of shade post-FB and root pruning with shade post-FB. By the end of the sampling period, berry ammonium concentrations had not declined to the same values as those in Season One.
Chapter Three - Field trial: vigour and light effects on BSN. Results

3.3.4.8 pH

In Season One regression analyses showed that linear trend lines fitted to the data were significantly different among treatments ($P<0.0001$). Treatments that were root pruned in Season One tended to have a berry pH close to the control at the beginning of the sampling period and a higher pH at the end (Figure 40). Any other treatment trends were difficult to determine due to the fluctuations in pH values throughout the sampling period (Figure 40).

Figure 39: Mean ammonium concentration (mg l$^{-1}$) of treatments from one to five weeks after 100% veraison in Season Two.
Chapter Three - Field trial: vigour and light effects on BSN. Results

Figure 40: pH of treatments for the six weeks from 100% veraison to harvest in Season One.

In Season Two, the only sampling time where significant differences among treatments could be found was three weeks after 100% veraison ($P=0.0394$). Heading back had significantly higher pH values than the control ($P=0.0032$) and any other treatment (Figure 41). Across the sampling period most treatments had a significant increase of pH. The increase was not significant for shade pre- or shade post-FB or the treatment of root pruning with shade post-FB. Root pruning alone did, however, have a highly significant increase in pH over the sampling period.

Figure 41: Mean pH of treatments one to five weeks after 100% veraison in Season Two.
There were no significant differences among treatments in the pH values of the berry juice in Season Three (Table 20).

3.3.4.9 Malic acid

In Season One regression analyses showed that linear trend lines fitted to the data were significantly different among treatments ($P<0.0001$). Treatments that were root pruned in Season One tended to have a lower malic acid concentration in the berry juice in Season One (Figure 42).

![Figure 42: Malic acid concentration (mg l$^{-1}$) of treatments from 100% veraison to six weeks after in Season One.](image)

In Season Two, malic acid concentration was significantly different among treatments, two ($P=0.0041$) and three ($P=0.0089$) weeks after 100% veraison. Vines that were root pruned in Season One continued to have a lower malic acid concentration in Season Two compared to all other treatments (Figure 43). This was significantly different from the control ($P=0.0089$) and all other treatments ($P\leq0.0007 - 0.0712$) two weeks after 100% veraison.
For all treatments sampled, except for the treatment of root pruning with shade post-FB, there was a highly significant decrease in malic acid in the berry juice over time. Although not significant, there was still a decline between the two sample dates for the treatment of root pruning with shade post-FB.

There was a significant difference ($P=0.0362$) among treatments in Season Three for malic acid concentration (Table 20). As in both Season One and Two, root pruning alone had significantly less malic acid in the juice sample than the control and most other treatments. There were no other significant differences among treatments.

3.3.4.10 Tartaric acid

In Season One regression analyses showed that linear trend lines fitted to the data were significantly different among treatments ($P<0.0001$). Treatments with root pruning, and at times heading back, tended to reduce tartaric acid concentration compared to the control (Figure 44). Generally the control had one of the higher tartaric acid concentrations in the berry juice compared to all other treatments. It was difficult to determine any other trends due to the lack of replication.
Chapter Three - Field trial: vigour and light effects on BSN. Results

Figure 44: Tartaric acid concentration (mg l⁻¹) of treatments from 100% veraison to six weeks after in Season One.

There were no significant differences in berry tartaric acid concentrations among treatments in Season Two. In relation to each other, root pruning and Extenday™ did tend towards the higher concentrations of tartaric acid and heading back tended to have lower concentrations (Figure 45).

Most treatments had a significant reduction in tartaric acid concentration over the sampling period. The reduction was not significant for the treatments of shade pre-FB and root pruned with shade post-FB.

In Season Three there was a significant difference ($P=0.0004$) among treatments (Table 20). Root pruning significantly decreased berry tartaric acid concentration compared to the control ($P=0.0017$) and all other treatments ($P<0.001 - 0.0604$). The Extenday™ treatment significantly increased the tartaric acid concentration even though this treatment was not reapplied in Season Three. Extenday™ increased tartaric acid concentration compared to the control ($P=0.0234$) and all other treatments ($P<0.0001 - 0.0021$).
3.3.5 Gas exchange

3.3.5.1 Season One

Statistically there were no significant differences among treatments for any values obtained from the light response curves in Season One (data not presented).

3.3.5.2 Season Two

At flowering in Season Two there were significant differences among treatments for transpiration rate \((P=0.0055)\) and stomatal conductance \((P=0.0081)\), but not for photosynthetic rate or internal CO\(_2\) concentration (Table 21).

The application of Extenday\textsuperscript{TM} resulted in a reduced transpiration rate and lower stomatal conductance of the vines. For both measurements, when Extenday\textsuperscript{TM} was applied as a main treatment it was not significantly different from the control (Table 21). It was significantly different from the treatment of heading back for both transpiration rate \((P=0.0021)\) and stomatal conductance \((P=0.0324)\) and, for stomatal
conductance, also from the treatment of root pruning \((P=0.0114)\). When Extenday\textsuperscript{TM} was applied with other main treatments (of root pruning and heading back), the interactions were only significantly different from heading back alone for transpiration rate \((P=0.0001)\), but with stomatal conductance the interaction treatments were significantly different from both main treatments heading back \((P=0.0098)\) and root pruning \((P=0.0052)\).

At the second measurement two weeks later there was no significant difference among the main treatments for transpiration rate, photosynthetic rate, stomatal conductance or internal CO\textsubscript{2} concentration (Table 21).

Four weeks later, at the third measurement prior to veraison, there were significant differences among main treatments for stomatal conductance only \((P=0.0479)\) (Table 21). The Extenday\textsuperscript{TM} treatment significantly increased stomatal conductance compared to all other treatments except heading back \((P\leq 0.0057 - 0.0774)\). Heading back also increased stomatal conductance but only significantly when compared to the shade pre-FB treatment \((P=0.0350)\).
### Chapter Three - Field trial: vigour and light effects on BSN. Results

Table 21: Means of gas exchange measurements carried out on field grown vines in Season Two. Measurement times are 1) full bloom, 2) two weeks post-FB, 3) pre-veraison. Means shown are least squares means due to unbalanced data sets.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Transpiration rate ($\mu$molm$^{-2}$s$^{-1}$)</th>
<th>Photosynthetic rate ($\mu$molm$^{-2}$s$^{-1}$)</th>
<th>Stomatal conductance ($\mu$molm$^{-2}$s$^{-1}$)</th>
<th>Internal CO$_2$ concentration ($\mu$mol m$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measurement 1 2 3</td>
<td>1 2 3</td>
<td>1 2 3</td>
<td>1 2 3</td>
</tr>
<tr>
<td>Control</td>
<td>2.0 ab</td>
<td>2.7 3.8</td>
<td>9.5 11.6 15.5</td>
<td>286 a</td>
</tr>
<tr>
<td>Root pruned</td>
<td>2.0 ab</td>
<td>2.6 3.5</td>
<td>9.0 10.3 15.7</td>
<td>309 a</td>
</tr>
<tr>
<td>Root pruned/Extenday$^\text{TM}$</td>
<td>1.8 m</td>
<td>8.9</td>
<td>231</td>
<td>m</td>
</tr>
<tr>
<td>Root pruned/shade pre-FB</td>
<td>2.0 m</td>
<td>7.8</td>
<td>268</td>
<td>no</td>
</tr>
<tr>
<td>Root pruned/shade post-FB</td>
<td>1.8 m</td>
<td>9.0</td>
<td>258</td>
<td>no</td>
</tr>
<tr>
<td>Heading back</td>
<td>2.1 a</td>
<td>2.8 3.8</td>
<td>9.6 13.7 16.0</td>
<td>295 a</td>
</tr>
<tr>
<td>Heading back/Extenday$^\text{TM}$</td>
<td>1.7 z</td>
<td>9.4</td>
<td>227</td>
<td>y</td>
</tr>
<tr>
<td>Heading back/shade pre-FB</td>
<td>1.9 yz</td>
<td>9.7</td>
<td>276</td>
<td>x</td>
</tr>
<tr>
<td>Heading back/shade post-FB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extenday$^\text{TM}$</td>
<td>1.8 b</td>
<td>2.6 3.6</td>
<td>8.4 10.8 17.2</td>
<td>247 a</td>
</tr>
<tr>
<td>Shade pre-FB</td>
<td>2.0 a</td>
<td>3.5</td>
<td>10.0</td>
<td>15.4</td>
</tr>
<tr>
<td>Shade post-FB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means in a column with a different letter are significantly different from each other at $P=0.05$ (LSMeans, SAS). * abc – main treatments, mno – comparisons of root pruning with main treatments, xyz – comparisons of heading back with main treatments.
3.3.6 Harvest

3.3.6.1 Bunch weight

In both Seasons One and Two there was a significant difference among treatments for bunch weight ($P=0.0026$ and $0.0202$, respectively). None of the main treatments alone were significantly different from each other or the control in Season One but in Season Two there was a difference between the main treatments and the control (Table 22).

In general, in Season One, root pruning reduced the weight of the bunch. This included root pruning combined with other treatments, except where root pruning was combined with Extenda/™ (Table 22). In this situation, the treatment combination resulted in bunches that were heavier than bunches from both the root pruning treatment alone ($P=0.0296$) and, although not significantly different, the Extenda/™ treatment alone and the control.

In Season Two, the mean bunch weight for the treatment of root pruning alone was only significantly different from the treatment of heading back alone ($P=0.0440$) where bunches were lighter in the root pruned treatment (Table 22). Of the treatment combinations with root pruning, only the combination of root pruning with shade post-FB significantly affected the bunch weight. This was significantly different compared to root pruning alone ($P=0.0475$) and shade post-FB alone ($P=0.0420$). Overall, when root pruned treated vines were compared to vines without root pruning, there was an overall significantly negative affect on bunch weight in both Seasons One ($P=0.0383$) and Two ($P=0.0449$).

Heading back treated vines did not have significantly different bunch weights compared to the control in Season One, but in Season Two they were significantly heavier ($P=0.0061$) (Table 22). In Season Two they were also significantly heavier than bunches from vines that were treated with shade post-FB alone ($P=0.0136$). Vines from the treatment combination of heading back with shade post-FB also had significantly lighter bunches than bunches from vines treated with heading back alone ($P=0.0100$).
### Table 22: Harvest measurements carried out on field grown vines in Seasons One and Two.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Season</th>
<th>Bunch weight (g)</th>
<th>Bunch length (cm)</th>
<th>Bunch width (cm)</th>
<th>Peduncle diameter (mm)</th>
<th>Total number of berries</th>
<th>Mean healthy berry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One</td>
<td>Two</td>
<td>One</td>
<td>Two</td>
<td>One</td>
<td>Two</td>
<td></td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td>142 a</td>
<td>152 b</td>
<td>13.0</td>
<td>14.1</td>
<td>8.7</td>
<td>8.2</td>
<td>131</td>
</tr>
<tr>
<td></td>
<td>mn</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>no</td>
<td>1.08 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.32</td>
</tr>
<tr>
<td><strong>Root pruned</strong></td>
<td>129 a</td>
<td>166 b</td>
<td>12.1</td>
<td>14.5</td>
<td>8.5</td>
<td>8.9</td>
<td>136</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.90 b</td>
</tr>
<tr>
<td><strong>Root pruned/Extenday™</strong></td>
<td>160</td>
<td>166 n</td>
<td>13.2</td>
<td>13.7</td>
<td>9.5</td>
<td>8.6</td>
<td>139</td>
</tr>
<tr>
<td></td>
<td>m</td>
<td>n</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.08 m</td>
</tr>
<tr>
<td><strong>Root pruned/shade pre-FB</strong></td>
<td>128</td>
<td>158 no</td>
<td>12.7</td>
<td>14.0</td>
<td>8.9</td>
<td>8.5</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.98 no</td>
</tr>
<tr>
<td><strong>Root pruned/shade post-FB</strong></td>
<td>113</td>
<td>123 o</td>
<td>12.7</td>
<td>13.2</td>
<td>8.3</td>
<td>7.8</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td>o</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.02 mn</td>
</tr>
<tr>
<td><strong>Root pruned/heading Back</strong></td>
<td>125</td>
<td>183 mn</td>
<td>11.7</td>
<td>13.9</td>
<td>8.5</td>
<td>9.0</td>
<td>127</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.97 no</td>
</tr>
<tr>
<td><strong>Heading back</strong></td>
<td>139</td>
<td>210 a</td>
<td>12.9</td>
<td>15.5</td>
<td>9.0</td>
<td>9.6</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>m</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.09 a</td>
</tr>
<tr>
<td><strong>Heading back/Extenday™</strong></td>
<td>181</td>
<td>186 xy</td>
<td>13.5</td>
<td>14.1</td>
<td>9.9</td>
<td>8.8</td>
<td>148</td>
</tr>
<tr>
<td></td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.21 x</td>
</tr>
<tr>
<td><strong>Heading back/shade pre-FB</strong></td>
<td>142</td>
<td>187 xy</td>
<td>13.4</td>
<td>15.2</td>
<td>8.7</td>
<td>9.3</td>
<td>127</td>
</tr>
<tr>
<td></td>
<td>y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.07 y</td>
</tr>
<tr>
<td><strong>Heading back/shade post-FB</strong></td>
<td>143</td>
<td>156 y</td>
<td>13.1</td>
<td>15.0</td>
<td>9.0</td>
<td>8.6</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.17 y</td>
</tr>
<tr>
<td><strong>Extenday™</strong></td>
<td>139</td>
<td>185 ab</td>
<td>12.3</td>
<td>14.2</td>
<td>8.8</td>
<td>9.0</td>
<td>118</td>
</tr>
<tr>
<td></td>
<td>m</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.15 a</td>
</tr>
<tr>
<td><strong>Shade pre-FB</strong></td>
<td>148</td>
<td>178 ab</td>
<td>13.2</td>
<td>14.9</td>
<td>9.0</td>
<td>8.9</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.16 a</td>
</tr>
<tr>
<td><strong>Shade post-FB</strong></td>
<td>150</td>
<td>165 h</td>
<td>12.9</td>
<td>14.5</td>
<td>9.1</td>
<td>11.2</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>m</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.18 a</td>
</tr>
</tbody>
</table>

* Means in a column with the same letter are not significantly different from each other at P=0.05 (LSMeans, SAS). * abc - main treatments, mno - root pruned with main treatments, xyz - heading back with main treatments.
The use of Extenda\textsuperscript{TM} alone did not significantly affect bunch weight compared to the other main treatments in either Season One or Season Two (Table 22). However, in Season One when Extenda\textsuperscript{TM} was combined with either root pruning or heading back as a treatment, it did significantly increase bunch weight compared to root pruning and heading back alone ($P=0.0296$ and $0.0065$, respectively) (Table 22). In the case of the treatment combination of heading back with Extenda\textsuperscript{TM}, bunch weight was also significantly greater than the control ($P=0.0106$). Consequently in Season One, when the treatments with Extenda\textsuperscript{TM} were compared to treatments without Extenda\textsuperscript{TM}, the treatments with Extenda\textsuperscript{TM} significantly increased the weight of bunches ($P=0.0070$) (Table 22).

The treatment combination of root pruning with shade post-FB significantly reduced the bunch weight compared to the control in Season One ($P=0.0360$) and shade post-FB alone in both Seasons One ($P=0.0123$) and Two ($P=0.0233$) (Table 22).

### 3.3.6.2 Bunch length

No main treatments or treatment combinations significantly affected the length of the bunches in either Seasons One or Two (Table 22). However, in Season Two overall, when all the vines that were root pruned were compared to vines that were not root pruned, there was a significant reduction in bunch length as a consequence of root pruning ($P=0.0300$).

### 3.3.6.3 Bunch width

There were no significant differences in the width of the bunches due to any treatment combination in both Seasons One and Two (Table 22). However, there was a trend for treatments with Extenda\textsuperscript{TM} to increase the width of the bunch, and when the treatments with Extenda\textsuperscript{TM} were compared to the treatments without Extenda\textsuperscript{TM} there was a significant increase in width ($P=0.0208$) as a consequence of the Extenda\textsuperscript{TM} treatment (Table 22).
3.3.6.4 Peduncle diameter

There was a significant difference in the diameter of the peduncle due to treatments in both Seasons One and Two ($P<0.0001$) (Table 22). Although peduncles from root pruned vines tended to be smaller than the control the difference was not statistically significant, but peduncle diameter was significantly less for root pruned vines compared to vines that had been headed back ($P=0.0001$) in both Seasons One and Two (Table 22). In Season One, root pruned vines also had significantly smaller peduncle diameters than Extenday™ treated vines ($P=0.0001$) (Table 22). In Seasons One and Two, treatment combinations with root pruning also reduced peduncle diameter compared to the main treatments of heading back ($P=0.0004$ and 0.0094, respectively), and shade post-FB alone ($P=0.0240$ and 0.0040, respectively), Extenday™ alone in only Season One ($P=0.0653$) and shade pre-FB only in Season Two ($P=0.0445$) (Table 22). In Season One only the vines in the treatment combination of root pruning with Extenday™ had a significantly different peduncle diameter compared to vines in the root pruning only treatment (Table 22). Overall, when vines that were root pruned were compared to vines that were not root pruned, root pruning significantly reduced the diameter of the peduncle in both Seasons One ($P<0.0001$) and Two ($P=0.0002$).

In both Seasons One and Two heading back significantly increased peduncle diameter compared to the control ($P=0.0148$ and 0.0001, respectively) and the treatments of shade pre-FB ($P=0.0436$ and 0.0132, respectively) and shade post-FB ($P=0.0777$ and 0.0054, respectively) (Table 22). In Season Two, heading back also increased peduncle diameter compared to the treatment of Extenday™ ($P=0.0044$). Peduncle diameter in the treatment combinations with Extenday™, shade pre-FB and shade post-FB were not significantly different from the main treatments alone in Season One, but in Season Two they were ($P=0.0017$, 0.0017 and 0.0177, respectively), although in both seasons they were not significantly different from heading back alone (Table 22). Overall, when vines that were headed back were compared to vines that were not, there was a significantly positive treatment affect of heading back on peduncle diameter in both Seasons One ($P=0.0128$) and Two ($P<0.0001$).
Extendal M did significantly increase peduncle diameter compared to the control ($P=0.0148$) and the treatments of shade pre-FB ($P=0.0398$) and shade post-FB ($P=0.0715$) in Season One (Table 22). Overall, when vines with Extendal M were compared with vines without Extendal M in Season One, there was a significant treatment affect ($P=0.0005$). There were no significant differences in peduncle diameter between the two shade treatments in either season (Table 22).

### 3.3.6.5 Total number of berries per bunch

There were no significant differences in the total number of berries per bunch due to any of the treatments or treatment combinations in Season One although in Season Two there was ($P=0.0487$) (Table 22). Root pruning did not significantly affect the total number of berries per bunch compared to any other main treatments (Table 22). The treatment combination of root pruning with shade post-FB did however significantly reduce the number of berries per bunch compared to root pruning alone ($P=0.0420$).

Only in Season Two did heading back significantly increase the number of berries per bunch compared to the control ($P=0.0090$), and the treatments shade pre-FB ($P=0.0294$) and shade post-FB ($P=0.0574$). The treatment combination of heading back with shade post-FB was also significantly different from heading back alone ($P=0.0182$) in Season Two, but not compared to shade post-FB alone (Table 22).

Although shade post-FB did not significantly change the total number of berries compared to the control (Table 22), when vines that had been treated with shade post-FB were compared to vines that had not, overall shade post-FB significantly reduced the number of berries per bunch in both Seasons One ($P=0.0510$) and Two ($P=0.0329$).

### 3.3.6.6 Mean healthy berry weight

There was a significant difference in the mean healthy berry weight among treatments in Season One ($P<0.0001$). However, in Season Two there was no significant difference in mean healthy berry weight due to any treatments (Table 22).
In Season One, root pruning significantly reduced the mean healthy berry weight compared to all other main treatments and the control ($P<0.0001 - 0.0002$). When combined with the treatments of heading back, shade pre-FB and shade post-FB, root pruning significantly reduced the mean berry weight compared to those main treatments alone ($P=0.0129$, 0.0003 and 0.0144, respectively) (Table 22). The treatment combinations of root pruning with Extenda™ ($P=0.0003$) and root pruning with shade post-FB ($P=0.0037$), had significantly higher mean healthy berry weight compared to the treatment of root pruning alone (Table 22). When vines that had been root pruned were compared to vines that had not, there was an overall significant reduction in berry weight due to root pruning ($P<0.0001$). Although as an individual treatment, root pruning did not significantly affect the mean berry weight compared to the control in Season Two, overall there was a significant reduction due to root pruning on mean healthy berry weight ($P=0.00662$).

Heading back alone did not significantly affect the mean healthy berry weight compared to the control or any other main treatments not already mentioned, in either season (Table 22). In Season One however, the treatment combination of heading back with Extenda™ had significantly higher mean healthy berry weight compared to heading back alone ($P=0.0339$) and the control ($P=0.0171$) (Table 22).

In Season One, when the vines that had been treated with Extenda™, shade pre-FB or shade post-FB were compared with vines that were not treated with those treatments, there was a significant overall effect on mean healthy berry weight ($P<0.0001$, $P=0.0661$ and $P=0.0005$, respectively).

### 3.3.7 Seed number and seed weight

There were no significant differences in mean seed number per berry or mean seed weight among any of the main treatments and treatment combinations (Table 23).
Table 23: Mean seed number per berry and mean seed weight for ‘Cabernet Sauvignon’ vines in Season Two (2003/2004).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean seed number per berry</th>
<th>Mean seed weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.22</td>
<td>42</td>
</tr>
<tr>
<td>Root pruned</td>
<td>1.22</td>
<td>43</td>
</tr>
<tr>
<td>Root pruned/Extenda™</td>
<td>1.23</td>
<td>41</td>
</tr>
<tr>
<td>Root pruned/shade pre-FB</td>
<td>1.30</td>
<td>39</td>
</tr>
<tr>
<td>Root pruned/shade post-FB</td>
<td>1.29</td>
<td>39</td>
</tr>
<tr>
<td>Root pruned/heading Back</td>
<td>1.40</td>
<td>38</td>
</tr>
<tr>
<td>Heading back</td>
<td>1.30</td>
<td>41</td>
</tr>
<tr>
<td>Heading back/Extenda™</td>
<td>1.23</td>
<td>43</td>
</tr>
<tr>
<td>Heading back/shade pre-FB</td>
<td>1.27</td>
<td>40</td>
</tr>
<tr>
<td>Heading back/shade post-FB</td>
<td>1.26</td>
<td>41</td>
</tr>
<tr>
<td>Extenda™</td>
<td>1.30</td>
<td>43</td>
</tr>
<tr>
<td>Shade pre-FB</td>
<td>1.31</td>
<td>43</td>
</tr>
<tr>
<td>Shade post-FB</td>
<td>1.29</td>
<td>40</td>
</tr>
<tr>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

*ns – means not significantly different at 5%

### 3.3.8 Bunch stem necrosis

#### 3.3.8.1 Season One

The incidence and severity of BSN was only measured twice between veraison and harvest in Season One. Due to the nature of the data from the first assessment, assumptions of normality and heterogeneity of variance were not met and none of the transformations accessed were able to adjust the raw data to meet these assumptions. Normal ANOVA analysis was therefore not possible. Comparisons were limited to non-parametric tests such as Friedman’s test for two-way designs. However, the second measurement did meet all assumptions and therefore multiple comparisons were able to be carried out.

By the time of the first assessment there was already a highly significant \((P<0.0001)\) difference among treatments for BSN incidence. All treatments with root pruning had
dramatically less BSN than the control and most of the other treatments (Table 24). This was still evident at the second assessment where the treatment of root pruning alone had significantly less BSN incidence than the control vines ($P=0.0019$) and all other main treatments ($P=0.0003 - 0.0369$). When root pruning was applied with heading back or shade post-FB, absolute BSN incidence tended to be slightly less than where root pruning was applied alone, but there was no significant difference between these interaction treatments and root pruning alone (Table 24). The interaction treatments were significantly different from heading back alone ($P=0.0506$) and shade post-FB alone ($P=0.0002$). The interaction treatments of root pruning with Extenda$^{TM}$ and root pruning with shade pre-FB had slightly higher BSN incidence than root pruning alone, although again not significantly different. The treatment of root pruning with Extenda$^{TM}$ had significantly less BSN incidence than Extenda$^{TM}$ alone ($P=0.0034$). When all treatments with root pruning were compared to treatments without root pruning the reduction in BSN was highly significant ($P<0.0001$).

Root pruning alone reduced absolute BSN incidence by 9% compared to the control at the second assessment. Adjusting for days after 50% veraison or standard maturity resulted in a similar absolute BSN incidence decrease of 8% compared to the control (Figure 46). The BSN incidence for interaction treatments with root pruning also tended to remain independent of the adjustments based on either time of development or maturity (Figure 46).
Chapter Three - Field trial: vigour and light effects on BSN. Results

Table 24: Unadjusted BSN scores (%) for the two assessments in Season One.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>13 March 2003</th>
<th>26 March 2003**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.5</td>
<td>11.4 a*</td>
</tr>
<tr>
<td>Root pruned</td>
<td>0.2</td>
<td>2.3 c</td>
</tr>
<tr>
<td>Root pruned/Extenday™</td>
<td>0.3</td>
<td>3.1 m</td>
</tr>
<tr>
<td>Root pruned/shade pre-FB</td>
<td>0.3</td>
<td>3.8 m</td>
</tr>
<tr>
<td>Root pruned/shade post-FB</td>
<td>0.1</td>
<td>1.0 m</td>
</tr>
<tr>
<td>Root pruned/heading Back</td>
<td>0.1</td>
<td>1.6 m</td>
</tr>
<tr>
<td>Heading back</td>
<td>1.0</td>
<td>6.4 b</td>
</tr>
<tr>
<td>Heading back/Extenday™</td>
<td>2.0</td>
<td>16.9 y</td>
</tr>
<tr>
<td>Heading back/shade pre-FB</td>
<td>1.2</td>
<td>8.0 xz</td>
</tr>
<tr>
<td>Heading back/shade post-FB</td>
<td>1.1</td>
<td>8.9 xz</td>
</tr>
<tr>
<td>Extenday™</td>
<td>1.6</td>
<td>13.8 a y</td>
</tr>
<tr>
<td>Shade pre-FB</td>
<td>0.3</td>
<td>6.5 b m</td>
</tr>
<tr>
<td>Shade post-FB</td>
<td>1.7</td>
<td>10.3 a n y</td>
</tr>
</tbody>
</table>

* Means within a column with a different letter are significantly different from each other at P=0.15 (LSMeans, SAS). abc - main treatments, mno - comparisons of interaction treatments that included root pruning with respective main treatments, xyz - comparisons of interaction treatments that included heading back with respective main treatments.

** Harvest occurred on 1 April 2003.

Heading back tended to significantly reduce actual BSN incidence (Table 24), compared to the control (P=0.0471) at the second assessment. At that point the heading back treatment had approximately 5% less actual BSN incidence than the control. When all heading back treatments were compared to those without heading back, there was a significant reduction in BSN incidence. When adjusted for days after 50% veraison and maturity, as with root pruning, adjusted scores had a similar BSN incidence reduction from the control as the raw data (Figure 46).
The interaction treatment of heading back with Extenday™ was significantly different from heading back alone ($P=0.0001$) and the control ($P=0.0422$) and was the only other interaction treatment to be so. It resulted in the highest BSN score of all treatments of 16.9% (when comparing treatments using the raw, unadjusted data; Table 24). When adjusted data were used however, this treatment was almost identical to the control (Figure 46). Actual BSN incidence for heading back with Extenday™ was 6% more than the control when using the raw data, but this difference disappeared for veraison and maturity adjusted means (Figure 46). The incidence of BSN for the treatment of heading back with Extenday™ was not significantly different from the treatment of Extenday™ alone when comparing the raw, unadjusted data.

Raw data indicated that Extenday™ alone was not significantly different from the control at the second assessment, although there did appear to be a slight increase in actual BSN incidence of nearly 2.5% (Table 24). Adjusting for days after 50% veraison resulted in there being a 3% decline in actual BSN incidence when Extenday™ was
applied (Figure 46). Adjusting for maturity however, showed no difference compared to the control (Figure 46).

Shade post-FB was also not significantly different from the control when analysing the raw unadjusted data (Table 24). This is obvious by there being only around 0.5% difference between means. This did not change for the maturity adjusted data (Figure 46).

In contrast, shade pre-FB did significantly reduce BSN incidence compared to the control ($P=0.0516$). This trend could also be seen at the first measurement (Table 24) and was apparent with unadjusted and adjusted data. The decrease in actual BSN incidence ranged between 3 and 4% (Figure 46).

### 3.3.8.2 Season Two

BSN incidence was considerably higher in Season Two than in Season One and the development of BSN increased progressively for all treatments between 100% veraison and harvest (Figure 47). Initial development tended to be slow except for the shade post-FB treatment. Incidence increased sharply for most treatments at around three weeks after 100% veraison. The application of shade pre-FB tended to delay the rapid increase in BSN incidence as did root pruning (that had been carried out in the previous year). This resulted in bunches in the root pruning with shade pre-FB treatment never exhibiting a rapid increase in BSN while assessments were being taken through until harvest. The shade post-FB treatment tended to promote a rapid onset of BSN.
Due to the nature of the data for the first two assessments, Friedman’s test was carried out on the data in order to determine if there were significant treatment differences. There was a significant difference among treatments at the first assessment on 2 March. By the second assessment on 9 March this difference had become highly significant and remained so for the following three assessments of BSN incidence where multiple comparison procedures were able to be carried out.

Root pruning tended to reduce BSN incidence compared to the control at the first two measurements (Table 25). This trend continued through to harvest for root pruning and was significantly different from the control, but only at the 15% level for the last two measurements (P=0.0434, 0.1303 and 0.1141 for the respective assessments). When root pruning was adjusted for days after 50% veraison and for standard maturity, the reduction in actual BSN incidence from the control due to root pruning increased from 4.5% for unadjusted data, to 8% and 10%, respectively (Figure 48).
Table 25: BSN incidence (%) for all treatments in Season Two.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.6</td>
<td>0.9</td>
<td>4.8 b*</td>
<td>6.0 b</td>
<td>14.5 bc</td>
</tr>
<tr>
<td>Root pruned</td>
<td>0.0</td>
<td>0.0</td>
<td>0.9 c</td>
<td>2.1 c</td>
<td>9.8 d</td>
</tr>
<tr>
<td>Root pruned/Extenda™</td>
<td>0.6</td>
<td>1.3</td>
<td>4.7 m</td>
<td>7.5 o</td>
<td>17.0 n</td>
</tr>
<tr>
<td>Root pruned/shade pre-FB</td>
<td>0.0</td>
<td>0.3</td>
<td>1.2 n</td>
<td>1.2 p</td>
<td>2.1 p</td>
</tr>
<tr>
<td>Root pruned/shade post-FB</td>
<td>1.0</td>
<td>1.8</td>
<td>3.9 m</td>
<td>9.1 o</td>
<td>16.4 n</td>
</tr>
<tr>
<td>Root pruned/heading back</td>
<td>0.0</td>
<td>0.0</td>
<td>1.1 n</td>
<td>6.5 o</td>
<td>14.4 n</td>
</tr>
<tr>
<td>Heading back</td>
<td>0.1</td>
<td>0.3</td>
<td>2.9 b</td>
<td>7.5 b</td>
<td>16.6 bc</td>
</tr>
<tr>
<td>Heading back/Extenda™</td>
<td>0.4</td>
<td>2.1</td>
<td>5.0 xy</td>
<td>10.6 yz</td>
<td>19.6 y</td>
</tr>
<tr>
<td>Heading back/shade pre-FB</td>
<td>0.0</td>
<td>0.1</td>
<td>3.1 y</td>
<td>4.4 yz</td>
<td>9.6 z</td>
</tr>
<tr>
<td>Heading back/shade post-FB</td>
<td>1.7</td>
<td>3.4</td>
<td>6.2 x</td>
<td>13.6 x</td>
<td>21.0 xz</td>
</tr>
<tr>
<td>Extenda™</td>
<td>1.1</td>
<td>2.4</td>
<td>5.6 b</td>
<td>9.9 a</td>
<td>18.1 b</td>
</tr>
<tr>
<td>Shade pre-FB</td>
<td>0.0</td>
<td>2.1</td>
<td>4.1 b</td>
<td>5.1 b</td>
<td>10.0 cd</td>
</tr>
<tr>
<td>Shade post-FB</td>
<td>0.8</td>
<td>3.8</td>
<td>12.4 a</td>
<td>17.0 a</td>
<td>27.7 a</td>
</tr>
</tbody>
</table>

Means within a column with a different letter are significantly different from each other at $P=0.15$ (LSMeans, SAS). *abc - main treatments, mno - comparisons of interaction treatments that included root pruning with respective main treatments, xyz - comparisons of interaction treatments that included heading back with respective main treatments.

** Harvest occurred on 1 April 2004.
When using the raw unadjusted data, interaction treatments that included root pruning decreased BSN incidence from the main treatments alone, although not significantly at the 15% level for Extenda™ and heading back (Table 25). Therefore, when all treatments with root pruning were compared against those without root pruning across the last three assessments, there was a significant decrease ($P=0.0004 - 0.0034$) in BSN incidence due to root pruning. As some of the other main treatments tended to increase BSN incidence compared to the control, not all interaction treatments with root pruning were significantly different from the control, or even less than the control (Table 25). For example, at the fourth assessment date, Extenda™ increased BSN incidence compared to the control, and the interaction treatment of root pruning with Extenda™ had a higher incidence of BSN than root pruning alone and the control, though less than Extenda™ alone.

The incidence of BSN was not affected by the treatment of heading back compared to the control (Table 25). There was also no significant difference in BSN incidence when all treatments with heading back were compared to all treatments without heading back over the last three assessments. Heading back appeared to respond additively with root pruning and with Extenda™. However, with the two shade treatments, heading back did not respond additively as shade post-FB and heading back both increased BSN incidence (in the last two assessments), whereas BSN incidence with heading back combined with shade post-FB was not more than shade post-FB alone (Figure 48).

When adjusted for days after 50% veraison and for standard maturity there were no consistent changes in trends for heading back. Adjusting for veraison resulted in a similar increase to the raw data (around 2-3%) in actual BSN incidence due to heading back. However, when adjusting for maturity there was no difference in BSN incidence between heading back and control vines (Figure 48).

Extenda™ and shade post-FB increased BSN incidence compared to the control (Table 25) and this was significantly different from the control for the Extenda™ treatment for the fourth assessment ($P=0.0832$) and for the last three assessments for the shade post-FB treatment ($P=0.0017-0.0203$).
Figure 48: Raw, veraison adjusted and standard maturity adjusted BSN incidence (%) of all treatments in Season Two at harvest.

* adjustment was unable to be made due to juice analyses not being carried out on these treatments.

When used in an interaction with either heading back or root pruning, both Extenday™ and shade post-FB increased BSN incidence compared to heading back or root pruning alone (Table 25). This was significant for both the Extenday™ and shade post-FB interaction treatments compared to root pruning alone at all assessments (Extenday™ interaction $P=0.0255 - 0.0685$, shade post interaction $P=0.0047 - 0.1148$), and significantly different compared to heading back alone at both the third and fourth assessments for the shade post-FB interaction ($P=0.1297$ and $0.1381$, respectively). For Extenday™, there was a significant increase in BSN incidence when all treatments with Extenday™ were compared with all treatments without Extenday™ at the third and fourth assessments ($P=0.0798$ and $0.0424$, respectively). For shade post-FB, there was a significant increase in BSN incidence at all assessment times when all treatments that included shade post-FB were compared with all treatments without shade post-FB ($P\leq 0.0001 - 0.0113$).
When BSN incidence for shade post-FB was adjusted for days after 50% veraison and for standard maturity, the increase compared to the control did not change (Figure 48). In contrast, adjusting the Extenday™ values for days after 50% veraison led to there being no difference from the control, whereas adjusting for standard maturity resulted in there being a 10% actual increase in BSN incidence compared to the control for the Extenday™ treated vines (Figure 48).

Shade pre-FB tended to reduce BSN incidence at all except the second assessment but these differences were not significantly different from the control values (Table 25). The interaction treatments with shade pre-FB and either root pruning or heading back were only significantly lower compared to either root pruning or heading back alone at the final assessment ($P=0.0733$ and $P=0.0887$, respectively). Hence there was a significant difference when comparing all treatments with shade pre-FB with those without shade pre-FB in the last assessment ($P=0.0135$).

When scores for BSN incidence for shade pre-FB were adjusted for days after 50% veraison and standard maturity, there was no change in the difference between the shade pre-FB values and those of the control. The shade pre-FB treatment reduced BSN by 4% in all three cases (Figure 48).

### 3.3.8.3 Season Three

The progression of BSN incidence from 100% veraison to one week before harvest showed an initial sharp increase in incidence for most treatments, a plateau between the second and third assessment (coinciding with cold and very wet conditions) and a sharp increase in BSN incidence beginning close to the fourth assessment (which was four weeks after 100% veraison) (Figure 49). Those treatments with root pruning, except root pruning with shade post-FB and root pruning with Extenday™, showed a slower rate of increase over the final period through to harvest.
Figure 49: Progression of BSN incidence of all treatments in Season Three from 100% veraison to harvest.

As the first three measurements were un-transformable and did not meet the assumptions required for an ANOVA, Friedman’s non-parametric test was carried out on these data. There was no significant difference among treatments for the first two assessments. However, there was a significant difference between treatments at the third assessment, with root pruned vines tending to have a lower BSN incidence than the other treatments and vines that had shade post-FB in the previous season having a higher BSN incidence (Figure 49 and Table 26).

By the time of the fourth assessment, which was four weeks after 100% veraison, there were some significant differences among treatments ($P=0.0928$) and this significance had increased by the time of the final assessment ($P=0.0283$) (Table 26).
Table 26: BSN incidence for Season Three from 100% veraison to one week before commercial harvest.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.8</td>
<td>3.5</td>
<td>7.1</td>
<td>12.2 a</td>
<td>m</td>
</tr>
<tr>
<td>Root pruned</td>
<td>1.4</td>
<td>3.6</td>
<td>4.3</td>
<td>9.0 ab</td>
<td>m</td>
</tr>
<tr>
<td>Root pruned/Extendy™</td>
<td>0.4</td>
<td>5.8</td>
<td>5.9</td>
<td>13.1 m</td>
<td>22.2 mn</td>
</tr>
<tr>
<td>Root pruned/shade pre-FB</td>
<td>1.1</td>
<td>9.3</td>
<td>10.1</td>
<td>13.3 m</td>
<td>24.2 m</td>
</tr>
<tr>
<td>Root pruned/shade post-FB</td>
<td>2.1</td>
<td>3.8</td>
<td>3.6</td>
<td>3.9 m</td>
<td>8.1 m</td>
</tr>
<tr>
<td>Heading back</td>
<td>0.9</td>
<td>3.3</td>
<td>6.7</td>
<td>9.8 ab</td>
<td>n</td>
</tr>
<tr>
<td>Heading back/Extendy™</td>
<td>1.6</td>
<td>5.8</td>
<td>12.0</td>
<td>15.6 xy</td>
<td>25.7 y</td>
</tr>
<tr>
<td>Heading back/shade pre-FB</td>
<td>0.6</td>
<td>3.4</td>
<td>7.0</td>
<td>12.0 y</td>
<td>23.5 x</td>
</tr>
<tr>
<td>Heading back/shade post-FB</td>
<td>1.5</td>
<td>7.4</td>
<td>11.2</td>
<td>19.3 wx</td>
<td>31.9 x</td>
</tr>
<tr>
<td>Extendy™</td>
<td>1.3</td>
<td>1.3</td>
<td>1.9</td>
<td>4.0 m</td>
<td>16.0 m</td>
</tr>
<tr>
<td>Shade pre-FB</td>
<td>1.8</td>
<td>6.6</td>
<td>11.4</td>
<td>14.4 m</td>
<td>22.1 ab</td>
</tr>
<tr>
<td>Shade post-FB</td>
<td>2.0</td>
<td>11.6</td>
<td>12.4</td>
<td>15.6 a</td>
<td>25.9 a</td>
</tr>
</tbody>
</table>

* Means within a column with a different letter are significantly different from each other at P=0.15 (LSMeans, SAS). * abc - main treatments, mno - comparisons of interaction treatments that included root pruning with respective main treatments, xyz - comparisons of interaction treatments that included heading back with respective main treatments. ** Commercial harvest occurred on 13 April 2005.
Chapter Three - Field trial: vigour and light effects on BSN. Results

Vines that were root pruned in Season One had lower BSN incidence than the control but were not significantly different from the control vines until the final assessment ($P=0.0992$) (Table 26). Root pruned vines did have significantly lower BSN incidence compared to the shade post-FB treatment at the fifth assessment ($P=0.1113$). The interaction treatment of root pruning with heading back was the only interaction treatment with root pruning that was significantly different from the control at both the fourth and fifth assessments ($P=0.0900$ and $0.0090$, respectively) (Table 26). Although the interaction treatments of root pruning with Extenday$^{TM}$ and root pruning with shade post-FB were not different from the control at any assessment, overall when all treatments with root pruning were compared to all treatments without root pruning, root pruned vines demonstrated a significantly lower BSN incidence at the final assessment ($P=0.0104$).

When root pruned vines were adjusted for days after 50% veraison, the BSN incidence decreased but not as much as the BSN incidence for the control vines. Hence, while the raw data showed a 10% decrease in BSN due to root pruning compared to the control means, when adjusted for veraison this decrease dropped to a 7% difference (Figure 50). When adjusted for berry maturity, the BSN incidence was again 10% less in root pruned vines compared to control vines, although the adjusted incidence was markedly lower in both treatments.

Heading back alone also had lower BSN incidence but was not significantly different from the control at any assessments (Table 26). The interaction treatment of root pruning with heading back was not significantly different from root pruning alone, although it did have significantly lower BSN incidence compared to heading back alone at the fifth assessment ($P=0.1047$). The interaction treatment of heading back with shade post-FB had significantly higher BSN incidence than the control at the fourth assessment ($P=0.1451$), and although still higher at the fifth assessment it was no longer significantly different (Table 26). For both the fourth and fifth assessment the heading back with shade post-FB interaction treatment was significantly higher than the main treatment of heading back alone ($P=0.0527$ and $0.0269$, respectively).
The 6% decrease in actual BSN incidence for the heading back treatment, compared to the control vines, was reduced to a 3% decrease for veraison adjusted means, and to a 1% difference with the maturity adjusted means (Figure 50).

The Extenda™ treatment had significantly lower BSN incidence than the control at the fourth assessment ($P=0.0924$) but, although the BSN incidence was still lower at the fifth assessment, it was no longer significant. The Extenda™ treatment also had significantly less BSN incidence when compared to the treatments of shade post-FB ($P=0.0182$), and shade pre-FB ($P=0.0926$) at the fourth measurement and a lower incidence compared to shade post-FB at the fifth measurement ($P=0.0616$). When applied in an interaction treatment with either root pruning or heading back, BSN levels rose to be similar to that of the control vines (Table 26).

Extenda™ treated vines originally had an 8% decrease in actual BSN incidence compared to the control using the raw data from the final assessment, but when adjusted for veraison, this became a 6% decrease. When adjusted for maturity there was no difference in BSN incidence from the control (Figure 50).

The main treatment of shade post-FB alone was not significantly different from the control at any assessment (Table 26). Shade post-FB was the only treatment at the fourth assessment to demonstrate a significant difference ($P=0.0439$) when all treatments with shade post-FB were compared to treatments without shade post-FB. By the fifth assessment this difference was more significant ($P=0.0202$).

When the raw data were adjusted for veraison, shade post-FB had a 3% increase in BSN incidence when compared to the control and this increase was increased further when the data were adjusted for maturity, to an 11% increase (Figure 50).

Shade pre-FB showed no significant difference in BSN incidence compared to the control (Table 26). Differences between the shade pre-FB and the control vine values were not significant irrespective of the basis of comparison (Figure 50).
**Figure 50:** Raw, veraison adjusted and standard maturity adjusted BSN incidence (%) of all treatments in Season Three one week prior to commercial harvest. * adjustment was unable to be made due to juice analyses not being carried out on these treatments.

**Season Three root pruned vines.** Vines that were root pruned in Season Three had lower BSN incidence than the control at all assessment times. This was significant at the first, second, fourth and fifth measurements \((P=0.0769, 0.0455, 0.0008\) and 0.0608, respectively) (Table 27). Friedman’s test was carried out on the data from the second assessment due to the data not meeting the required statistical assumptions.

**Table 27:** BSN incidence (%) from vines root pruned in Season Three and Season Three control vines assessed from 100% veraison to one week before commercial harvest.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.8 a</td>
<td>4.8 a</td>
<td>7.5</td>
<td>15 a</td>
<td>26 a</td>
</tr>
<tr>
<td>Root pruned</td>
<td>0.0 b</td>
<td>0.0 b</td>
<td>3.8</td>
<td>4.8 b</td>
<td>10 b</td>
</tr>
</tbody>
</table>

Means within a column with a different letter are significantly different from each other at \(P=0.15\) (LSMeans, SAS). Friedman’s test was carried out for the means on the 10 March 2005.
Chapter Three - Field trial: vigour and light effects on BSN. Results

When these vines were compared with vines that had been root pruned in Season One and with the original control vines, the first two assessments did not meet the required statistical assumptions and therefore Friedman’s test was carried out. No significant differences among treatments were detected.

For the last three assessments, the two control groups were not significantly different from one another (Figure 51). The two root pruned groups were also not significantly different from each other, even though vines root pruned in Season Three tended to have lower BSN incidence than vines root pruned in Season One (Figure 51).

There was no difference in the date of 50% veraison between vines that were root pruned in Season Three and the control vines (20 February 2005) (Figure 16). In Season Three the control group reached 50% veraison between 19 and 20 February 2005, which is close enough for the assumption to be made that there was no difference in veraison between the two control groups. However, vines that were root pruned in Season One, reached 50% veraison on 18 February 2005 but any possible difference among treatments would account for only 1-2 % BSN incidence and no adjustment was considered necessary.

![BSN Incidence Graph](image)

**Figure 51:** BSN incidence (%) from 100% veraison to one week before commercial harvest in Season Three. LSD bars are at 5% significance level.
Season Comparison. The main treatments were adjusted for a standard maturity of 1.8 across all three seasons in order to compare them (Figure 52). Control vines demonstrated a higher incidence of BSN in the second season, as did most other treatments except shade pre-FB where BSN incidence in Season Three was equal to that of Season Two. Season One had the lowest incidence for all treatments although Seasons One and Three were very similar in BSN incidence for the control and the Extenday™ treatment.

![Figure 52: BSN incidence (%) of the main treatments adjusted to a common maturity ratio of 1.8 in three seasons. Note: root pruning was only carried out in Season One. All other treatments were applied only in Seasons One and Two.](Image)
3.4 Discussion

Root pruning dramatically reduced the vigour of vines for the three seasons after it was carried out. Although heading back of the vines reduced the ECN, vines filled the canopy area to the similar degree as control vines and therefore initial vegetative growth must have been very vigorous. There were no measurable differences in vigour of vines treated with Extenda™ but any differences may have been obscured by the trimming that took place throughout the growing season. Shaded vines also did not have any significant difference in vigour compared to the control vines.

The incidence of BSN was dramatically reduced by root pruning in all three seasons that it was measured. Heading back of the vines only tended to increase BSN incidence in the already high vigour season of Season Two. Generally the Extenda™ treatment increased the incidence of BSN. Shade pre-FB reduced BSN incidence in the two seasons that it was applied, while shadepost-FB increased the incidence in Season Two and Three, although it was not applied in Season Three. In Season One the interaction treatment of heading back with shade post-FB increased the incidence of BSN compared to the control vines, and vines that were only headed back, indicating that shade post-FB may have also increased BSN incidence in Season One.

There were no significant correlations between nutrient concentrations in the petiole, rachis or berry and BSN incidence for any of the treatments. Root pruning however did increase quality attributes of the berry juice by generally increasing the Brix concentration, reducing TA and reducing berry size.

3.4.1 Vegetative growth

For the three seasons following root pruning there was a significant reduction in the development of the vine canopy as measured by LLN, PIL and PG (Figures 4 – 9 and Tables 2 – 7). Other studies on grapevines have also found a decrease in vegetative growth due to root pruning (Mika and Krzewinska, 1995; Ferree et al., 1999). Root pruning in Season One was the only treatment for all three seasons that resulted in the
approximate point quadrat values that Smart and Robinson (1991) recommended of LLN 1.0 – 1.5 or less, PG 20 – 40% and PI less than 10%. This treatment therefore lead to better light penetration into the canopy and greater air movement throughout the canopy. Also, the amount of trimming required to control the vines was dramatically reduced and leaf plucking was not required. In Season One, the trimmer removed very little, if any, of the canopy and, although not measured, visually there were very few laterals. Although the actual number of growing points per cane measured in Season Two was not significantly lower in root pruned vines than in the control, as there was extensive trimming throughout the season on the control, it is difficult to ascertain whether or not this was an accurate measurement of the number of growing points. In Season Two, LLN was half that of the control, and PIL was 20% less than that of the control, indicating that lateral growth had been reduced as ECN was not significantly lower than the control but LLN and PIL were. It is probable that the reduction in vine maintenance required resulted in lower production costs.

Hunter (2000) states that laterals have an important role to play in berry growth and there should be 12–13 lateral shoots per main cane and four leaf layers in the canopy. This LLN value is well above what Smart and Robinson (1991) recommend and also well above what this study found to be adequate to produce quality grapes. Also, as only those laterals nearest the bunch export assimilates to the bunch, 12–13 laterals appears to be a very large number of laterals to maintain on a vine. However, laterals may play an important role in bunch development, but this study indicates that a much smaller number would be required than that which Hunter suggests.

It is well known that there is an equilibrium between shoots and roots with the manipulation of either resulting in an increase or decrease in growth to restore the balance (Richards, 1983). The reduction in vine growth due to root pruning was therefore directly related to this phenomenon. There are many ways in which roots and shoots can interact to bring about this balance and a reduction in total water and nutrient uptake are two possibilities where this may occur (Richards, 1983) as both root restriction and root pruning can affect some nutrient concentrations in the leaves (Richards and Rowe, 1977a). The canopy that resulted after root pruning was probably
the size of canopy that was able to be sustained with the water and nutrients provided by the reduced root volume.

It would be expected that as root volume increased over the successive years following root pruning, that the vigour of the shoots would also have increased, but water stress may also have played a role in restricting shoot growth in Seasons One and Three. In Season Two, which was a much wetter year, the vigour of the root pruned vines was much higher than in either Seasons One or Three. Leaf water potential assessments (data not shown) also indicate that in Season One the vines were under more water stress than in Season Two. The Extenda™ treatment did tend to increase vine vigour in both Seasons One and Two but, due to the extensive mechanical trimming carried out in both seasons, it is difficult to determine exactly how much more vigour had been induced. The interaction treatment of Extenda™ combined with root pruning resulted in more vegetative growth than when the vines were root pruned alone, but vines in this interaction treatment were still not as vigorous as the control vines.

Assessments of leaf water potential (data not shown) indicate that Extenda™ vines were also under less water stress than the control vines. Soil moisture assessments (data not shown) also indicate that soil moisture was higher under the Extenda™ mulch, which corresponds with studies showing that mulching decreases soil water loss (Richards, 1983). Many studies have found that a reduction in vegetative growth is correlated with water stress (Redl, 1984b; Stevens et al., 1995). Redl (1984c) found that shaded leaves were less stressed than leaves in full sun, and that shoots further away from the trunk were under more water stress than shoots closer to the trunk. Kliewer et al. (1983) found that shoot elongation was reduced in vines that were under water stress even before leaf pre-dawn water potentials detected such a stress. They also found that although final shoot length in irrigated vines was longer than that of non-irrigated vines, the main increase in foliage density of the irrigated vines was due to lateral shoot growth, indicating that this shoot growth was limited in the water stressed vines. McArtney and Ferree (1999a) were also unable to extract xylem exudates from root pruned vines when they were able to from non-root pruned vines, indicating that root pressure was lower in the root pruned vines. Ferree et al. (1999) found that as the
amount of root pruning increased, stomatal conductance and transpiration decreased and wilted leaves increased, indicating water stressed vines. However, Richards and Rowe (1977b) found that the effects of root treatments, such as root restriction or root pruning, on shoot growth, could not be explained solely in terms of water or nutrient uptake.

Studies indicate that shoot growth is also controlled through hormonal changes in the plant (Richards and Rowe, 1977b; Richards, 1983). It is thought that the decrease in vegetative growth is due to an increase in ABA due to water stress, and the subsequent reaction of the plant to high ABA concentrations (During et al., 1996; Davies et al., 2000 and references therein). Cytokinins and GAs may also be involved as they are thought to be produced to some degree in the root apex (Richards and Rowe, 1977b; Kliwer et al., 1983). There are clear relationships between cytokinins and shoot growth (Richards and Rowe, 1977b; Richards, 1983) - the higher the number of root tips, and therefore the higher the amount of cytokinin production, the higher the number of leaves (Richards, 1983). Roots from plants that have been root pruned may reach their original length again quickly, but the original root number takes longer to attain (Richards and Rowe, 1977a). Therefore, even in the seasons after root pruning, root pruned plants probably have a high ratio of young white roots to older woody roots, but the total number of root tips is probably reduced. Therefore, there would be less cytokinin activity in the plant, thus reducing shoot growth. However, Arnold and Young (1991) did not find a correlation between cytokinin concentration in the xylem and the reduced shoot growth of apples due to root pruning. In Arnold and Young’s (1991) study, root pruning was carried out during the growing season. Cytokinin in the xylem sap in apples has been found to be high before budbreak and to decrease shortly thereafter (Belding and Young, 1989; Young, 1989). Therefore root pruning during the growing period may not affect cytokinin in the xylem whereas root pruning during the dormant stage may. The reduction in shoot growth due to root pruning during the growing season may be due to the effect of other plant growth regulators such as ABA.

In both Season One and Two, even by the first point quadrat assessment, the differences in vegetative growth between the headed back and control vines were minimal (Figures 4 – 9 and Tables 2 – 7). Therefore the initial growth of headed back vines must have
been very vigorous for these vines to fill the same canopy area as the control as they had only half the number of shoots to achieve this vegetative development overall. However, between the first and second assessment (near flowering and four weeks later), the rate of canopy development appeared to be either the same as or less than that of the control, indicating that during this period vines had slowed their growth compared to growth prior to the first assessment. This finding concurs with McArtney and Ferree (1999a) who found on grapevines that the effect of cane number on shoot elongation rate had disappeared by bloom. In the interaction treatment of heading back combined with root pruning, point quadrat assessments indicate that these vines had the same canopy development as vines that were only root pruned. Again, shoot growth would have been faster on vines in this interaction treatment prior to bloom compared to vines that were only root pruned, due to there being fewer shoots. Point quadrat assessments for the interaction treatment of heading back with Extenday™ indicate that vine growth was generally the same as for heading back alone, except for the second assessment in the second season when this interaction treatment had more vegetative growth than heading back alone. This difference had disappeared by the third assessment. As mentioned earlier, due to the trimming that took place during the season, it is difficult to demonstrate that vines with Extenday™ were more vigorous than the control vines.

McArtney and Ferree (1999a) found that the rate of shoot elongation was decreased with an increasing number of shoots per vine. For non-fruiting shoots Stewart et al. (1996) found that the leaf area of shoots on canes pruned to a short dormant length, was the same as that obtained from shoots on canes pruned to a long dormant length. However, for fruiting canes, the leaf area was much larger on shoots from short canes. This is in keeping with the point quadrat results obtained in the current study for vines that were headed back.

Although not significantly different from the control, shaded vines tended to have slightly more vegetation at the last point quadrat measurement in Season One. The effect of shade on grapevine morphology appears to be contradictory in the literature. Schultz and Mathews (1993) found that individual leaf area was not affected by neutral
shade applied over the growing season although there was a trend for shade to decrease leaf area. Specific leaf area was increased and internode length and diameter were decreased. Morgan et al. (1985) also found an increase in specific leaf area although they found a significant decrease in individual leaf area due to a reduction in light quantity over the growing season. Keller and Koblet (1995a) found that individual leaf area was decreased by shade although total leaf area was increased due to the increase in leaf number. In contrast, CarTechini and Palliotti (1995) found that individual leaf area was significantly increased by the application of shade, but total leaf area was reduced. Internode length was increased and internode diameter decreased.

Shading has also been found to influence the development of lateral leaves and shoots. Keller et al. (1998) found that shade around veraison increased the number of lateral leaves and shoots. The increase in lateral shoots was more obvious at lower soil nitrogen availability. Keller and Koblet (1995a) also found an increase in the number of leaves and laterals when shade was applied during bloom. However, CarTechini and Palliotti (1995) found a decrease in axillary shoots per cane due to shade. Smart et al. (1988) also applied shade treatments to grapevines around veraison but found no effect on total or mean leaf area, specific leaf weight or leaf area to fruit weight ratio. However, as reported by Smart et al. (1988), growth had essentially ceased by the time of treatment application.

The difference in results among studies for vine morphology assessments may be due to an interaction between light intensity and temperature or nutrient supply. Buttrose (1968) found that in general, at 25 and 30°C, as the light intensity increased, leaf area per plant and individual leaf area increased. However, at 20°C, leaf area per plant was the greatest at their lowest light intensity. It then decreased before again increasing as the light intensity increased. Also, lateral growth was highest at lower light intensities at 20 and 30°C, but at 25°C, the higher light intensity increased lateral growth. Keller and Koblet (1995a) found winter pruning weights increased with increasing light intensity only at lower nitrogen application rates, but at higher rates increased light intensity had no effect on main shoot weight, but decreased lateral shoot pruning weight.
Although in the current study assessments of individual leaf area, vine leaf area, specific leaf area and internode length and diameter were not carried out, it is still possible to estimate how shade affected canopy growth. There were no significant differences in any of the point quadrat measurements among the shade treatments and the control in any season that assessments were taken, although in Season One at the last assessment shaded vines tended to have more vegetative growth than the control. Shade, whether it was pre- or post-FB, reduced the number of effective canes per vine in the second season, although not the first. This is probably a carry-over effect from the season prior as other studies have found a reduction in bud break in the season following the application of shade (Morgan et al., 1985). As the ECN was reduced, but point quadrat determined values were not, it can be stipulated that the shaded vines had a higher leaf area per cane and this filled the canopy space at the same rate as the control. This is in contrast to other studies that found total leaf area per cane was reduced in the year after shading due to fewer and smaller leaves (McArtney and Ferree, 1999b). However, McArtney and Ferree (1999b) used 80% shade cloth from flowering to veraison, while in the current study 50% shade cloth was used for only three weeks.

The pruning weight for shaded vines was also less than that of the control vines and vines that were shaded post-FB had a significantly lower growing point weight than the control. This, combined with point quadrat measurements, indicates that the leaf area may not have been dissimilar to the control thus suggesting that the specific leaf area may have been increased for shade post-FB vines, which is in accordance with Schultz and Mathews (1993) and Morgan et al. (1985). In keeping with Keller et al.'s (1998) results, shade post-FB may also have increased the number of growing points per vine and per cane in the season that shade was applied, although this was not significant and due to the amount of trimming that took place, it is difficult to determine if there was in fact any difference.

Studies have found that a decrease in light quantity decreases the overall dry weight of the vine (Kliewer et al., 1972), which was also found in the current study with the reduction in overall pruning weight. Gu et al. (1996) found that 60% shading of young ‘Pinot noir’ vines around flowering significantly reduced the dry matter accumulation in
the current season’s shoots so that, although two weeks prior to anthesis there was a 30% difference in dry weight between shaded and un-shaded vines, by two weeks post-anthesis this had increased to 50%. Shading has been found to increase the nitrate concentration in the shoots and, although ammonium concentration was also increased in shaded vines, this difference had almost disappeared by two weeks post-anthesis (Gu et al., 1996). Smart et al. (1988) also found an increase in nitrate and ammonium concentration in the leaves due to shade applied around veraison. Both of our shade treatments may therefore have affected the dry weight, and the nitrate and ammonium concentrations in the current season’s shoots. However, the rate at which dry weight and ammonium concentrations increased was greater post-anthesis compared to pre-anthesis in the study by Gu et al. (1996). Our shade post-FB treatment may therefore have affected the dry weight and nutrient concentration of the shoots more than our shade pre-FB treatment, which is also evident in that shade post-FB tended to reduce magnesium and calcium concentrations in the petiole samples.

Although some of the literature may be contradictory, it is obvious that shade decreases the dry weight of the vine and influences the partitioning of assimilates. It is how this change in partitioning is exhibited that can vary among studies. Therefore shade, whether pre- or post-FB, possibly affected the partitioning of assimilates in the current study by increasing leaf area and the growth of lateral shoots. Once the shade cloth had been removed there is a possibility that the relative growth rate of leaves, and/or canes and/or laterals etc. may have been subsequently reduced for a further period of time. For the shade pre-FB treatment this reduction would have occurred over the time immediately after flowering, which may account for the difference in BSN incidence (discussed later) between the control and shade pre-FB treated vines.

The change in assimilate partitioning caused by shade has been reported to increase the sink strength of vegetative growth when the shade is applied around flowering, which results in a reduction in the current season’s inflorescence dry weight (Keller and Koblet, 1995a). Not only is the partitioning of assimilates affected by shade, but the rate of photosynthesis, and therefore the rate of assimilate production, is also reduced by shade. Although Iacono et al. (1995a) state that shade did not significantly decrease
photosynthesis, in fact it did at the 10% probability level as the $P$ value they obtained was 0.055. Kinet (1977) suggested that the depletion in photosynthesis that occurs when tomato plants are moved to adverse light conditions would affect the inflorescence first before vegetative growth, which supports Keller and Koblet's (1995a) results. However, a transfer to favourable light conditions and an increase in photosynthesis would first favour vegetative growth (Kinet, 1977). If this was the case it might have been expected that the shade pre-FB treatment in the current study would decrease the number of berries due to an increase in competition for assimilates between vegetative and reproductive sinks. However, berry number was not affected by shade pre-FB and the lack of effect may have been due to the shade treatments not being applied over the main time of flowering.

3.4.1.1 Vegetative growth and BSN

There was a significant positive correlation between canopy development as measured by the point quadrat method and BSN incidence. This was significant across all three seasons (Figure 53). LLN was determined to be a better measurement of vine vigour than other measurements such as PG, and it was therefore this assessment of canopy development that was used in the correlation with BSN. With PG, once vines filled the canopy space, PG values did not change although vine growth may have continued. The assessments approximately three weeks after flowering were also chosen as these were a good indicator of the vine vigour around flowering. Although later point quadrat assessments did produce slightly better linear fits than the assessment used, the R² value was not increased by a great deal (Appendix 4). This increase in linear fit may have been due to the continued vine growth through out the season in Season Two (Figure 4), which may have enhanced the difference in vigour among treatments.
Although Season Three initially had much higher canopy development than either Season One or Two, canopy development slowed and was similar to the canopy development of Season One from flowering to harvest. Therefore the relative growth rate would still have been less than in Season Two, which had a much higher canopy development after flowering than either of the other two seasons. BSN incidence in Season Two was also higher than that of either Season One or Three and, although BSN incidence for root pruned vines was the same in Seasons Two and Three, the trend was for the root pruning effect to decrease over successive years. Therefore, it is suggested that if canopy development after flowering was the same in Season Two as in either Season One or Three, that BSN incidence would have been lower, and therefore, there would have been a more gradual increase in BSN over the three years. At the end of Season Three, vines that were root pruned in that season generally had the same amount of vegetative growth in that season as vines that had been root pruned in Season One (noting that the intensity of root pruning in Season Three was lower), but at the first assessment date they had significantly less canopy development (hence indicating slower early-season vegetative development in Season Three). However, BSN incidence was slightly lower for vines that were root pruned in Season Three compared
to vines that were root pruned in Season One. As both canopies were still developing between the first and second assessment it is difficult to determine what the rate of canopy development may have been immediately after flowering as there was a seven week interval between assessments. Vines root pruned in Season One may have attained their final canopy area earlier than vines root pruned in Season Three, which may account for the slight increase in BSN incidence between the two treatments.

Although Capps and Wolf (2000) did not find a significant difference in LLN or PG when comparing treatments that did influence the incidence of BSN, their point quadrat values for the Winchester vineyard were much lower than those obtained from our experiment. The highest LLN of 1.2 that they obtained in 1997 and 1998 was close to the LLN obtained around flowering for our root pruned treatment in Seasons One and Three. For their Leesburg vineyard, LLN and PG values (2.2 – 3.2 and 0 – 4%, respectively) were closer to our control values in Seasons One and Three and the root pruned values in Season Two. However, at the Leesburg vineyard they experienced a very low BSN incidence although the vines appeared to be similar to our vines, and more vigorous than those in the Winchester vineyard.

However, Capps and Wolf (2000) measured the canopy only once, 60 days after bud burst. This would have been close to flowering, although it is difficult to determine exactly when as they do not mention the exact time of flowering and only stated that by the time that they measured the canopy, it had reached full development. It appears that most of the growth in the Leesburg vineyard occurred before the canopy measurements as it was pruned to 17 nodes per cane only once, at least one month after the first trim on the Winchester vineyard. The Winchester vineyard was however, pruned to 17 nodes twice in 1997 and 1998, and three times in 1996. As no further canopy measurements were taken it is impossible to determine how vigorous the canopy growth was after flowering, but the increase in trimming incidence in the Winchester vineyard demonstrates that while early point quadrat measurements may have shown a lower vigour in the Winchester vineyard compared to the Leesburg vineyard, canopy growth continued after flowering in the Winchester vineyard, but not the Leesburg.
Importantly, in the season that the highest vigour was demonstrated through vines being trimmed three times, BSN incidence was also the highest.

Moreno and Pavez (2000) claim that BSN was positively correlated with excessive growth during the veraison to harvest period. However, on studying their figures of canopy leaf area development from berry set to harvest, it is clear that on sites where further canopy development occurred after berry set, the majority of the growth occurred between berry set and veraison, not veraison and harvest. On these sites BSN incidence was typically high. However on other sites where minimal growth occurred between fruit set and veraison, even if the final canopy leaf area was high, BSN incidence was low.

Holzapfel and Coombe (1995) found that their minimally pruned vines had a lower BSN incidence than their spur pruned vines. They also comment that shoots on the minimally pruned vines developed at half the rate as those on the spur pruned vines, and that shoot elongation did not slow on the spur pruned vines until a month after the minimally pruned vines. Although they state that shoot elongation continued on minimally pruned vines until after flowering, according to their reported results the rate of increase had decreased by the time of flowering. Keller et al. (2001) also found a positive relationship between BSN incidence with both the mean lateral shoot length and the number of lateral leaves at the end of flowering. Theiler (1979) recommended that long canes should be left after pruning as short canes resulted in a high incidence of BSN. Results from the current study are therefore consistent with other studies, in indicating that BSN incidence is correlated with the growth of vines after the flowering period.

The mechanism behind the increase in BSN due to the increase in vigour may simply be the increase in shade around the fruiting zone. Shade has been implicated in the incidence of a similar disorder, EBSN (Jackson and Coombe, 1988; Jackson, 1991; Gu et al., 1996) as well as BSN itself (Perez and Gaete, 1986; Koblet et al., 1997). Similarly, in the current study shade post-FB was found to increase BSN incidence in the second season with a carry over affect into the third season. In the first season it did
not appear that shade post-FB increased BSN incidence, which would possibly indicate that shade post-FB only affects BSN incidence in the year after it was applied. However, the interaction treatment of heading back with shade post-FB did tend to have more BSN than heading back alone in the first season and therefore there may also be some effect of shade in the season in which shade was applied. Shade pre-FB decreased BSN incidence in the two seasons that it was applied but had no carry over affect into Season Three. However, if the increase in BSN incidence was purely the effect of shade on the bunches, the Extenday™ treatment, which increased the PPF values around the fruiting zone but also increased vigour, would have decreased the incidence of BSN. In fact, generally this treatment increased the incidence of the disorder. Therefore, there must be some other mechanism, other than a decrease in PPF values around the fruiting zone after flowering, which is influencing the incidence of BSN.

As already discussed, shading affects the partitioning of assimilates to different parts of the vine, and heading back of canes resulted in individual shoot growth being more vigorous prior to flowering. However, although vines that were headed back followed a similar pattern in canopy development in both seasons, the development of BSN was different between Seasons One and Two. Although individual shoots were more vigorous in the heading back treatment, there was no difference in BSN incidence when compared to the control vines when adjusted for maturity in Season One. Season One results are in contrast to findings by Redl (1984a), which indicate that the shorter the cane or spur length, the higher the BSN incidence. When the BSN values for vines that were headed back in the current study were not adjusted, or were adjusted for days after veraison, BSN incidence was slightly higher than the control in Season Two, but lower than the control in Season One. Vines that were root pruned and headed back followed a similar pattern with BSN incidence in Season One being lower than in root pruned only vines, and in Season Two BSN incidence was higher than for vines that were only root pruned. In fact in Season Two BSN incidence for these vines was very similar to that of the control. Initially in Seasons One and Two the vines in the treatment combination of heading back with Extenday™ appeared to have a higher BSN incidence than the control, heading back alone, and the Extenday™ treatment. However once adjusted, this interaction treatment showed no difference in BSN incidence to the control in Season One, but BSN incidence still appeared to be slightly
higher than the control in Season Two and generally higher again than heading back alone and Extenday™ in both seasons. Therefore heading back only appeared to have increased BSN incidence generally in the already high vigour season. Therefore, the general canopy development rate alone was not a good predictor of the incidence of BSN.

Redl (1984b) found that a training system with a high vine trunk height resulted in high vine water stress and weaker vine growth, but a higher BSN incidence was also exhibited. Redl (1984c) also demonstrated that canes further away from the trunk experienced higher water stress at dawn and dusk, which again lead to a higher BSN incidence. In contrast, in the current experiment treatments that resulted in lower vine vigour, possibly due to high water stress, resulted in a reduced BSN incidence. Root pruned vines were already demonstrating higher dawn, midday and dusk water deficits after flowering compared to the control (data not presented). Vines with the Extenday™ treatment had lower water deficits indicating less water stress. In Redl’s (1984c) study, for well watered vines the midday water deficit was higher for canes closer to the trunk indicting greater water stress, and in vines in dry soil there was very little difference in the water deficits of canes close to or far away from the trunk. Therefore if the midday, rather than dawn or dusk assessments are used for Redl’s (1984c) study, it would appear that canes with higher midday water deficits demonstrated less BSN incidence, which is consistent with our findings. Also, these experiments by Redl (1984c) occurred in the summer, possibly after fruit set had occurred, and therefore it cannot be determined what the water potential of canes around flowering was.

Redl (1984c) also found that shaded leaves were under less water stress than fully exposed leaves. The shade post-FB treatment in the current study had a higher incidence of BSN. However, water potential measurements were not carried out on shade treatments for the current study, and therefore a relationship between water potential and BSN incidence can not be determined for the shaded vines. Vines that were headed back in Season One may have experienced more water stress due to the increase in canopy area per cane and then the subsequent dry weather in Season One.
But unfortunately water potential assessments were not carried out on headed back vines in Season One to assess such an impact. Whereas in Season Two, due to the wet weather, vines that were headed back may not have experienced any water stress, which was confirmed by water potential assessments in Season Two (data not shown).

In Redl’s (1988) study the training system with the high trunk height also had cane length pruned to a shorter length (a lower number of buds), and yet had the same number of leaves per cane. Redl (1984a) found that by pruning to a lower number of buds per cane, BSN incidence increased, even though the total number of buds per vine was the same as that on the vines with a higher number of buds per cane. Individual buds were therefore probably more vigorous on the canes that had a lower number of buds. Results therefore indicate that there is a very strong correlation between the individual vigour of shoots immediately after flowering and the incidence of BSN. However, how the vigour of shoot is influencing the disorder is still not known, but may include the partitioning of assimilates, the water potential of the shoot and/or some other mechanism within the vine.

3.4.2 Berry quality

Root pruning has been found to decrease the number of berries per bunch in some studies (McArtney and Ferree, 1999a) but not others (Ferree et al., 1999), and in the current study in neither the year of root pruning (Season One) nor the following season were there any significant differences in berry number due to root pruning (Table 22). McArtney and Ferree (1999a) found that there was a positive correlation between leaf area per cane at bloom and berry number per bunch. It is difficult to determine what the leaf area of root pruned vines was in relation to control vines in the first season as point quadrat assessments were not carried out until after flowering. However, in the second season at bloom, point quadrat assessments indicate that root pruned vines had a similar leaf area to control vines and hence a difference in berry number would not have been expected.
Increasing cane number, which decreases individual cane growth, has also been found to decrease berry number per bunch (McArtney and Ferree, 1999a). In Season Two the number of berries per bunch was increased by heading back of the canes, although there was no difference in the first season. As point quadrat assessments indicate similar total leaf areas between headed back and control vines at bloom, and the headed back vines had fewer canes, this would indicate that headed back vines had a higher leaf area per cane. Also, the amount of assimilates exported to a bunch is decreased in vines when the dormant cane length is increased (Stewart et al., 1996), which may account for the higher amount of berry set.

Mulching has also been found to increase berry weight (Richards, 1983) but there were no significant differences in berry weight or number due to the Extenday™ treatment, although there was a trend for heavier berries. This may be due to the increase in vigour of these vines and the possible increase in internal canopy shading. Studies have found that shade decreases cluster fresh weight. Gu et al. (1996) found that this was due to the reduction in the number of berries per bunch, and that shading had an effect on berry set rather than berry fresh weight. Other studies have also found a reduction in berry set due to low PPF, but unlike Gu et al. (1996), they did find a reduction in berry fresh weight due to low PPF (May and Antcliff, 1963; Morgan et al., 1985; Archer and Strauss, 1989; Hummell and Ferree, 1997). Smart et al. (1988) found a decrease in berry weight, but not a reduction in berry number, due to a reduction in light quantity from veraison to harvest. Although, in these studies if a difference in berry weight was found due to shade, berry weight tended to be reduced, whereas some other studies have found that berry weight can be increased due to shade (Crippen and Morrison, 1986; Reynolds et al., 1986). Crippen and Morrison (1986) determined this to be due to a higher water content in shaded berries. The decrease in berry set and/or berry weight can be explained by competition between the apical shoot growth and inflorescence development in the lower light regimes (Leonard et al., 1983). Shading greatly reduces the export of assimilates to apples three and five weeks after bloom which can affect fruit set and fruit size (Tustin et al., 1992; Grappadelli et al., 1994). Shading also reduces the partitioning of assimilates to permanent vine structures in grape vines (Vanden Heuvel et al., 2002). Further discussion on competition between vegetative and reproductive sinks is provided in Chapter Four.
In contrast however, in the current study there were no significant differences in final berry number per bunch or berry fresh weight among the shade treatments and the control. This may be due to the fact that some cultivars are more sensitive to shade around flowering than others (Ferree et al., 2001). Conversely, shaded leaves of tomato and pepper plants were found to export more than they were able to fix for short periods of time after shade was applied (Ho, 1979; Grange, 1987), while fully irradiated leaves may not export all the assimilates that are fixed (Ho, 1979). The photosynthetic rate and export rate of the pepper plant increases to maximum levels within days of moving plants from low to high irradiance conditions (Grange, 1987). In the current study shade was not applied over the 50% flowering period, and therefore adequate assimilates may have been imported into the developing inflorescences and berries, which prevented a reduction in both fruit set and cell division.

There was also no effect on yield (data not presented) in either the first or second season due to shade even though the ECN was reduced in the second season. This lack of carry over effect from the previous season on fruitfulness is contradictory to what other authors have found (May and Antcliff, 1963; Morgan et al., 1985). May and Antcliff (1963) found that fruitfulness (% fruitful shoots and clusters per cane) was only reduced if shade was applied for at least four weeks during early summer, while Morgan et al. (1985) found the flower number to be reduced by lower PPF after this time. It is difficult to determine when these studies applied shade in relation to flowering as the time of flowering was not noted. Keller and Koblet (1995a) found reduced fruitfulness due to shade over the three week flowering period. In the previous three studies, light intensity was reduced by between 65 – 75%, whereas in the current study, light intensity was only reduced by 50%. Also the shade applied in the current study may not have been applied long enough over the critical time to affect inflorescence and flower initiation, which may explain why in the current study neither shade treatment affected the following season’s fruitfulness.

Wine quality aspects were not detrimentally affected by root pruning. In fact, the resulting juice from the root pruned vines lead to enhanced wine quality as, for example, the Brix concentration was higher (Figures 26 and 27, and Table 20). Other studies
have found an increase in Brix concentration in apples due to root pruning (Ferree, 1992; Mika and Krzewinska, 1995) but not in grapes (Ferree et al., 1999). This may be caused by the decrease in mean berry size due to root pruning, as smaller berries attain higher Brix concentrations (Dreier et al., 2000). Other studies have also found root pruning to decrease fruit size (Ferree, 1992; Mika and Krzewinska, 1995; Ferree and Knee, 1997b; Ferree et al., 1999), and although this may be detrimental for some crops, for wine grapes it is beneficial to wine quality. The decrease in berry size may be due to higher berry temperatures during the day which decrease turgor pressure and consequently berry size (Crippen and Morrison, 1986; Reynolds et al., 1986).

Over the time in which juice samples were assessed TA, tartaric acid and malic acid were generally lower in root pruned vines even in the third season after root pruning (Figures 28, 29, 42 - 45). In contrast McArtney and Ferree (1999a) found that root pruning of grape vines increased the TA in berries. The decrease in the current study may have been caused by the increase in water stress that root pruned vines experienced. Stevens et al. (1995) found that water stress decreased both TA and malic acid concentrations, but did not find a correlation with Brix concentration, tartaric acid or potassium concentrations with water stress. Potassium concentration did tend to be increased or remain the same as the control in the juice from root pruned vines (Figures 36 and 37). However, as berries from root pruned vines were more mature and potassium concentration tends to increase through to maturity (Dry and Coombe, 2004), this may be due to the maturity of the berries and once this is taken into account the potassium concentration may in fact be lower than the control at the same maturity ratio. The decrease in TA, tartaric and malic acids may also have been due to the advanced maturity of the berries as these quality assessment values also generally decline as the season progresses (Dry and Coombe, 2004). Overall therefore, the main effects due to root pruning may have been caused through bunches on root pruned vines being more mature than those on control vines.

Studies have shown that shading can reduce the Brix, TA and tartaric acid concentration and increase the pH, potassium and calcium concentration in berries at harvest (Smart et al., 1988; Iacono et al., 1995b; Hummell and Ferree, 1997; Keller et al., 1998 and many
more). Shade effects on these berry composition attributes appear to be due to shading of the leaves, and not the bunches themselves (Morrison and Noble, 1990). Shading of leaves has been found to reduce the translocation of assimilates to apple fruit (Grappadelli et al., 1994) and would therefore affect processes dependant on assimilate supply. This may be why Crippen and Morrison (1986) did not find a significant difference in Brix, TA and pH for bunches shaded by the canopy. Shading of bunches does however, affect anthocyanin development and total phenol content whereas shading of the leaves does not (Morrison and Noble, 1990). In contrast, no significant difference was found between our shade treatments and the control except in the first week after veraison when shade pre-FB reduced TA (Figure 29). However, this difference was no longer significant from two weeks after veraison until harvest. The lack of significant difference was possibly due to the fact that other studies looked at the effect of shade over different periods of berry growth (for example, over the entire period from flowering to veraison) compared to our study. Further canopy development after the shade treatments had been removed would have resulted in assimilate supply to the bunches not being affected from veraison onwards. However, a reduction of assimilates to developing bunches immediately after berry set may affect early berry growth and therefore possibly BSN incidence.

The Extenda™ treatment, while increasing the reflected light from the ground, did not affect the Brix, TA, pH and malic acid concentrations. However, calcium and magnesium concentrations were generally increased, and potassium concentration decreased in the berry due to the use of Extenda™ mulch. These differences may be due to how light intensity affects vegetative and berry development differently as discussed above, caused by the increased vigour and therefore the increased number of shaded leaves within the canopy, even though light intensity around the fruit may have been increased. Carbonneau (1997) reported that as the vigour of a vine increases, as measured by cane length, the onset of veraison is delayed. Excessive pruning also leads to a decrease in berry maturity or wine quality (Carbonneau, 1997). Although the onset and date of 50% veraison were not delayed in the current study due to the application of Extenda™, the rate of veraison development was slower compared to the control in both seasons where the treatment was applied (Figures 13 and 14, and Tables 10 and 11). The application of Extenda™ also tended to reduce soil temperature by 2°C
Chapter Three - Field trial: vigour and light effects on BSN. Discussion

during the day and night and studies have shown that nutrient uptake can be affected by soil temperature (Behboudian et al., 1994). Tartaric acid was not affected in either Season One or Two, yet in Season Three, when Extenday™ was not reapplied, tartaric acid concentration was increased in the berry (Table 20). This may be due to carry over effects of cane and leaf growth from the previous year (McArtney and Ferree, 1999b).

If the increased vigour in Season Two detrimentally affected cane growth in Season Three, then during Season Three there may have been less internal canopy shading that may then have increased the tartaric concentration in the berries.

Schupp and Ferree (1987) found that cork spot in apple was reduced by root pruning and this may have been due to an increase in calcium concentration in the fruit. They also found that magnesium and potassium concentration in the fruit was reduced by root pruning. In contrast, in the current study root pruning resulted in calcium concentration being reduced in the berry, and either decreased or increased in the juice, depending on the season. Ferree and Knee (1997a) found no difference in fruit calcium when apple trees were root pruned at bloom. In the current study magnesium concentration was increased in the berry in some samples and increased in the juice in others due to root pruning. However, when potassium concentration was similarly affected, it was reduced in both berry and juice samples in vines that had been root pruned.

The nutrient concentration of berries can be influenced by many factors. Seed number per berry has been found to affect the calcium, magnesium and potassium content, but not concentration (Boselli et al., 1995). However, there were no significant differences in seed number per berry in the current study (Table 23). Berry transpiration has been directly correlated with berry nutrition for potassium and calcium, but not magnesium (Boselli et al., 1998). Berry transpiration has also been found to increase as leaf transpiration decreases (Boselli et al., 1998), and therefore treatments that affect leaf transpiration rate due to, for example, water stress or a change in light quantity, may affect berry nutrition. Root pruning, which has been found to decrease leaf transpiration (Ferree et al., 1999), may increase berry transpiration. Nutrients may also be affected by the different volume and/or type of root depending on the age and type of root that is most abundant.
Root pruning grape vines during the winter months therefore potentially has many beneficial effects on grape quality and consequently wine quality mainly through advancing the maturity of the berries and decreasing individual berry size. The other treatments of heading back, ExtendaTM and shade all potentially have detrimental affects on berry quality although these appeared to be minimal in the current study. Any detrimental affects of ExtendaTM may possibly be avoided if it is laid down near to or after veraison so that it influences berry ripening rather than early vine growth.

3.4.3 Root growth

Using the synthetic representation of root distribution presented by Giulivo and Pitacco (1996), it is obvious that by root pruning 25 cm on either side of the trunk to a depth of 60 cm, a very high percentage of roots were cut from the vine. This representation indicates that the highest density of roots was contained between 40 – 90 cm from the trunk and at a depth of 25 – 35 cm. Ferree (1994) also noted that new root growth after root pruning tended to occur close to the cut site, with very little occurring 15 cm away. Jordan (1985) suggested that root pruning may limit the uptake of nitrogen due to the reduced root volume, and therefore may reduce the incidence of BSN in this way. In the current study ammonium concentration was lower in juice samples from root pruned vines in keeping with this hypothesis.

Nutrient uptake occurs in different types of roots at different rates. Potassium uptake is higher in young white roots than in woody roots with the reverse being true for calcium (Kliewer et al., 1983). Root pruned plants probably have a higher ratio of young white roots to older woody roots, but the total number of root tips is probably reduced. Ferree (1994) found the number of roots <1 mm in diameter to be lower in root pruned apple trees. However, there had been some growth of these roots as less than half the number of roots were >1 mm in diameter for root pruned trees compared to the control. In both the current and Ferree’s (1994) study, the canopy was reduced in relation to the reduced root volume. Therefore the ratio of white roots to foliage may have either increased or may possibly have been the same as for the control vines but as the amount of reduction in vegetative growth was not noted in Ferree’s (1994) study this can not be determined.
Partitioning of assimilated carbon to the root system is higher in root pruned vines (McArtney and Ferree, 1999a). Therefore the reduction in shoot elongation due to root pruning may be due to the root system being a more competitive sink for the assimilated carbohydrates than the shoot system, as well as being due to a reduction in the stored carbohydrates in the root system (McArtney and Ferree, 1999a). In both the current and McArtney and Ferree’s (1999a) study although shoot growth still increased after bloom, the rate at which shoots on root pruned vines grew was less than that of shoots on vines that were not root pruned.

Most studies indicate that root growth does not begin until near flowering (Richards, 1983; Dry and Coombe, 2004), and has its first peak at flowering and its second peak around harvest, with very little growth before or during budburst or in mid summer (van Zyl, 1988). However, some fine root growth has been found to occur in ‘Concord’ grapes between bud break and bloom (Bates et al., 2002). As reduced light intensities reduce root growth and increase the shoot:root ratio (Richards, 1983), shading around the time of flowering dramatically reduces root growth (Gu et al., 1996). In Gu et al.’s (1996) study they found that at anthesis there was a 20% difference in dry weight of young roots between shaded and un-shaded vines, and two weeks later, due to little root growth in shaded vines, there was an 80% difference in root dry weight.

In our study we found little root growth prior to flowering (data not shown) confirming previous work (Gu et al., 1996) and suggesting that treatments may have affected root growth in our vines at a similar time to other studies. As most of the shade effect on root growth was post-flowering in other studies (Gu et al., 1996), we can assume that our treatment of shade pre-FB would have had only a minor effect on root growth. However, our treatment of shade post-FB would have had an effect on root growth with the possibility of rapid root growth only occurring once the shade cloth had been removed.

Shade post-FB generally led to an increase in BSN incidence in the year after shade had been applied. Post-FB is an important time for not only the current season’s crop, but also the following season’s crop. This is due to inflorescence initiation occurring
around this time and a reduction in root growth would therefore be detrimental as the uptake of some nutrients would be limited. This in turn would reduce the availability of nutrients needed for further shoot growth, the developing berries, and the developing inflorescence. A reduction in light intensity also directly affects the actual initiation of inflorescences for the following season (Smart and Robinson, 1991).

As the vines in our experiment were cane pruned in the winter, many of the reserves available to the developing bunch early in the next season were dependent on the cane that was formed the season before. Therefore, an altered carbohydrate and nutrient reserve may have influenced the following season’s crop, not only during the inflorescence initiation of the current season, but also during the early stages of shoot and inflorescence growth in the following season.

3.4.4 Nutrient analyses

In Scienza and Fregoni’s (1978) study although BSN incidence was higher on less vigorous vines, the calcium concentration was higher and potassium concentration lower in the apices of the growing canes, compared to the more vigorous vines. In our study, magnesium concentration in the petioles was reduced in root pruned vines, but there was no consistent trend for either calcium or potassium concentrations (Figures 17 – 19). Other studies on root pruning have found contradictory results for leaf nutrition. Results reported include an increase in potassium concentration with no effect on calcium concentration in the leaves for peach (Richards and Rowe, 1977a), an increase in calcium with no affect on potassium concentration in the leaves of apple (Mika and Krzewinska, 1995), and a decrease in potassium concentration with no affect on any other nutrients in apple leaves (Baugher et al., 1995). As there was no evidence in Season One of a change in potassium concentration in the petiole samples, in that particular season potassium uptake may not have been affected by root pruning, or petiole samples may not be a good indicator of this change. Therefore there is a possibility that changes in potassium and calcium concentrations in the berry and juice are due to some other mechanism other than potassium and calcium uptake from the soil.
Shading increases nitrogen and decreases phosphorus in leaves, but there is no affect on leaf potassium, calcium, and magnesium (Iacono et al., 1995b). Although not statistically significant, shade post-FB in the current study tended to reduce petiole magnesium and calcium concentrations after the onset of veraison, but not before. Therefore it is suggested that as the statistics indicate, there is no difference in these nutrients due to the shade post-FB treatment. Shade pre-FB also had no affect on nutrient concentrations in the petioles.

Sharma et al. (2003) found petiole calcium and magnesium concentrations to be decreased and potassium concentrations to be increased in the leaf opposite BSN affected bunches. Other studies have found that an increase in the petiole K/Mg ratio leads to an increase in BSN incidence (Delas et al., 1976; Nahdi et al., 1993). However, there were no significant correlations between petiole nutrition and BSN incidence in the current study. This is in accordance with Christensen and Boggero (1985) who found no differences in petiole nutrition analyses between high and low incidence vineyards.

Smart et al. (1988) found that shade increased peduncle magnesium and decreased peduncle potassium concentration leading to a significant decrease in the K/Ca, K/Mg and K/(Ca+Mg) ratios in the peduncle. In contrast, our study found that shade pre- and post-FB tended to increase potassium concentrations in the peduncle and the rachis. Magnesium concentration tended to be decreased in the rachis for shade post-FB, and in the peduncle for shade pre-FB, although none of these were significantly different from the control (Figures 23 – 25). Increased ratios resulted from these changes in cation concentrations in the rachis and peduncle.

Sharma et al. (2003) found that in both the upper and lower portion of the rachis, calcium and magnesium concentrations were lower in BSN affected bunches compared to healthy bunches. There was no significant difference in potassium concentration. In the current study, a week prior to the onset of veraison in the first season, calcium was possibly the only nutrient with a correlation with BSN incidence as indicated by the results for root pruned vines. In these vines, rachis calcium concentration and BSN
incidence were both generally lower than vines which had not been root pruned. This result is opposite to the results of some studies (Haub, 1986; Suneel et al., 1999) but is consistent with others (Redl, 1983; Boselli et al., 1995). Once veraison onset had occurred, there was a negative correlation with potassium concentration and the K/Mg and K/Ca ratios and BSN. It is difficult to determine if these correlations were the cause of, or a consequence of BSN, because even though BSN symptoms may not have been visible at this time, it is possible that the disorder was already present, and therefore nutrient concentrations may have already been affected by changes occurring within the bunch.

In the third season when post-FB samples were taken, there were no significant differences in either the berry or the rachis nutrients or nutrient ratios between the control and the root pruning treatments, although the root pruning treatments did significantly reduce BSN incidence (Tables 14-19). Generally, root pruning tended to reduce potassium, calcium and magnesium in the berry and rachis resulting in consistent ratios among the treatments. By the time the pre-veraison samples were taken in the third season both root pruning treatments were found to reduce rachis potassium concentration. Root pruning in the third season increased rachis and berry magnesium concentration, but root pruning in the first season did not, in neither the year of pruning nor for the two seasons following.

Alleweldt and Hifny (1972) suggested that analyses of the total calcium concentration of the peduncle was not a good indicator to use to determine if BSN was related to calcium because the disorder may be localised. They found that even in the early stages of BSN development, that the cell walls of the affected tissue had a higher calcium concentration compared to healthy tissue. This would appear to be in contradiction to other studies that found that an increasing K/Ca ratio in BSN affected tissue even though visual symptoms had not appeared (Feucht et al., 1975).

The current study found no correlations between any of the nutrients analysed in the petiole, rachis and/or berry samples and BSN incidence. These and the contradictory results of other studies therefore suggest that BSN incidence is not correlated with an
imbalance in the nutrients analysed, or that the time in which samples are taken is not a good indicator of the predisposition of the berries to the disorder or, as Alleweldt and Hifny (1972) suggest, the bulk sampling methods that are normally employed are not good indicators of the disorder.

### 3.4.5 Xylem development

It has been suggested that any restriction in xylem development of the peduncle may result in decreased amounts of calcium moving into the berry resulting in increased BSN incidence (During and Lang, 1993). It has been shown that shade can reduce xylem development in vines and therefore the hydraulic conductivity is reduced (Schultz and Mathews, 1993). This effect of shade on xylem development is obvious early in the development of petioles, but later in internodes (Schultz and Mathews, 1993). However, most of the xylem development has occurred in the peduncle by the time of flowering (Theiler and Coombe, 1985) and therefore, of the two shade treatments likely to have affected xylem development, it was the shade pre-FB that might have done so. However, in our study this treatment decreased the incidence of BSN.

Lovisolo and Schubert (1998) suggested that water stress results in smaller shoot xylem vessel size with lower cross-sectional areas and decreased shoot hydraulic conductivity. It therefore might be expected that vines that were root pruned may have decreased shoot hydraulic conductivity leading to increased BSN incidence, and yet these vines had a reduction in BSN incidence. However, Mapfumo et al. (1993) found that water stress has only a minor effect on the structure and axial resistance of grapevine roots. Further work would need to be carried out to determine whether or not the treatments applied in the current study did affect shoot and peduncle xylem development and whether or not this correlated with the incidence of BSN.

### 3.4.6 Plant growth regulators

Nutrient translocation is also controlled by plant growth regulators. Gibberellic acid reduces calcium uptake from a nutrient solution, and reduces the accumulation of
calcium in fruit (Saure, 2005). Therefore, processes that reduce the biosynthesis of GAs, or suppress GAs and their action, promote the translocation of calcium to the fruit. Root pruning is one such technique that is said to reduce the action of gibberellins (Saure, 2005). The reduction in gibberellins may also be a factor in the reduction in vegetative growth in root pruned vines. IAA is also thought to induce the preferential movement of calcium to the shoot apex even though the transpiration rate there is much lower than in older leaves (Mengel and Kirkby, 1987). IAA is also considered to be important in assimilate supply to developing sinks (Bangerth, 1989), and Porter (1981) suggested that endogenous ABA in plant organs could serve as an important factor in the directional control of assimilate transport in plants. It therefore appears that plant growth regulators are important in the ability of a reproductive sink to attract nutrients and assimilates.

ABA has often been implicated in BSN incidence (Baldachino et al., 1987b; Holzapfel and Coombe, 1998) and is generally considered a stress response hormone. It is known that when vines experience water stress that ABA accumulates in roots, leaves and the xylem sap that results in stomatal closure (Loveys, 1984; Lauer and Boyer, 1992; Stroll et al., 2000). However, root pruned vines had higher water stress and had a lower incidence of BSN.

### 3.4.7 Summary

Our treatment of shade pre-FB did not increase the incidence of BSN but shade post-FB did. This result occurred mainly in the season after the treatment was applied, although there is a possibility that it may have also affected BSN incidence in the current season as well. It therefore appears that there may be a critical time after flowering, whether it be in the current season or following season, in which bunches may be predisposed to BSN. Inflorescence initiation occurs in grape vines in the season prior to flowering and occurs throughout the summer, depending on environmental conditions. Shade post-FB may therefore have been affecting this process. However, other studies have also found that environmental conditions prior to flowering can have a detrimental effect on berry quality and increase BSN incidence. Therefore the hypothesis of a critical time needs to be further investigated.
If BSN incidence is influenced by the competition between vegetative and reproductive sinks, the potential sink strength of the bunch is important. As sink strength is considered a product of sink size and sink activity (Ho, 1988), the final cell number and size within each berry can influence the sink strength. Factors that affect this, such as plant growth hormone concentrations and environmental conditions, may therefore play an important role in the incidence of BSN. Further chapters will therefore be investigating the possibility of a critical time for the predisposition of BSN, and how the manipulation of sink strength through plant growth regulators, canopy manipulation and environmental conditions affects BSN incidence.
4 Source-Sink Relationships and BSN

4.1 Introduction

In this group of experiments, treatments were applied to test the hypothesis that BSN incidence was a result of competition between vegetative and reproductive sinks. A sink is defined as a plant organ that is a net importer, while a source is defined as a plant organ that is a net exporter (Ho, 1988). In relation to carbohydrates in grape vines it has been reported that leaves begin to export, and therefore become a source rather than a sink, once they have reached 50% of their final size (Hale and Weaver, 1962; Williams, 1996). Although mature leaves are the major source of assimilates, other aerial organs containing chlorophyll can also contribute. In the grapevine these can include stems and the grape cluster (Williams, 1996). Although phloem transport can occur over large distances, sinks are usually supplied with assimilates by a nearby source (Warldaw, 1990). Sinks include expanding shoot tips and leaves less than 50% fully expanded, buds, flowers, fruits, stems and roots. Assimilates fixed by source organs are then competed for by the sinks and it is the ability of a sink to import these assimilates relative to other sinks which is the sink strength (Ho, 1988), although the term competitive ability may be a better term in a competitive situation. Measurement of sink strength purely through absolute growth rate or net accumulation of dry matter can greatly under-estimate the actual sink strength of an organ as the proportion of imported assimilate used for respiration can be substantial (Ho, 1988). In grapes specific berry respiration can be as high as 600 μg CO₂ g⁻¹ fresh wt h⁻¹ early in berry development, decreasing to 40 μg CO₂ g⁻¹ fresh wt h⁻¹ at fruit maturity. However, grape berries are also capable of photosynthesis while they contain chlorophyll and reported rates of CO₂ assimilation can be as high as 600 μg CO₂ g⁻¹ fresh wt h⁻¹ shortly after anthesis. This drops to less than 10 μg CO₂ g⁻¹ fresh wt h⁻¹ close to fruit maturity (Williams, 1996 and references therein).

The most critical determinant of actual sink strength is the intrinsic ability of a sink to receive or attract assimilate. This intrinsic ability has been termed potential sink strength (Ho, 1988). Potential sink strength is generally considered to be the product of sink size and sink activity, these being defined as the physical constraint and
physiological constraint upon the sink organ’s assimilate import, respectively (Ho, 1988). The number of cells in the sink may be considered a suitable measurement of sink size (Ho, 1988). Sink activity is related to the metabolic activity of the sink during development as the import of assimilates can be affected by factors that affect the metabolic rate within the sink including temperature and metabolic inhibitors (Ho, 1988). Therefore factors affecting cell division and final cell number and the metabolic processes within a sink will affect the potential sink strength. However, one must also consider the situation where the sink is small (and therefore, by definition sink strength is possibly low), but the ability of a sink to attract its carbohydrate requirement is high, for example, a single berry (Woolley, personal communication).

When assimilates are limiting due to low source strength (e.g. low numbers of leaves, low photosynthetic activity) competition among sinks may be amplified. However, generally the priority of assimilate partitioning is consistent within each stage of plant development, but will change throughout plant development. For example the priority for assimilates may change from roots > young leaves > inflorescence to fruit > young leaves > flowers > roots once fruit have set (Ho, 1992). Grapevines tend to have a similar priority to this for assimilates and assimilates are preferentially partitioned to vegetative growth early in the season (Hale and Weaver, 1962). Inflorescences tend to have low sink strength (or competitive ability) and only increases after 70% flowering (Glad et al., 1992). A reduction in assimilate supply to the inflorescences results in a decrease in berry set and in some instances it has been suggested that this reduction in assimilate supply leads to an increase in EBSN (Caspari and Lang, 1997).

Most literature indicates that grape berries are strong sinks, and compete effectively with other sinks for assimilates. Hunter and Visser (1988a) found that 97% of fixed assimilate is transported from basal leaves to the bunch at fruit set (Figure 54). However, very little assimilate is transported to bunches from either the middle or apical leaves at this time. Once berries are approximately 8-10 mm in diameter, 92-98% of the fixed assimilate is transported from all leaves on the cane to the developing bunches (Hunter and Visser, 1988a). This indicates that bunches increase their competitive ability for assimilates as berry development progresses.
The competition between vegetative and reproductive growth can be altered by increasing or decreasing the competitive ability of particular sinks. In order to reduce the competition to the developing flower, various techniques such as root restriction, shoot topping at the correct time, or applying plant growth regulators such as gibberellins and cytokinins to the bunch or inflorescence, have been used (Smart and Robinson, 1991; Ho, 1992). The application of GAs have been found to divert assimilates from the shoot apex to the flower (Quinlan and Weaver, 1970; Leonard et al., 1983). The increase in assimilates may therefore increase the competitive ability of the berry by supporting metabolic processes, or conversely the increase in assimilate transport may be the result of an increase in the competitive ability of the sink.
Gibberellin application has been shown to directly decrease BSN in grapes (Allleweldt and Hifny, 1972; Beetz and Bauer, 1983; Haub, 1983; Donna, 1985; Theiler and Coombe, 1985), although it can increase berry shatter (Ben-Arie et al., 1998). Theiler and Coombe (1985) suggest that the development of BSN is due to low concentrations of gibberellins during berry set. GA$_3$ is often applied to bunches in order to increase berry size in seedless grapes (Raven et al., 1981; Gianfagna, 1995; Ben-Arie et al., 1998) although it has been reported that seeded varieties often do not respond favourably to this practice. Internode elongation is also associated with gibberellic activity where plant mutants, which demonstrate low gibberellin-like activity, tend to be dwarf types (Reid and Howell, 1995). It has been established that the biologically active gibberellins are 3β-hydroxylated C$_{19}$GAs, and it is some of these gibberellins that are required for stem elongation. 3β-hydroxylated C$_{19}$-gibberellins include GA$_1$, GA$_3$, GA$_4$ and GA$_7$ (Sponsel, 1995) and plants with low concentrations of these gibberellins and high concentrations of gibberellins such as GA$_{20}$ have reduced stem elongation (Reid and Howell, 1995). The increases in berry size and stem elongation are due to gibberellins stimulating both cell division and cell elongation (Davies, 1995b). In a berry this increase in cell division and cell elongation results in the berry achieving a stronger potential sink strength due to the increase in potential sink size. If this increase in potential sink strength is realised the competitive ability of the sink has increased and therefore the sink must attract increased amounts of assimilates (Ho, 1988).

Through work carried out on apical dominance, it has been shown that the production of the auxin, IAA, in the developing apical bud is responsible for inhibiting axillary shoot growth (Phillips, 1975). Sink strength has also been attributed to the concentration of auxins in the fruit and the dominance of some fruit appears to be mediated by the amount of IAA produced by the seeds (Ho, 1992). It is suggested that the import of assimilates is regulated by the efflux of auxin from the fruit (Ho, 1992) and the rapid growth rate of the grape berry after fruit set has been correlated with high endogenous auxin concentrations in the berry (Coombe, 1960; Bertrand and Weaver, 1972; Niimi et al., 1977). It is thought that stronger sinks, due to factors such as higher seed numbers or earlier fruit set, inhibit polar transport of auxin from other sinks (Bangerth, 1989).
The partitioning of assimilates between vegetative and reproductive growth is regulated by the competition between the vegetative and reproductive growth (Ho, 1984). Here too IAA is thought to be one of the controlling mechanisms, with the dominant sink producing higher amounts of IAA, which in turn regulates assimilate and nutrient supply to the subordinate sink (Bangerth, 1989). As IAA is synthesised in the apical bud (Snow, 1929; Thomas, 1983) the more apical buds on a cane, the more competition a bunch has for nutrients and assimilates.

In vigorous vineyards the problem of excessive growth can be exacerbated by canopy management techniques. When removal of shoot tips occurs through trimming while the vine is still actively growing, apical dominance is lost and additional lateral growth is stimulated (Hunter, 2000). Vigorous vine growth can be increased by trimming of those laterals resulting in further lateral growth (Smart and Robinson, 1991). Therefore a vigorous vine, if not correctly managed, can end up with multiple apical points and a dense canopy.

The correlation between vine vigour and the incidence of BSN has been demonstrated in Chapter Three, with a positive relationship between BSN incidence and LLN, PIL and the number of growing points per vine. Other authors have also indicated that high vigour vines tend to have a higher incidence of BSN (Bolay et al., 1965; Delas et al., 1976; Cline, 1987; Moreno and Pavez, 2000). BSN incidence does not appear to be solely related to the resulting shade within high vigour vine canopies. Instead it appears that it may be related to the increased competition between vegetative growth and reproductive growth and it is this increased competition that has a direct effect on the disorder (see Chapter Three).

As studies have found that a related disorder EBSN may be caused by an inadequate carbon supply to the developing inflorescence (Caspari et al., 1998) BSN may also be caused by an inadequate supply to the developing bunch, brought about by increased competition between the vegetative and reproductive sinks. The following set of experiments were undertaken to further investigate this hypothesis.
4.2 Materials and Methods

4.2.1 Plant material

Seven-year-old field grown ‘Cabernet Sauvignon’ grape vines on SO4 rootstock were used for this study. Vines were situated on a different area of the Montana vineyard at Moteo to that used in the experiments described in Chapter Three. However, spacing of vines, vine layout within bays, irrigation and commercial practices were the same as for those vines.

4.2.2 Treatments

4.2.2.1 Canopy manipulation

There were six blocks of vines due to the soil fertility gradient in that area of the vineyard. Two vines within a block were chosen at random for treatment application (Appendix 1). To manipulate growing point number, and therefore apical bud number, laterals were removed from one of the vines as soon as lateral growth began and removal continued throughout the growing season. The other vine did not have any canopy manipulation other than that normally carried out during common commercial practice (control).

4.2.2.2 Plant growth regulators

Twelve bunches per vine were chosen at random and totally immersed in one of four different treatment solutions. Each treatment solution was applied to three bunches per vine. Treatments were applied as soon after fruit set as possible and were applied only once.

Treatments were:

- Gibberellic acid (GA_3) \( 50 \text{ mg l}^{-1} \)
- \( \beta \)-indole acetic acid (IAA) - auxin \( 200 \text{ mg l}^{-1} \)
- 1-naphthylphthalamic acid (NPA) – an anti auxin \( 200 \text{ mg l}^{-1} \)
• Control (water)

Solutions were made up by dissolving the plant growth regulator in 0.88 specific gravity ammonium hydroxide that was then evaporated using a vacuum rotary evaporator. The residue was then re-dissolved in reverse osmosis (RO) water. The pH of the resulting solution was checked to ensure that it was neutral, and then made up to 3 litres with RO water and 0.1% (v/v) Tween was added. The control solution consisted of 0.1% (v/v) Tween dissolved in 3 litres of RO water.

4.2.3 Statistical analyses

Data were analysed using a blocked split-plot design. This was due to the canopy manipulation treatment being applied to the entire vine, and then the plant growth regulators being randomly applied to individual bunches within each vine. The vine to which each canopy manipulation treatment was applied was chosen at random. The canopy manipulation was therefore classed as the main plot, with the plant growth regulators being classed as the split-plot. LSMeans were used to determine if the treatments were significantly different from each other in order to obtain P-values for pre-planned paired comparisons. In the case of the vigour measurements, only the canopy manipulation treatments could be investigated, so therefore data were analysed using a randomised block design (RBD).

4.2.4 Measurements

4.2.4.1 Vigour

Point quadrat analyses. The point quadrat method was used to measure canopy development during the growing season as described in Chapter Two. Leaf layer number (LLN), percentage gaps (PG) and percent interior leaves (PI) were determined from the point quadrat assessments as for the vigour/light trial vines. Vines were only assessed twice throughout the growing season. The first assessment was carried out immediately after the removal of laterals from the vines, which was one week after
Chapter Four - Field trial: Source sink relationships and BSN. Materials and Methods

flowering. The second assessment was carried out one month later at the beginning of veraison.

**Growing point number.** During the following winter dormant stage, growing point number per vine and pruning weights were measured as described in Chapter Two. Measurements were carried out on the two vines allocated to the canopy manipulation treatments of control (laterals retained) and laterals removed.

4.2.4.2 Veraison development

The progress of veraison development was measured using the method described in Chapter Two for Season Two. All experimental bunches were visually scored at each measurement date. The mean veraison development for each block of each treatment was plotted using Origin™ software, version 7.0 (Microcal Software Inc, Northampton, MA, USA) and a sigmoid curve fitted to each block. The day on which 50% veraison occurred, and the veraison development rate for each block, was then determined using the data provided for the sigmoidal curve by the Origin software. These values were then statistically analysed using an ANOVA carried out in SAS using the GLM procedure to determine if there were significant differences among treatments.

4.2.4.3 Berry juice analyses

Berry samples were taken from three of the six blocks immediately prior to harvest for juice analyses. Samples were taken from all treatment combinations. Analysis of the juice was carried out as described in Chapter Two.

4.2.4.4 Harvest measurements

Measurements were carried out at harvest time in all six blocks, on all individual bunches that had been treated with plant growth regulators as well as the bunches that had been nominated to be control bunches. Harvest measurements carried out were as described in Chapter Two.
4.2.4.5 Bunch stem necrosis

All bunches treated with plant growth regulators on both the control vines and the vines with the laterals removed were visually scored for BSN incidence as described in Chapter Two. Bunches were scored four times from 100% veraison to harvest with the scores being approximately one week apart.
Chapter Four - Field trial: Source sink relationships and BSN. Results

4.3 Results

4.3.1 Vigour

4.3.1.1 Point quadrat analyses

Removing laterals from vines reduced the measured canopy development of the vines by significantly reducing LLN ($P=0.0002$, 0.0040) and PI ($P<0.0001$, 0.0039) and increasing PG ($P=0.0044$, 0.0250), compared to the control at both measurement times (Table 28).

Table 28: Mean leaf layer number, percent interior leaves and percentage gaps of control and vines where laterals had been removed.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaf Layer Number</th>
<th>Percent Interior Leaves</th>
<th>Percentage Gaps</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(LLN)</td>
<td>(PI)</td>
<td>(PG)</td>
</tr>
<tr>
<td>Measurement 1</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Laterals retained</td>
<td>3.8 a</td>
<td>4.5 a</td>
<td>49.2 a</td>
</tr>
<tr>
<td>Laterals removed</td>
<td>2.3 b</td>
<td>2.8 b</td>
<td>27.3 b</td>
</tr>
<tr>
<td>Measurement 2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Laterals retained</td>
<td>4.5 a</td>
<td>55.3 a</td>
<td>2.0 b</td>
</tr>
<tr>
<td>Laterals removed</td>
<td>2.8 b</td>
<td>36.9 b</td>
<td>9.6 a</td>
</tr>
</tbody>
</table>

Means within a column with a different letter are significantly different from each other at $P \leq 0.05$ (LSMeans, SAS)

4.3.1.2 Dormant canopy measurements

As there was no difference between the number of canes per vine and the number of effective canes per vine, the data presented are based on the total number of canes per vine. Manipulating the vine canopy did not significantly affect the total number of canes per vine. It did however, significantly affect the total pruning weight ($P=0.0176$), growing point number ($P=0.0006$), cane weight ($P=0.0124$), growing point weight ($P=0.0032$) and the number of growing points per cane ($P=0.0012$). Except in the case of growing point weight that was increased, the removal of laterals reduced all other significantly affected variables (Table 29).
**Table 29: Mean total cane number, total vine pruning weight, total growing point number, cane weight, growing point weight and growing point number per cane for control, and vines with laterals removed in Season Two.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total cane number</th>
<th>Total winter pruning weight (kg)</th>
<th>Total growing point no.</th>
<th>Mean cane weight (g)</th>
<th>Mean growing point weight (g)</th>
<th>Mean growing point no. per cane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laterals retained</td>
<td>22</td>
<td>4.1 a</td>
<td>78 a</td>
<td>191 a</td>
<td>52 b</td>
<td>3.9 a</td>
</tr>
<tr>
<td>Laterals removed</td>
<td>22</td>
<td>3.3 b</td>
<td>36 b</td>
<td>150 b</td>
<td>92 a</td>
<td>1.8 b</td>
</tr>
<tr>
<td></td>
<td><strong>ns</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means within a column with a different letter are significantly different from each other at $P \leq 0.05$ (LSMeans, SAS).

ns – not significantly different.

**4.3.2 Veraison development**

Veraison development followed a typical sigmoid curve over time (Figure 55). Veraison development began in some treatments in early February 2004 and continued for a month until early March 2004 by which time most bunches had reached 100% veraison. The canopy manipulation treatment of removing laterals with the application of the plant growth regulator, NPA, never reached 100% veraison during the sampling period.
Chapter Four - Field trial: Source sink relationships and BSN. Results

There were no significant differences in the date of 50% veraison for either the vines with laterals removed or the control vines. There was also no significant difference in the veraison development rate between the two treatments (Table 30).

There was however, a significant overall difference in the date of 50% veraison amongst plant growth regulator treatments ($P=0.0014$) but there was no interaction between the plant growth regulators and canopy manipulation treatments. Bunches that were treated with GA$_3$ reached 50% veraison earlier than the other plant growth regulator treatments of IAA and NPA ($P=0.0025$ and 0.0003, respectively) (Table 30). Overall, bunches treated with IAA or NPA were also significantly slower in reaching 50% veraison compared to the control ($P=0.0801$ and 0.0159, respectively) (Table 30).

There was also a significant difference in the rate of veraison development ($P=0.0212$) amongst the plant growth regulator treatments. When GA$_3$ was applied to bunches, the veraison rate was faster than for either of the other hormone treatments, IAA and NPA.
Table 30: Mean time of 50% veraison and mean veraison development rate for bunches treated with plant growth regulators and vines that have retained their laterals or vines that have had their laterals removed.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Laterals retained</th>
<th>Laterals removed</th>
<th>Overall mean</th>
<th>Laterals retained</th>
<th>Laterals removed</th>
<th>Overall mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>52.8 ab</td>
<td>54.5 ab</td>
<td>53.7 x</td>
<td>2.2 b</td>
<td>2.8 ab</td>
<td>2.5 x</td>
</tr>
<tr>
<td>GA₃</td>
<td>51.7 a</td>
<td>53.3 a</td>
<td>52.5 x</td>
<td>2.9 a</td>
<td>2.6 ac</td>
<td>2.7 x</td>
</tr>
<tr>
<td>IAA</td>
<td>54.2 b</td>
<td>56.8 bc</td>
<td>55.5 y</td>
<td>1.9 bc</td>
<td>2.0 c</td>
<td>1.9 y</td>
</tr>
<tr>
<td>NPA</td>
<td>54.0 b</td>
<td>57.3 c</td>
<td>55.7 y</td>
<td>2.1 bc</td>
<td>2.0 c</td>
<td>2.0 y</td>
</tr>
<tr>
<td>Overall mean</td>
<td>53.2 x</td>
<td>55.3 x</td>
<td>2.3 x</td>
<td>2.3 x</td>
<td>2.3 x</td>
<td>2.3 x</td>
</tr>
</tbody>
</table>

Means within a column and within a row with a different letter are significantly different from each other at \(P \leq 0.05\) (LSMeans, SAS).

*abc - applies to individual hormone and lateral means, xyz - applies to overall hormone and lateral means.
Chapter Four - Field trial: Source sink relationships and BSN. Results

(P=0.0080 and 0.0159, respectively) (Table 30). IAA and NPA treated bunches also had a slower veraison development rate when compared to the control (P=0.0511 and 0.0906, respectively).

4.3.3 Berry juice analyses

There were no interaction effects between the canopy manipulation and the application of plant growth regulators. The removal of laterals from the vines did not significantly affect any of the berry juice quality aspects (Table 31).

Application of the plant growth regulators, GA3, NPA and IAA, significantly affected the titratable acidity (P=0.0384), the brix/TA ratio (P=0.0889), potassium concentration (P=0.0332) and malic acid concentration (P=0.0316).

The application of the plant growth regulators GA3 and IAA increased the titratable acidity in the juice compared to the control (P=0.0446 and 0.0672, respectively) and the application of NPA (P=0.0157 and 0.0230, respectively), but they were not significantly different from each other (Table 31).

Using the Brix/TA ratio, bunches that were treated with GA3 were significantly less ripe than either the control (P=0.0616) or the bunches treated with NPA (P=0.0395). Bunches treated with IAA were only significantly less ripe than bunches treated with NPA (P=0.0661) (Table 31).

The application of all plant growth regulators resulted in juice with a higher potassium concentration compared to the control (P=0.0098 - 0.0258), but they were not significantly different from each other (Table 31).
Table 31: Harvest juice quality measurements carried out on juice from vines with canopy manipulation and with the application of plant growth regulators to bunches.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Brix (°)</th>
<th>TA (g l⁻¹)</th>
<th>B/TA (ratio)</th>
<th>pH</th>
<th>Malic acid (g l⁻¹)</th>
<th>Tartaric acid (g l⁻¹)</th>
<th>Calcium (mg l⁻¹)</th>
<th>Magnesium (mg l⁻¹)</th>
<th>Potassium (mg l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Canopy manipulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laterals retained</td>
<td>18.85</td>
<td>12.8</td>
<td>1.5</td>
<td>3.27</td>
<td>4.4</td>
<td>6.5</td>
<td>28.6</td>
<td>54.6</td>
<td>2359</td>
</tr>
<tr>
<td>Laterals removed</td>
<td>16.85</td>
<td>13.2</td>
<td>1.3</td>
<td>3.21</td>
<td>3.3</td>
<td>7.5</td>
<td>34.8</td>
<td>56.6</td>
<td>2298</td>
</tr>
<tr>
<td>(b) Hormone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>18.5</td>
<td>12.3</td>
<td>1.50 bc</td>
<td>3.25</td>
<td>2.4 b</td>
<td>6.3</td>
<td>29</td>
<td>54</td>
<td>2049 b</td>
</tr>
<tr>
<td>GA₃</td>
<td>17.4</td>
<td>13.9 a</td>
<td>1.25 d</td>
<td>3.23</td>
<td>6.6 a</td>
<td>7.4</td>
<td>33</td>
<td>56</td>
<td>2465 a</td>
</tr>
<tr>
<td>NPA</td>
<td>17.8</td>
<td>11.8 b</td>
<td>1.53 ab</td>
<td>3.26</td>
<td>2.5 b</td>
<td>7.0</td>
<td>30</td>
<td>54</td>
<td>2424 a</td>
</tr>
<tr>
<td>IAA</td>
<td>17.7</td>
<td>13.7 a</td>
<td>1.29 cd</td>
<td>3.22</td>
<td>3.9 b</td>
<td>7.5</td>
<td>35</td>
<td>59</td>
<td>2377 a</td>
</tr>
</tbody>
</table>

Means within a column with a different letter are significantly different from each other at $P \leq 0.07$ (Lsmeans, SAS).

$ns$ – not significantly different.
The application of GA$_3$ significantly increased the malic acid concentration in the juice compared to the control ($P=0.0105$) and both the plant growth regulators IAA ($P=0.0515$) and NPA ($P=0.0109$) (Table 31).

### 4.3.4 Harvest measurements

There were no significant interactions between canopy manipulation by the removal of laterals and plant hormone application, or canopy manipulation alone for any of the harvest measurements compared to the retention of laterals (Table 32).

<table>
<thead>
<tr>
<th>Table 32: Harvest measurements on bunches from canopy manipulated vines and from bunches treated with plant growth regulators.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment</strong></td>
</tr>
<tr>
<td>(a) Canopy manipulation</td>
</tr>
<tr>
<td>Laterals retained</td>
</tr>
<tr>
<td>Laterals removed</td>
</tr>
<tr>
<td>ns</td>
</tr>
<tr>
<td>(b) Hormone</td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>GA$_3$</td>
</tr>
<tr>
<td>NPA</td>
</tr>
<tr>
<td>IAA</td>
</tr>
<tr>
<td>ns</td>
</tr>
</tbody>
</table>

Means within a column with a different letter are significantly different from each other at $P\leq0.05$ (LSMeans, SAS)

*ns* – not significantly different

Bunch weight was significantly affected by the application of plant growth regulators ($P=0.0432$). Bunches treated with GA$_3$ were significantly heavier than control bunches ($P=0.0095$) and bunches treated with either NPA ($P=0.0567$) or IAA ($P=0.0321$) (Table 32). This may be caused by the mean berry weight being significantly affected by the application of hormones ($P<0.0001$). Berry weight for GA$_3$ treated bunches was heavier than the control, IAA and NPA treated bunches ($P<0.0001$) (Table 32). IAA
and NPA treated bunches also had significantly smaller berries than the control ($P=0.0401$ and 0.0423, respectively) (Table 32).

### 4.3.5 Seed number and seed weight

There were no significant differences in seed number or seed weight due to the removal of laterals, application of plant growth regulators or the interaction of the two (Table 33).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean seed number per berry</th>
<th>Mean seed weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Canopy manipulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laterals retained</td>
<td>1.27</td>
<td>38</td>
</tr>
<tr>
<td>Laterals removed</td>
<td>1.22</td>
<td>37</td>
</tr>
<tr>
<td>ns</td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td>(b) Hormone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.26</td>
<td>37</td>
</tr>
<tr>
<td>Ga$_3$</td>
<td>1.22</td>
<td>39</td>
</tr>
<tr>
<td>IAA</td>
<td>1.25</td>
<td>36</td>
</tr>
<tr>
<td>NPA</td>
<td>1.28</td>
<td>38</td>
</tr>
<tr>
<td>ns</td>
<td></td>
<td>ns</td>
</tr>
</tbody>
</table>


*ns* – means not significantly different at $P \leq 0.05$

### 4.3.6 BSN incidence

Due to the nature of the data for all assessments, assumptions of normality and heterogeneity of variance for BSN incidence were not met, and none of the transformations accessed were able to adjust the raw data to meet these assumptions. Normal ANOVA analysis was therefore, not possible. Also, no suitable non-parametric tests are available for split-plot experimental designs, so no statistical analyses could be performed. Therefore only trends of means can be discussed.
The removal of laterals did not influence the final BSN incidence assessed for bunches that were treated with NPA or GA$_3$. However, the development of BSN was different. For NPA treated bunches on vines with laterals retained, bunches obtained what appears to be their maximum BSN incidence quickly, while bunches on vines with laterals removed had only just begun to develop BSN at the last assessment. The reverse occurred for GA$_3$ treated bunches. The removal of lateral shoots did tend to increase BSN incidence for bunches that were not treated with any plant growth regulator, and reduce the BSN incidence for IAA treated bunches. For IAA treated bunches the difference in BSN incidence between vines with laterals retained and laterals removed was small compared to the difference between the control bunches on each canopy manipulation treatment (Figure 56).

![Figure 56: BSN incidence from 100% veraison to harvest in Season Two for canopy manipulated vines of laterals retained (control) and laterals removed and plant growth regulator dipped bunches of NPA, GA$_3$, IAA and control.](image)

In the vines with laterals retained the application of IAA to the bunches did not influence BSN incidence (Figure 56). NPA treated bunches had a slightly lower BSN incidence than the control bunches, with GA$_3$ treated bunches having a much lower BSN incidence than either the control or the other hormone treated bunches. This trend
was also apparent for vines with laterals removed, with GA$_3$ treated bunches having the lowest BSN incidence (Figure 56). For the IAA and NPA treated bunches however, there was no difference in BSN incidence between the two, although both had a lower BSN incidence than the control bunches (Figure 56).
Chapter Four - Field trial: Source sink relationships and BSN. Discussion

4.4 Discussion

In this group of experiments it was hypothesised that competition between vegetative and reproductive growth for carbohydrates and/or essential nutrients during the early stages of berry development resulted in bunches that were predisposed to BSN. This hypothesis has also been suggested by other authors. Kasimatis (1957) found that waterberry was most prevalent on thoroughly thinned, vigorous vines. They found that it was due to stresses caused by the competition between the fruit and vegetative parts for materials in short supply. Therefore the removal of lateral shoots, should have reduced the number of growing points in the canopy and the consequent vegetative competition, and would then have resulted in a reduction of BSN. However, this was not the case. Although there was a difference in BSN incidence due to the removal of laterals, on those bunches that had not been treated with a plant growth regulator, instead of a reduction in BSN there was a slight increase. Redl et al. (1984) also found that in some varieties, although not significantly different, the removal of ‘unnecessary shoots’ tended to result in an increase in BSN incidence. This effect, however, was not consistent across all varieties or all seasons.

It appears unlikely that in vines that did not have their lateral shoots removed, that assimilates were limiting after anthesis. By the time of berry set individual cane length and leaf development was such that there was likely to be adequate assimilate production. Hunter and Visser (1988a) found that at berry set 97% of assimilates from basal leaves were transported to the bunch. Although at this time only 5 and 17% of assimilates were transported to the bunch from middle and apical leaves respectively, it is likely that the basal leaves are able to provide the necessary assimilates to the bunch. However, in Hunter and Visser’s (1988a) study the vigour of the vine was not reported and it is possible that in high vigour vines the percentage of assimilates exported to the bunch may be affected. Studies have shown that defoliation, in the form of leaf or lateral removal, stimulated further vine growth (Hunter and Visser, 1990; Hunter, 2000) and other studies have shown that defoliation also changes the partitioning of assimilates, with less transported to the bunch and more transported to the apical leaves (Hunter and Visser, 1988b). Hale and Weaver (1962) also found that on a vigorous cane assimilate transport towards the shoot tip occurred in preference to transport to the bunch at the end of flowering. Therefore, it appears likely that the vigour of the vine
can influence assimilate partitioning. In the current study, assimilate transport to the actively growing apical bud may have occurred in preference to transport to the bunch on vigorously growing vines. However, further research into how the vigour of the vine may affect the partitioning of assimilates is required in respect to the predisposition of bunches to BSN.

Dry weight accumulation is initially slow after berry set on a per berry basis (Harris et al., 1968; Williams and Biscay, 1991) but the relative growth rate in relation to the initial berry weight is fast, which may indicate high reproductive sink activity and therefore a high competitive ability for assimilates. In studies where canes were girdled below the bunch at 50% flowering to isolate the cane from the rest of the vine, individual dry weight of the berry was not increased although berry set was and therefore overall bunch weight was also increased (Caspari et al., 1998). Therefore it would appear that at FB the production of assimilates is not limiting but that the low competitive ability of the inflorescence with the rest of the vine may affect berry set. This may also be the case immediately after berry set when the bunches may not have increased in their competitive ability sufficiently to compete with the rest of the vine for assimilates. The initial dry weight accumulation pattern in grape berries appears to differ depending on the number of seeds (Cawthon and Morris, 1982), the environmental conditions (Harris et al., 1968), and by comparing studies, among cultivars (Harris et al., 1968; Cawthon and Morris, 1982; Zhang et al., 2003), which may be due to how these factors affect the competitive ability of the bunch. Therefore, assimilates may still become a limiting factor after berry set in certain circumstances, but further research into this area is required before any relationship between BSN and possible limiting assimilates can be determined.

It therefore appears that the availability of assimilates may not have been a concern in vines that did not have their laterals removed, but that the competitive ability of the bunch immediately after berry set, for assimilates and/or nutrients, could have influenced the incidence of BSN. Dominance among sinks can often be observed very early in the development of reproductive sinks where competition for assimilates is unlikely due to the low demand of the sinks for assimilates at this time (Bohner and
Bangerth, 1988). Removal of the dominating organ leads to increased growth rates of the limited organ. The dominant organ does not appear to be determined by position on the plant, rather the sequence of development relative to each other (Bangerth, 1989). Primigenic dominance (PD) has been suggested as a name for this occurrence with apical, medial and basal dominance as special cases of primigenic dominance (Bangerth, 1989).

Through work carried out on apical dominance it is now generally accepted that the auxin IAA is the hormonal signal controlling dominance (Snow, 1929; Thomas, 1983). However, exactly how this plant growth regulator works is still not fully understood. With PD it is thought that the dominant organ exports higher quantities of IAA, increasing the concentration of IAA at the junction of the subordinate organ preventing IAA transport of that organ. The reduction or inhibition of IAA out of the subordinate organ may then reduce organ growth through a number of ways. It has been suggested that a reduction in vascular differentiation may occur and therefore the supply of assimilates, water and nutrients would be reduced. IAA may also have a direct effect on assimilate transport with an increase in the amount of assimilates being directed to the dominant organ that has higher IAA diffusion rates. The inhibition of IAA may cause an abscission of the organ, and finally, the build up of IAA in the subordinate organ due to the inhibition of IAA export may affect the hormone-mediated sink affects (Bangerth, 1989). Therefore, vegetative sinks may have had dominance over the developing reproductive sinks early after berry set through PD.

If PD was occurring in the vine, removal of the growing points should have decreased the incidence of BSN as there would have been less dominating vegetative organs. However, in these vines the incidence of BSN was increased. In this case the availability of assimilates may have been limiting for the developing bunches. Although some studies have found that partial defoliation can increase the photosynthetic activity of the remaining leaves (Iacono et al., 1995a) and therefore they are able to adequately compensate for the loss of the other leaves (Hunter and Visser, 1988b, 1988c, 1989), this may not have occurred in the current study. Hunter (2000) found that treatments where laterals were removed did not increase the photosynthetic
activity of the remaining leaves. Ollat and Gaudillere (1998) also found that when they manipulated the canopy by the removal of leaves, apical buds and lateral growth after anthesis, that the remaining leaves did not compensate photosynthetically. The decrease in available assimilates in Ollat and Gaudillere’s (1998) study resulted in a reduction in early berry development that would also suggest that these berries were detrimentally affected by the defoliation. It therefore appears that leaf removal may cause the remaining leaves to compensate photosynthetically, but lateral removal has no such effect. As it was the laterals that were removed in the current study, it is expected that the remaining leaves did not therefore increase in their photosynthetic ability.

However, Caspari and Lang (1997) did not find that the remaining leaves compensated for the leaves removed. The removal of even one leaf off a cane was found to decrease fruit set, mean berry weight and cluster weight. However, it would appear that Caspari and Lang (1997) began their removal of leaves with those closest to the bunch and these leaves, along with the laterals in this area, are vital to the developing bunch for assimilate supply, especially in the early stages of bunch development (Hunter and Visser, 1988a, 1988b; Caspari et al., 1998). Therefore their removal would affect bunches more than random leaf removal, which would leave a higher percentage of young leaves in the lower canopy close to the bunch, including leaves on laterals, providing the developing bunch with more assimilates. This theory is supported by other studies that found the removal of laterals decreased juice soluble solids in grapes (Vasconcelos and Castagnoli, 2000). As all laterals were removed in the current study, including those close to the bunch, and the literature indicates that the remaining leaves may not have compensated for the removal, it is probable that the supply of assimilates available for the developing bunch were reduced in the lateral removal treatment, thus affecting early berry development as also found by Ollat and Gaudillere (1998).

The removal of the laterals may also have increased the PD of vegetative growth over the reproductive sinks. Lateral and leaf removal has been found to have a stimulatory role in vine growth with increased vegetative growth through further lateral growth (Hunter and Visser, 1990; Hunter, 2000), an increase in leaf mass (Hunter and Visser, 1990) and the greater expansion of remaining leaves. Lateral removal also results in a
higher degree of compensatory growth than leaf removal alone (Hunter, 2000). Kinet et al. (1985) found in tomato that when there is a limited amount of assimilates available that most of the assimilates are imported into the young developing leaves to the detriment of the developing inflorescences. In the current study although lateral growth did not appear to be stimulated due to the removal of lateral shoots, the mean growing point weight was increased. This would suggest that the vegetative growth was increased. If the reproductive sinks were effective in their ability to compete for the reduced assimilates available in the lateral removal treatment it is suggested that there would not be any evidence of an increase in vegetative growth, and berry development would not be detrimentally affected. However, the date at which 50% veraison occurred was later for vines that had lateral shoots removed, indicating that lateral removal was detrimentally affecting berry development prior to veraison.

Studies have also shown that the competition among sinks is amplified when there is limited assimilate supply (Ho, 1988). In tomatoes when assimilate supply is low the initiating truss only obtains assimilate after the demand by the apex and young leaves has been met (Kinet, 1977). With limiting assimilate supply, bunches with the greatest competitive ability would have been able to effectively compete with subordinate bunches for the limited assimilates available. Field observations showed that when a cane was fully defoliated, bunches subtending this cane shrivelled and failed to ripen. It was therefore assumed that little assimilate movement occurred among canes as otherwise bunches may have been able to obtain assimilates from other canes on the vine. However, literature indicates that this is in fact may not be the case (Quinlan and Weaver, 1970; Stewart et al., 1996; Vanden Heuvel et al., 2002). In the current study bunches that were submersed in plant growth regulators, with the possibility of increasing the competitive ability of that bunch, did not show an increase in BSN incidence due to the removal of laterals. Plant growth regulators have been shown to play an important role in the translocation of assimilates (Lucas and Madore, 1988). Exogenous IAA and gibberellins are known to stimulate assimilate translocation to the site of application of these compounds (Gianfagna, 1995). Defoliation can alter the equilibrium of endogenous growth regulators through either the removal of a potential site of synthesis or indirectly through altering the source/sink relationship (Candolfi-Vasconcelos et al., 1994). Therefore, hormone treated bunches may have attracted
assimilates from canes where the bunches were not treated with a plant growth regulator, further decreasing the assimilate availability of that bunch and increasing the incidence of BSN.

### 4.4.1 Plant growth regulators

Although it was noted by some authors that seeded grape varieties do not respond favourably to the application of GAs (Coombe, 1973; Bhullar and Dhillon, 1974; Gianfagna, 1995), in this study berry size was significantly increased by GA₃ application as has been previously found in seedless cultivars (Miele et al., 1978). As the application occurred after berry set, there were no detrimental effects on berry number per bunch as has been found when GA₃ is applied during bloom (Miele et al., 1978). In Japan the seeded cultivar ‘Delaware’ is treated with GAs to increase berry size as well as to induce parthenocarpic fruit development (Gianfagna, 1995). Parthenocarpic fruit development however, did not occur with the GA₃ treatment in the current study. In fact, seed number and weight were not significantly affected by any of the plant growth regulator treatments. This again was probably due to the timing of the treatments in relation to berry set.

The GA₃ treated bunches tended to have a lower ripeness ratio than the control with higher TA and, although not significantly different, a lower Brix concentration. This result is consistent with other studies (Gianfagna, 1995; Ben-Arie et al., 1998). Ben-Arie et al. (1998) found that the application of GA₃ delayed ripening in grapes, although not significantly. In lemons GAs are used to delay ripening so that the availability of fruit is higher during the time that demand is high (Gianfagna, 1995). However, the date of 50% veraison and the rate at which veraison progressed was generally not statistically influenced by the application of GA₃ post berry set compared to the control. Where GA₃ did affect veraison development rate in vines where laterals were retained, the rate was increased. Therefore the reduction in maturity at harvest was not due to a delay in the onset of veraison or the rate at which it progressed. Bunches treated with GA₃ did have significantly larger berries than control bunches and as small berries must store less sugar than large berries to reach the same sugar concentration (Ollat and Gaudillere, 1998; Dreier et al., 2000), the reduction in the maturity ratio due to GA₃
application may be purely due to GA₃ application increasing berry size. Some studies have found an increase in Brix concentration due to GA₃ application (Miele et al., 1978), but as the application occurred during flowering thus resulting in reduced berry set, there would have been less competition for assimilates among berries within a bunch. In the current study there was no affect on berry set and therefore competition among berries within a bunch would not have decreased. If anything, due to the increase in berry size, competition may have increased, which also indicates that assimilate production was not limiting.

Although the final BSN incidence for GA₃ treated bunches was the same between vines that had laterals removed or retained, the development differed. In vines where the laterals were retained there was very little incidence of BSN until the final measurement, whereas for vines where laterals were removed the incidence of BSN reached its final value early on and then did not increase further. This may be due to the fact that for GA₃ treated bunches the BSN incidence was generally low, and one bunch at 100% severity results in a large relative increase in the BSN incidence assessment. On vines with their laterals retained one bunch exhibited 100% severity at the last measurement increasing the incidence, whereas for vines with laterals removed, a few bunches demonstrated low incidences initially, but never worsened.

The reduction in final BSN incidence due to GA₃ application is in accordance with other studies (Allleweldt and Hifny, 1972; Beetz and Bauer, 1983; Haub, 1983; Theiler and Coombe, 1985). Theiler and Coombe (1985) found that not only did the application of GA₃ reduce BSN incidence but it also increased the cross-sectional area of metaxylem. They suggested that BSN development was due to a deficiency in gibberellins in the berries during berry set. The application of an cytokinin and/or gibberellins to aborting tomato inflorescence causes cellular activity to resume in the ovules (Kinet et al., 1985). Although not significantly different from the control, GA₃ treated bunches tended to have larger peduncle diameters, which may indicate increased cell, including xylem, development within the peduncle. This, however, requires further investigation. Gibberellin biosynthesis can occur in all growing, differentiated tissue and there is undeniable evidence that developing fruits and seeds are sites of
gibberellin biosyntheses (Radley, 1958; Phinney and West, 1960 and references there in; Davies, 1995a). It is also generally accepted that immature organs such as stem apical regions, expanding leaves (Sponsel, 1995) and root apices (Richards, 1983) are also sites for gibberellin biosynthesis. The biosynthesis of gibberellins begins in immature seeds shortly after anthesis and at this stage the GAs are qualitatively and quantitatively similar to those in vegetative tissue (Sponsel, 1995). It is thought that the presence of GAs in the seed may be important for the early development of the seed soon after fertilisation in many crops (Garcia-Martinez, 1998) and it is the immature seed that contains the highest concentrations of GAs (Raven et al., 1981).

For Theiler and Coombe’s (1985) hypothesis that BSN development is due to a deficiency in gibberellin concentration in the berries during berry set, to be robust, it could be assumed that only the application of gibberellins around berry set would influence the incidence of BSN as this is the time of peduncle development. However, other authors have found that application of gibberellins over the entire period between berry set and veraison also decreases BSN incidence (Hifny, 1971; Beetz and Bauer, 1983) but exactly when the application is most effective is not conclusive. Beetz and Bauer (1983) found that in one year the application of GA3 when berries were 7 mm in diameter was more effective than when GA3 was applied either when berries were 4 mm in diameter or during the lag stage before veraison. Yet in the following year, they found the opposite with applications that occurred when berries were 4 mm in diameter or during the lag stage, being equally effective in reducing BSN incidence, and both were more effective than when GA3 was applied when berries were 7 mm in diameter. Interestingly, Alleweldt and Hifny (1972) found that GA3 only reduced the incidence of BSN if it was applied directly to the rachis in a lanolin paste. Irrespective of application time, if bunches were sprayed with gibberellins in an aqueous solution, the incidence of BSN was not affected. However, Haub (1983) found that spraying bunches with gibberellins when the berries were between 4 and 7 mm in diameter (approximately 20 days after flowering) did reduce BSN incidence.

Even when gibberelin is applied directly to the rachis just prior to veraison, stronger collenchyma cell walls of the cortex, activation of the cambium and the development of
numerous, non-lignified xylem cells occurs (Alleweldt and Hifny, 1972). In natural rachis growth cambium and xylem cell development has ceased by the end of bloom and all other cell development has ceased by the time of veraison (Theiler and Coombe, 1985). These results would therefore suggest that application of gibberellin reactivates xylem and cambium cell development and either reactivates or enhances the development of other cells such as the collenchyma. Gibberellins have also been found to be important for cambial growth in other plant species and thought to be involved in the regulation of cambial growth (Siebers and Ladage, 1973; Li and Cui, 1985; Wang et al., 1995) through cambial cell division (Ridoutt et al., 1996). It has been suggested that the elongation of differentiating secondary xylem fibres in woody angiosperms is dependent on GA1 levels in the cambial region (Ridoutt et al., 1996).

The increase in cell division and expansion in the rachis may therefore lead to the decrease in BSN incidence. It would appear that Theiler and Coombe's (1985) hypothesis of BSN being caused by a deficiency in gibberellin in the developing berry during berry set fitted with the findings of During and Lang (1993) of more susceptible cultivars tending to have a restriction in the xylem system. During and Lang (1993) hypothesised that it was this restriction that limited xylem flow and therefore nutrients such as calcium to the developing berry, and this predisposed bunches to BSN. Xylem and phloem differentiation are controlled by auxin concentrations, with high concentrations leading to xylem differentiation and low concentrations leading to phloem differentiation (Aloni, 1995). However, gibberellins also play a role in vascular differentiation. Generally, high IAA and low gibberellin concentrations result in xylem differentiation and low IAA and high gibberellin concentrations lead to phloem differentiation (Digby and Wareing, 1966). However, if IAA concentrations are high, and gibberellin is very low and is the limiting substance, the application of gibberellin may result in the differentiation of xylem. This may have been the case in Theiler and Coombe's study (1985). A deficiency in gibberellin may therefore lead to a reduction in xylem development and the restriction within the xylem. Cultivars susceptible to BSN may therefore be more likely to have a deficiency in gibberellins during berry set especially since potential concentrations of interacting growth regulators are genetically determined (Ho, 1988). However, the increase in xylem development due to the application of gibberellins may not be responsible for reducing the incidence of BSN.
Instead, the exogenous gibberellin may be decreasing BSN incidence through some other plant response to gibberellin. This may include the response of phloem differentiation to gibberellin application. Varying ratios of IAA and GA₃ have been found to influence phloem fibres. Aloni et al. (1990) found that high IAA/low GA₃ induced short phloem fibres with thick secondary walls that were lignin rich. Low IAA/high GA₃ promoted long phloem fibres with thin walls. Therefore, the response of phloem differentiation to GA₃ application to the rachis may also influence the predisposition of a bunch to BSN incidence.

With the increase in strength of the collenchyma cell walls of the cortex, further cell development of the cambium and xylem and a reduction of BSN incidence due to gibberellin application near veraison, it may be considered that BSN is caused by weak cells or restricted cell development. As the initial symptoms of BSN are the stomata, epidermis and hypodermis cells becoming necrotic (Delas et al., 1976; Theiler, 1976; Brendel et al., 1983) the difference between these initial symptoms (primary) and further symptom development (secondary symptoms) may be the difference in the cell development of these areas in the peduncle. It is suggested that strong collenchyma cell walls may prevent the further spread of the necrosis to the phloem and therefore prevent the secondary symptoms of BSN developing, or that the cortex cells are indicative of what may be occurring in other cells.

Studies have shown that application of GA₃ to a plant part increases the amount of assimilates transported to that plant part (Quinlan and Weaver, 1970; Jahnke et al., 1989). Drier et al. (1998) found that GA₃ treated bunches incorporated approximately twice as much insoluble carbohydrates on a per berry basis than the untreated bunches. The increased competitive ability of a sink due to gibberellin application may be due to an increase in cell division and therefore the increase in cell number of the berry, or it may be due to an increase in phloem unloading. Either way, an increase in assimilate and nutrient transport to the berry would have occurred (Ho, 1992). This increase, (in assimilate and nutrient supply) may have influenced cellular generation in the rachis and/or seed and therefore the predisposition to BSN may be affected. However, it is difficult to determine if any of these ideas are plausible until it is known whether it is
the rachis/peduncle, the berry or the seed, which is predisposing the bunch to the initial symptoms of BSN development. Further research is therefore required in this area.

The effect on BSN of applying GA₃ to the bunches, may not be directly due to gibberellins. The application of gibberellins has been found to increase endogenous auxin activity in fruit such as mandarins (Garcia-Martinez and Garcia-Papi, 1979) and grapes (Bertrand and Weaver, 1972). It is thought that GA₃ causes a more efficient release of IAA because GA₃ has been found to stimulate the basipetal transport of IAA in legumes (Jacobs and Case, 1965; Tamas, 1995).

IAA treated bunches also tended to have a lower BSN incidence when laterals were removed, but not when laterals were retained. Theiler and Coombe (1985) found that the application of α-naphthalene acetic acid (NAA – an auxin-like compound) and 2-chloroethyltrimethyl ammonium (CCC – a gibberellin inhibitor) did not reduce the incidence of BSN when applied during flowering, even though the application of NAA to de-berried peduncles did prevent abscission and peduncle death. The application of IAA also prevented peduncle death of de-berried bunches but only when treatments were applied just after fruit set. However, Theiler and Coombe (1985) found in their study, that unlike the application of GA₃, NAA did not change the thickness or tissue area of the peduncle, and these assessments were not looked at in the IAA treatments.

In contrast, Alleweldt and Hifny (1972) did find a reduction in BSN incidence due to the application of IAA directly to the rachis 43 days after flowering (prior to veraison). They also found that early applications of NAA (as a spray in an aqueous solution) reduced the number of bunches with secondary symptoms, although not primary, but that later applications did not have an effect. As for GA₃, the late application of IAA direct to the rachis did result in stronger collenchyma cell walls of the cortex and activation of the cambium and when applied with GA₃ also resulted in stronger walled parenchyma cells, sclerenchyma cells and lignified metaxytem. This is because auxins stimulate cell enlargement, cell division and vascular tissue differentiation (Davies, 1995a). In the current study IAA application did tend to increase peduncle diameter, although not significantly compared to the control. Therefore, although not assessed in
the current study, increased cell division and/or expansion may have occurred, which is in keeping with Alleweldt and Hifny’s (1972) results.

Auxins are used in horticulture to prevent fruit drop (Gianfagna, 1995). When auxins are applied to apples during the mid stages of fruit growth, abscission is delayed or prevented (Southwick et al., 1953). It is thought that auxins maintain the abscission zone in a non sensitive state so that it does not respond to ethylene therefore preventing abscission (Reid and Howell, 1995). However, the relationship between auxin and abscission is complex and not fully understood as the application of exogenous auxin at certain times can in fact increase the rate of abscission (Davies, 1995a). For example, auxin-like substances applied directly after fruit set in apples can increase fruit drop and are therefore used as fruit thinning treatments (Ebert and Bangerth, 1981; Gianfagna, 1995). Ebert and Bangerth (1981) also found that the application of an auxin-like substance after fruit set decreased the diffusible gibberellin peak content but that it had varying effects on the extractable gibberellin concentration, again demonstrating the complexity of the interaction between growth regulators. The relationship between auxin and senescence is also complex in that in some species exogenous auxin prevents senescence yet in other species it can promote it (Dangl et al., 2000).

The concentration of endogenous auxin in grape berries increases to a maximum during the lag stage of berry growth. Not long after this maximum is attained auxin concentrations are undetectable or very low in the berry (Alleweldt and Hifny, 1972; Bhullar and Dhillon, 1974; Zhang et al., 2003). Therefore, it is possible that the endogenous concentrations of auxin are preventing symptoms of BSN occurring in predisposed berries until after auxin concentrations have declined, resulting in the characteristic appearance of the first BSN symptoms not long after veraison. In this case auxin may be acting in a similar manner as it does when used to prevent fruit drop. The application of IAA direct to the rachis in Alleweldt and Hifny’s (1972) study may therefore have resulted in a reduction in BSN incidence due to either the increase in cell growth and integrity, or the continued inhibition of BSN symptoms through mechanisms similar to auxin inhibition on abscission and senescence. The lack of any control of BSN due to late sprayings of NAA (Alleweldt and Hifny, 1972) may be due
to the auxin being applied primarily to the berry surface, as at that stage in bunch development the berry size would have resulted in bunch closure and consequently limited spray access to the rachis. Alternatively the sensitivity to auxin may become less as endogenous concentrations of auxin decline, and it is also possible that the effect of auxin applications may change once auxin concentrations in the berry are undetectable. Application of the auxin-like compound, benzothiazole-2-oxyacetic acid (BTOA) after veraison and after the drop in endogenous auxin concentration has been found to induce BSN like symptoms (Holzapfel and Coombe, 1998). BSN symptoms were directly related to the timing of the application of BTOA, with fewer symptoms developing the earlier the application (Holzapfel and Coombe, 1998). This may be similar to a response already mentioned in apples where auxin application results in thinning when applied at fruit set and the prevention of abscission when applied later in fruit growth.

The drop in endogenous auxin concentration in the berry may also be one of the internal triggers within the berry for ripening to begin (Davies et al., 1997). The application of BTOA and NAA, causes a delay in the onset of ripening (Coombe and Hale, 1973; Davies et al., 1997; Yakushiji et al., 2001). The results from the current study for IAA application are consistent with these findings. The application of IAA caused a delay in veraison, a decrease in the veraison development rate, and although not significantly different from the control, a decrease in the maturity ratio at harvest through a lower Brix concentration and a significantly higher TA.

As the application of IAA only reduced BSN incidence compared to the control when laterals had been removed (and therefore there were fewer apical buds), the concentration of the IAA thus applied (or the frequency of the application), may not have been enough to increase the competitive efficiency of the bunch with the apical buds in the treatment where the laterals were retained. When IAA was applied early in the bunch’s development an increase in sink competitive ability may have occurred. It is thought that this increase may have occurred because auxin increases the flow of assimilates to the fruit (Ho, 1992) through PD (Bangerth, 1989). As IAA is readily metabolized (Theiler and Coombe, 1985; Gianfagna, 1995), and as it was only applied
once, it may have influenced the sink strength of the berry only while it was still present within the berries. Once the exogenous IAA was transported out, endogenous IAA concentrations may have returned to normal. Thomas (1983) found that the inhibitory effect of IAA on the growth of axillary buds of Brussels sprouts was slowly lost and IAA had to be reapplied to prevent further axillary bud growth. As well, if the concentration was not high enough to significantly increase the efflux of auxin from the berry compared to the efflux of auxin from the apical bud, then an increase in assimilate translocation to the bunch would not occur.

NPA treated bunches had a very similar final BSN incidence compared to the IAA treated bunches, although the development of BSN incidence differed between vines with laterals retained and removed. The similarity in BSN incidence was unexpected as NPA, as an auxin transport inhibitor, is likely to increase BSN incidence if low auxin concentrations were partly responsible for BSN incidence. Applications of NPA, even at veraison, have been found to decrease the anthocyanin content and Brix concentration and increased TA (Yakushiji et al., 2001). In contrast in the current study NPA delayed veraison and slowed the veraison development rate, but only for bunches on vines with laterals removed. At the time of harvest there were no significant differences in Brix concentration, TA or the maturity ratio compared to the control. However, for vines with laterals removed the Brix concentration and TA were slightly lower in NPA treated bunches compared to the control (Appendix 5). All of these assessments indicate that bunches treated with NPA on vines with laterals removed, may have been delayed in their maturity compared to the control and compared to NPA treated bunches on vines with laterals retained. This may explain the difference in BSN incidence development for NPA treated bunches on vines with laterals retained and removed. It is possible that the BSN incidence for NPA treated bunches on vines with laterals removed at the same maturity as NPA treated bunches on vines with laterals retained may have been higher. Application of 2,3,5-triiodobenzoic acid (TIBA) has been found to delay the onset of ripening when applied at the beginning of the lag stage but does not affect the Brix concentration at harvest (Coombe and Hale, 1973; Yakushiji et al., 2001). When TIBA was applied two weeks prior to veraison there was an increase in ethylene concentration in the berry, which gave a similar result to when BTOA was applied (Coombe and Hale, 1973). Treatments that delayed veraison also delayed the increase in ABA
concentration in the berry (Coombe and Hale, 1973). However, it appears that NPA only had an affect on bunches when assimilates may have been limiting. However, a reduction in maturity would not account for the lack of difference in BSN incidence between IAA and NPA treated bunches as NPA treated bunches either had a significantly higher maturity ratio than IAA treated bunches (on vines with laterals retained) or the maturity appeared to be similar (on vines with laterals removed) (Appendix 5).

Although the application of auxins and auxin transport inhibitors after fruit set appear to have resulted in a similar result, the way in which it was achieved was probably different. Delayed maturity due to IAA application was possibly due to an increase in IAA concentrations around veraison. Although IAA is readily metabolised, and the exogenous IAA would probably have broken down by the time of veraison, the effect of IAA on the early development of the berry may have resulted in increased concentrations of endogenous IAA. In contrast the application of NPA may have affected early berry development by reducing the berries competitive ability, therefore reducing assimilate and nutrient movement into the berry and causing veraison to be delayed. However by Stage III, when auxin concentrations are naturally low, the NPA would not have had an effect if applied at this time.

NPA has been shown to not only inhibit auxin transport directly, but also to affect cellular events such as auxin metabolism (Yoshida et al., 2005). NPA has been shown to exhibit slight auxin activity (Keitt and Baker, 1966). Vascular differentiation has been found to occur after plant parts have been treated with NPA (Casson and Lindsay, 2003), and stimulation of cell division in the quiescent centre of the root cap has also occurred (Ponce et al., 2005). However, although cell division of tobacco cells was inhibited by NPA, it was also inhibited by large amounts of NAA (Petrasek et al., 2002). NPA reduces basipetal polar auxin transport (Thomson et al., 1973) by inhibiting the auxin efflux carrier and reducing auxin transport out of cells. However, auxin uptake continues (Lomax et al., 1995) and therefore accumulation of auxin in the cells occurs (Casimiro et al., 2001). Both the IAA and NPA treated berries were significantly smaller than the control berries, which is consistent with other studies on
kiwifruit (Currie, 1997). Therefore the accumulation of IAA in the berry, due to either increased exogenous IAA or a reduction in the polar transport and an increase in endogenous IAA, may have been super-optimal and may have decreased cell division or reduced expansion processes. Also, the reduction in polar transport may decrease the assimilate transport into the berry, preventing optimum berry growth (Bangerth, 1989).

Hegele et al. (2004) found that the reduction in IAA diffusion from mango leaves caused by NPA application was only moderate, and two days later there was a sharp rise in diffusible IAA. However Currie (1997) found IAA transport was inhibited for at least 21 days when NPA was applied to kiwifruit pedicels. It is difficult to determine how long the NPA remained active in the current study as assessments were not carried out to measure this. If the effect of NPA was short-lived, once the efflux carrier was no longer inhibited, the accumulated IAA may have been transported back out of the berry, again increasing PD and therefore decreasing BSN incidence. Although there may have then been an increase in assimilate transport to the berry due to the increase in auxin efflux, as the cell number would have been detrimentally affected the potential competitive ability of the sink would have been reduced (Ho, 1992).

The mechanisms behind the delay in veraison onset for NPA treated bunches compared to the control bunches maybe similar to that for IAA treated bunches. A delay in the reduction of IAA and/or the increase in ABA may have occurred. This may be due to an effect on early bunch growth processes that may include interactions with other endogenous growth regulators. Although only gibberellins and IAA have been discussed in relation to BSN it is possible that other growth regulators may also be involved as cytokinins are known to improve fruit set (Weaver et al., 1965) and the start of berry formation is accompanied by intense cytokinetic activity (Calo et al., 1996).

Coombe (1960) suggested that GAs may be involved in the growth mechanism during Stage I of seedless but not seeded cultivars as the responsiveness of berries to exogenous GA₃ was correlated with their endogenous concentrations of GAs. Seedless cultivars of grapes tended to have higher concentrations of endogenous GAs than seeded cultivars. Coombe (1960) also found that berry growth was correlated to
meristematic activity in the seed and auxin concentrations during Stage I in seeded but not seedless cultivars, and suggested that in seedless cultivars growth was controlled by gibberellin concentrations or a combination of gibberellins and auxins. As ‘Cabernet Sauvignon’ grapes, a seeded cultivar, increased in berry size due to GA₃ application in the current study it would appear that gibberellins are involved in the berry growth of this cultivar, and may therefore have been limiting for these bunches. It is also possible that the GA₃ application increased the meristematic activity in the developing seeds that then produced more auxins. It is well known that in seeded fruit, auxin from the fruit is related to the seed number (Ho, 1992). It is also possible that the effect of auxin on BSN was limited compared to the GA₃ affect, as often the effect of one hormone is dependant on the presence of one or more other hormones (Davies, 1995b).

In conclusion, it is difficult to determine from the current study exactly how the various hormones are reducing the incidence of BSN. There is also the possibility that changes in the hormones themselves affect the nutrient balance of the bunch, or that the nutrient balance of the vine or bunch is affecting the endogenous growth regulators. Further research is required to answer these questions that may include the effects of girdling, extraction of endogenous hormones and work with carbon-14.
5 Controlled Environment Studies

5.1 Introduction

Environmental conditions at particular times in the development of the grape berry have been implicated in the development of BSN. However, the literature is often conflicting. For example, rain events around veraison have been thought to increase BSN incidence (Brechbuhler, 1987; Redl, 1987; Baldacchino-Reynaud, 2000) and yet other studies have found no causal effect (Hartmair, 1975; Boselli et al., 1986, 1987; Holzapfel and Coombe, 1995). Other times that have been suggested as critical growth periods in which conditions can influence the incidence of BSN, are before flowering (Holzapfel and Coombe, 1995), during flowering (Theiler, 1983, 1986b; Theiler and Muller, 1986) and after flowering during the first growth phase of berry growth (Boselli et al., 1987; Baldacchino-Reynaud, 2000).

In field trials using shade cloth, it was found that the time immediately after fruit set was critical in the determination of BSN incidence, as a reduction in light quantity during this time increased the incidence of BSN (see Chapter Three). Also, plant growth regulators applied during this time affected the incidence of BSN (see Chapter Four). Therefore it seems likely that during the first phase of berry growth, when cell division and expansion are occurring, bunches can become predisposed to the incidence of BSN. However, as trials were carried out in the field, it is difficult to be certain that the incidence of BSN was not also influenced by other environmental factors.

Controlled environment (CE) rooms can be used to control desired environmental conditions in order to determine whether plant responses are due to treatments or to some other external influence. Jordan (1985) found that vines grown in controlled environment rooms with high relative humidity conditions (80%) developed significantly more BSN incidence than vines grown in low relative humidity conditions (40%). Rain events, which increase the humidity, possibly reduce the uptake of nutrients into the vine due to a reduction in transpiration. High vigour vines, another proposed causative of BSN incidence (Bolay et al., 1965; Theiler, 1975a; Cline, 1987; Moreno and Pavez, 2000), might also increase the humidity conditions around the
fruiting zone. In Jordan’s (1985) study, vines were grown in CE conditions for the entire duration of the bunch development and therefore any timing effect could not be determined. Also, vines within each relative humidity (RH) treatment were supplied with different nutrient solutions that varied in their calcium and potassium concentrations, although Jordan (1985) found that these different nutrient concentrations made no difference to the subsequent incidence of BSN.

It has often been reported that the incidence of BSN is caused by a deficiency in calcium or magnesium and has therefore been considered to be a similar disorder to other calcium disorders, such as bitter pit and watercore in apples, and blossom end rot in tomatoes and peppers (Boselli and Fregoni, 1986; Haub, 1986). Tipburn in vegetables is also another disorder considered to be due to a deficiency in calcium ions (Saure, 1998). High humidity has often been correlated with a high incidence of tipburn (Palzkill et al., 1980; Barta and Tibbitts, 1986) as it was thought that a low transpiration rate under such conditions lead to reduced calcium transport. However, a high evaporative rate has also been found to increase tipburn (Collier and Wurr, 1981). It has been hypothesised that low transpiration at night time was actually beneficial as it increased the calcium transport to the young apices and to young leaves due to the root pressure flow increasing the concentration of calcium ions in the xylem sap (Palzkill et al., 1976). Later studies however, excluded root pressure as the main driving force for the import of calcium (Geijn and Smeulders, 1981; Ho and Adams, 1989). Therefore alternative explanations for the influence that humidity has on tipburn, other than changes in calcium, are needed (Saure, 1998). This may also be the case with BSN incidence.

Other studies have suggested that BSN development is due to a reduction in the xylem development of the peduncle of susceptible cultivars resulting in a decrease in sap flow and, consequently in the flow of important nutrients such as calcium into the bunch (During and Lang, 1993). Sap flow sensors, whether heat balance or Granier-type sensors, have often been used to measure sap flow in vines to determine water usage and to improve crop water management (Ginestar et al., 1998; Braun and Schmid, 1999; Lovisolo et al., 2000). The heat balance method has been shown to be very sensitive,
recording fluctuations in sap flow to within 10 minutes (Braun and Schmid, 1999). The
Dynamax® stem flow gauges (Dynamax Inc., Houston, TX, USA) have also been found
to be accurate to within 5 to 10% of the daily value of transpiration (Lascano et al.,
1992) but, unlike the Granier method, they are non-invasive. Therefore the Dynamax®
stem flow gauge system was used in this study to measure sap flow in the cane and the
peduncle, and also to determine whether the incidence of BSN is likely to be caused by
a simple reduction in nutrient supply into the bunch.

The other aims of the experimental work described in this chapter were to re-evaluate
the results that Jordan (1985) obtained, and to determine whether there was a critical
time during which environmental conditions predispose bunches to BSN incidence.
The removal of laterals was carried out on some vines to further investigate the findings
from previous studies (as described in Chapter Four).
5.2 Materials and Methods

5.2.1 Plant material

Controlled environment experiments were carried out on potted vines in the 2004/05 season. Four-year-old ‘Cabernet Sauvignon’ vines on SO4 root stock were used. Vines were potted into 40 L pots two winters prior to the 2004/05 growing season, using a potting mix of 1:1:1 pumice:bark:peat. While not in the CE rooms vines were maintained in a covered Perspex greenhouse until the danger of spring frost was past. They were then moved to a sheltered outdoor standing area prior to flowering. Vines were irrigated and fertigated daily during the growing season with half strength Hoagland’s solution (Appendix 6). The spray schedule for pests and diseases was as used in the field experiments (Appendix 2).

During the dormant season vines were spur pruned to a system similar to VSP (vertical shoot positioning) and trained onto a double T aluminium frame. The cordons were 0.5 m each in length with approximately 5 buds per cordon and were trained to the lower arm of the frame (Figure 57). During the growing season, the current season’s canes were trained up strings that were attached between the upper and lower arms of the frame. Canes were trimmed when they reached the upper arm at approximately 1.0 m in height.
5.2.2 Experimental design

The CE rooms located at HortResearch, Palmerston North were used in this experiment. Four CE rooms were used and all rooms were set at 23/11°C day/night temperatures for the entire length of the experiment. Day length was 16 hours with an 8 hour night. Photosynthetic photon flux (PPF) was set at $600 \pm 15 \, \mu\text{mol m}^{-2} \, \text{s}^{-1}$, which is considered to be close to optimum for photosynthesis on grapevines (Jackson and Lombard, 1993). Temperature and vapour pressure deficit (VPD) changes were ramped over a 120 minute period within the 16 hour day period so that maximum temperature and VPD were obtained for 12 of the 16 hours (Figure 58). Two CE rooms were used for each humidity treatment: A) constant high RH treatment of 80% (corresponding to day/night VPD conditions of 0.57/0.27 kPa) and B) constant low RH treatment of 40% (corresponding to day/night VPD conditions of 1.68/0.79 kPa). Each CE room was classed as a block and therefore there were two blocks per humidity treatment.
Figure 58: Diagram of how conditions changed and related to each other in the CE rooms using the low humidity room as an example. Day/night VPD conditions for the high humidity room were 0.57/0.27.

After budburst, when the inflorescence number per vine was able to be determined, vines were randomly allocated to one of two lateral treatments; 1) laterals were left on the vines and 2) laterals were removed from the vines on a weekly basis. The inflorescence number per vine was variable across the vines that were available so vines were initially grouped by inflorescence number, and then re-allocated from these groups to the lateral treatment groups on a random basis. This was to ensure that similar inflorescence numbers were achieved in each lateral group, and that the distribution of inflorescences across the vines was also similar, i.e., each treatment group had the same number of vines from each inflorescence group.

Each lateral treatment group was then divided into three stage treatment groups; i) vines to be placed in CE rooms three weeks prior to flowering for three weeks (Stage One), ii) vines to be placed in the CE rooms for three weeks immediately post-flowering (Stage Two) and iii) vines to be placed in CE rooms three weeks prior to veraison for three weeks (Stage Three) (Figure 59). There were approximately 18 inflorescences per stage treatment, distributed across five vines. Appendix 7 details the exact distribution of
inflorescences in each group. The three treatments therefore formed a split-split-plot design with humidity as the main plot, and lateral and development stage being the split plots.

![Diagram showing treatment stages, physiological stages, and dates of measurement and assessments carried out on potted vines during the 2004/05 season.](image)

**Figure 59:** Time line indicating treatment stages, physiological stages, and dates of measurement and assessments carried out on potted vines during the 2004/05 season.

While in the CE rooms, potted vines were irrigated four times a day and fertigated once a day to run off using Hoagland’s solution (Appendix 6). Vines that were never placed in the rooms, but left in the standing out area for the entire length of the experiment were used as indicator control plants. Due to the limited number of suitable vines for controls, the data obtained from these vines could not be used in the statistical analyses of any data.

### 5.2.3 Leaf area and growing points

Leaf area per cane on each cane with an inflorescence or bunch was determined by measuring the widest part of each leaf on that cane. This was carried out at three separate times at monthly intervals; 1) the beginning of Stage One, 2) after flowering at
the beginning of Stage Two and 3) at the beginning of Stage Three (Figure 59). Throughout the season leaves were collected from similar vines not involved in the experiment and the diameter at the widest part of these leaves was measured. Leaves were then placed through a leaf area meter to obtain an exact leaf area for each diameter measurement and a relationship was determined (Appendix 8). Leaf diameters from experimental vines were converted to leaf area using this relationship, and total leaf area per cane was determined. Growing points (GP) per cane were counted at the same time that leaf diameters were measured. It was assumed that there was always a minimum of one GP due to the apical bud at the top of the cane.

5.2.4 Photosynthesis

Gas exchange measurements were carried out on leaves on the potted vines while they were in the CE rooms using a CIRAS-1 Portable photosynthesis system (PP systems, United Kingdom). The leaves that were measured were the first ones above a bunch on the corresponding side to the bunch. Measurements were carried out at 700 μmol m⁻² s⁻¹. Three replications per treatment and two duplicates per replicate were measured in each room. The first measurement was carried out on the vines that were in the rooms at the end of Stage Two (i.e. approximately three weeks following flowering) (Figure 59). Vines in all four rooms were measured resulting in two rooms per humidity treatment being measured. The second measurement was carried out on the vines that were in the rooms at the end of the Third Stage (i.e. approximately two weeks prior to 50% veraison) (Figure 59). As described in Chapter Two, Tr, Gs, Pn and internal CO₂ concentration, were measured at each assessment and statistically analysed using a split plot model and SAS software.

5.2.5 Sap flow measurements

Measurements of sap flow through peduncles and up canes were taken using commercial versions of a stem flow gauge. These consisted of Dynamax Dynagage Microsensors, models SGA2 and SGA5, and a Dynamax Stem Flow Gauge, model SGA9. For mechanical specifications of these gauges see Appendix 9. The data from the gauges were logged using a CR10x data logger programmed using Dynamax
Flow32™ version 3.2 software and the data from the gauges were also analysed using the Flow32™ version 3.2 software. Placement of sensors onto vines was carried out as per the Dynamx Dynagage Installation and Operation Manual. The stem heat balance theory is detailed in Appendix 10. Basically the gauges measure the sap flow by dividing the residual energy (after adjusting for radial heat conducted through the gauge to the ambient and the vertical or axial heat conducted through the stem) by the temperature increase between two points due to a heater, and the heat capacity of water. This is because a Watt (1 joule/s) can be converted to a flow rate (g/s) when divided by 4.186 joules/gram-degC (heat capacity of water), and the temperature increase in °C (for further details see Baker and Bavel, 1987).

Two bunches per CE room were assessed at any one time and assessments began immediately after flowering at the beginning of Stage Two (Figure 59). Depending on peduncle diameter, the two bunches were chosen from one cane with laterals and one cane without laterals where possible. Sensors were placed around the peduncle of the bunch being assessed and also on the cane just distal to the assessed bunch. Sensors were alternated between the high and low RH CE rooms, and were left on vines for approximately five days at any one time. Data were then graphed on a common axis to compare peduncle sap flow between RH treatments.

For a period of three days one vine was placed on a loadcell to measure water use and soil temperature was measured using a 101 probe and CR10x data logger in order to check sap flow data obtained from the Dynagages. The pot was wrapped in plastic to prevent evaporation from the soil.

5.2.6 BSN incidence

BSN incidence on potted vines was assessed as described in Chapter Two. Assessments began two weeks after 100% veraison on 14 March 2005, and were carried out a total of three times, approximately one week apart (Figure 59). By the time assessments began there was, unfortunately, extensive damage to bunches by birds and fungal infections.
5.3 Results

5.3.1 Leaf area

There were no significant differences in leaf area due to the interaction of the RH treatment and the removal of laterals. For assessment one, the removal of laterals significantly decreased the leaf area ($P=0.0681$) but this difference had disappeared by the second assessment and was also not significantly evident for assessment three (Table 34). Overall, there were no significant differences in leaf area at any of the three assessments due to the humidity treatment, or the stage at which they were placed in the rooms. However, there were significant differences in leaf area due to the interaction of the lateral treatment and the stage at which vines were placed in the rooms during the last assessment ($P=0.0784$) (Table 34). There were no significant differences in leaf area among the stages when the vines were placed in the CE rooms if the laterals were not removed. However, for the last assessment, if the vines had their laterals removed, vines placed in the CE rooms during Stage Two had a significantly higher leaf area than either vines placed in the CE rooms during Stage One ($P=0.0090$) or Stage Three ($P=0.0219$). Stages One and Three were not significantly different from each other (Table 34). During Stage One, removing the laterals significantly reduced the leaf area ($P=0.0605$). This was also significant during Stage Three but only at the 15% level ($P=0.1143$).
Table 34: Mean leaf area per cane for canes with laterals retained or removed, and placed in the CE rooms at the three different stages 1) pre-flowering, 2) post-flowering and 3) pre-veraison. Assessments were carried out on 5 November (early Stage One), 5 December (early Stage Two) and 5 January (early Stage Three).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Laterals retained</th>
<th>Laterals removed</th>
<th>Overall mean</th>
<th>Laterals retained</th>
<th>Laterals removed</th>
<th>Overall mean</th>
<th>Laterals retained</th>
<th>Laterals removed</th>
<th>Overall mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>740 a</td>
<td>635 a</td>
<td>688 x</td>
<td>1258 a</td>
<td>1124 a</td>
<td>1191</td>
<td>1512 a</td>
<td>1175 b</td>
<td>1344</td>
</tr>
<tr>
<td>2</td>
<td>656 a</td>
<td>620 a</td>
<td>638 x</td>
<td>1014 a</td>
<td>1212 a</td>
<td>1113</td>
<td>1515 a</td>
<td>1742 a</td>
<td>1629</td>
</tr>
<tr>
<td>3</td>
<td>651 a</td>
<td>619 a</td>
<td>635 x</td>
<td>978 a</td>
<td>1088 a</td>
<td>1033</td>
<td>1561 a</td>
<td>1220 a</td>
<td>1390</td>
</tr>
<tr>
<td>Overall mean</td>
<td>690 x</td>
<td>624 y</td>
<td>1103</td>
<td>1138</td>
<td>ns</td>
<td>1520</td>
<td>1382</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

Within an assessment date, means within a column and within a row with a different letter are significantly different from each other at \( P \leq 0.08 \) (Lsmeans, SAS).

abc - applies to individual stage and lateral means
xyz - applies to overall stage and lateral means
For vines with laterals, the rate of leaf area increase between assessments one and two was significantly higher for vines placed in the CE rooms during Stage One ($P<0.0001$) compared to vines placed in the rooms during Stages Two and Three (Figure 60). This rate for Stage One vines became significantly lower between assessments two and three ($P<0.0001$). There was no difference in leaf area development rate between Stages Two and Three over the three assessments.

The rate at which the leaf area per cane increased between the first and second assessment for vines without laterals, was not significantly different between Stages One, Two or Three. However, between the second and third assessment there was a significantly higher rate for vines placed in the CE rooms during Stage Two compared to the other stages ($P<0.0001$) (Figure 60).

![Figure 60: Mean leaf area for canes with laterals retained or removed, and placed in the CE rooms pre-flowering (Stage One), post-flowering (Stage Two) and pre-veraison (Stage Three).](image)

### 5.3.2 Growing point number (GPN)

There was no significant difference in GPN as a result of the RH treatment. Removing the laterals significantly reduced the GPN per cane for the last two assessments.
(\(P=0.004\) and \(P=0.0065\), respectively) (Table 35). There were no treatment differences for the first assessment as there were no laterals present and it was prior to vines being placed in the rooms for Stage One.

At the second assessment, which was taken at the beginning of Stage Two, after flowering, there was a significant difference in GPN per cane for the stages in which the vines were placed in the CE rooms (\(P=0.0732, F=3.69\)). However, this is not reported as there was a large significant interaction effect between the stage in which vines were placed in the CE rooms, and the removal of laterals on the canes (\(P=0.0229, F=6.28\)) (Table 35). Therefore, main treatment effects are not statistically reportable.

There were no significant differences in GPN as a result of the stage in which vines were placed in the CE rooms for canes that had had their laterals removed. However, for canes that did not have their laterals removed, the stage in which the vines were placed in the rooms did make a significant difference. Vines that were placed in the rooms during Stage One, had a higher GPN than the vines placed in the CE rooms during either Stages Two (\(P=0.0139\)) or Three (\(P=0.0039\)) (Table 35). Although there was an overall lateral treatment effect, this was only significant for Stages One (\(P=0.0004\)) and Two (\(P=0.0924\)).

At the third assessment, which was taken at the beginning of Stage Three, although there was a significant difference in GPN due to the stage in which vines were placed in the CE rooms (\(P=0.0059, F=10.43\)), only the significant interaction effect of the stage and the removal of the laterals can be reported due to \(P\) and \(F\) values obtained in the analyses (\(P=0.0044, F=11.5\)). For vines that did not have laterals removed, Stage Three had significantly fewer laterals than either Stage One (\(P=0.0188\)) or Stage Two (\(P=0.0065\)) (Table 35). For vines that did have laterals removed, vines that were placed in the CE rooms during Stage One had significantly fewer laterals than vines placed in the CE rooms during Stage Two (\(P=0.0008\)) and Stage Three (\(P=0.0106\)). As in the second assessment, even though there was a significant overall difference between the lateral treatments, this was only significant for vines placed in the CE rooms during Stages One (\(P<0.0001\)) and Two (\(P=0.0307\)).
Between the first and second assessments and for vines with laterals there was a significantly different rate in the increase of GPN between the three stages in which vines were placed in the CE rooms ($P<0.0001$). Vines placed in the CE rooms during Stage One had a faster rate of increase than either Stage Two or Three (Figure 61). Between the second and third assessment, the rate at which GPN increased was significantly higher for vines placed in the CE rooms during Stage Two compared to vines placed in the CE rooms during either Stage One or Three ($P<0.0001$) (Figure 61). Stage One had the lowest (actually negative) rate.

There was no stage treatment effect on the rate of increase of GPN for vines without laterals between assessments one and two. However, there was a significant difference among stages for GPN increase between assessments two and three ($P<0.0001$). As in vines with laterals, Stage Two had a faster rate of increase in GPN than either Stage One or Three, with Stage One having the lowest (actually negative) rate (Figure 61).
Table 35: Mean growing point number per cane for canes with laterals retained or removed, and placed in the CE rooms at the three different stages 1) pre-flowering, 2) post-flowering and 3) pre-veraison. Assessments were carried out on 5 November (early Stage One), 5 December (early Stage Two) and 5 January (early Stage Three).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Laterals retained</th>
<th>Laterals removed</th>
<th>Overall mean</th>
<th>Laterals retained</th>
<th>Laterals removed</th>
<th>Overall mean</th>
<th>Laterals retained</th>
<th>Laterals removed</th>
<th>Overall mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>7.4 a</td>
<td>1.3 c</td>
<td>4.3 x</td>
<td>6.5 a</td>
<td>0.8 c</td>
<td>3.7 x</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>4.0 b</td>
<td>1.7 c</td>
<td>2.9 x</td>
<td>7.3 a</td>
<td>5.1 b</td>
<td>6.2 x</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2.7 bc</td>
<td>2.1 c</td>
<td>2.4 x</td>
<td>4.1 b</td>
<td>3.7 b</td>
<td>3.9 x</td>
</tr>
<tr>
<td>Overall mean</td>
<td>1</td>
<td>1</td>
<td>ns</td>
<td>5.0 x</td>
<td>1.7 y</td>
<td>6.1 x</td>
<td>3.1 y</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Within an assessment date means within a column and within a row with a different letter are significantly different from each other at $P \leq 0.08$ (LSMeans, SAS).

abc - applies to individual stage and lateral means

xyz - applies to overall stage and lateral means
Figure 61: Mean number of growing points per cane for canes with laterals retained or removed, and placed in the CE rooms pre-flowering (Stage One), post-flowering (Stage Two) and pre-veraison (Stage Three).

### 5.3.3 Photosynthesis

There were no significant differences in Tr, Gs, Pn due to the interaction of the RH treatment and the removal of laterals (Appendix 11). There was a significant difference between humidity treatments for Tr ($P=0.0554$) for the first assessment (end of Stage Two) with the low humidity treatment having a higher Tr (Table 36). However, at the second assessment at the end of Stage Three, Tr was not affected by the humidity. Pn was also influenced by the humidity in the first assessment, but only at the 15% level ($P=0.1512$). However, at the second assessment, Gs ($P=0.0769$) and Pn ($P=0.0818$) were both significantly affected (Table 36).
Chapter Five - Controlled environment studies: Results

Table 36: Means of gas exchange measurements carried out on potted vines in Season Three for vines placed in CE rooms at either high (80%) or low (40%) RH. Assessment times are during 1) Stage Two – post-flowering and 2) Stage Three – pre veraison. Values are means of vines with and without laterals.

<table>
<thead>
<tr>
<th>Relative Humidity</th>
<th>Transpiration rate (Tr) (μmol m⁻² s⁻¹)</th>
<th>Stomatal conductance (Gs) (μmol m⁻² s⁻¹)</th>
<th>Photosynthetic rate (Pn) (μmol m⁻² s⁻¹)</th>
<th>Internal CO₂ concentration (Ci) (μmol mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Assessment 1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>High</td>
<td>0.72 b</td>
<td>0.80</td>
<td>118</td>
<td>93 a</td>
</tr>
<tr>
<td>Low</td>
<td>1.05 a</td>
<td>0.79</td>
<td>78</td>
<td>50 b</td>
</tr>
<tr>
<td></td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

Means within a column with a different letter are significantly different from each other at $P \leq 0.10$ (LSMeans, SAS).

ns – not significantly different at 10%

Removal of laterals significantly reduced internal CO₂ concentration in the first assessment ($P = 0.0850$), but no other variable was affect by the removal of the laterals at either assessment (Table 37).

Table 37: Means of gas exchange measurements carried out on potted vines in Season Three for canes with or without laterals removed. Assessment times are during 1) Stage Two – post-flowering, 2) Stage Three – pre veraison. Values are means for vines in high and low RH treatments.

<table>
<thead>
<tr>
<th>Laterals removed</th>
<th>Transpiration rate (Tr) (μmol m⁻² s⁻¹)</th>
<th>Stomatal conductance (Gs) (μmol m⁻² s⁻¹)</th>
<th>Photosynthetic rate (Pn) (μmol m⁻² s⁻¹)</th>
<th>Internal CO₂ concentration (Ci) (μmol mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Assessment 1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Yes</td>
<td>0.86</td>
<td>0.82</td>
<td>89</td>
<td>70</td>
</tr>
<tr>
<td>No</td>
<td>0.91</td>
<td>0.78</td>
<td>105</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

Means within a column with a different letter are significantly different from each other at $P \leq 0.10$ (LSMeans, SAS).

ns – not significantly different at 10%
5.3.4 Sap flow

5.3.4.1 Peduncle sap flow

Low humidity vs high humidity. Sap flow sensors measured the flow through the peduncle during day and night conditions in the CE rooms. During Stage Two the night flow appeared to be marginally higher than during the day (Figure 62). When the lights came on there appeared to be a quick peak in flow through the peduncle that gradually declined to the day sap flow rate soon after constant temperature and VPD conditions were obtained. In the evening peduncle flow rates declined when temperature and VPD changes began. When the lights were turned off peduncle sap flow reached its minimum value but increased quickly again to night flow rates when conditions again became constant. The peak and trough measured by the sensors are possibly a consequence of the way in which the sensors measure sap flow and how this is affected by the change in sap flow rate due to the change in conditions.

![Figure 62: Twenty-four hour cycle of sap flow through the peduncle of bunches from low (40%) and high (80%) RH treatments with similar leaf areas, and the temperature change in the CE rooms during the early stages of Stage Two (immediately post-flowering). VPD changes follow an identical pattern to that of temperature change. Green arrow indicates lights on, black arrow indicates lights off.](image-url)
Chapter Five - Controlled environment studies: Results

When canes with similar leaf areas in the low and high RH treatments were compared at the beginning of Stage Two, the low RH treatment appeared to have lower peduncle sap flow during both the day and the night compared to the high RH treatment (Figure 62). However, during the initial days of Stage Two there was still extensive peduncle growth and therefore it is difficult to determine whether or not both peduncles were at a similar physiological stage. Other factors, other than CE room humidity, may have therefore affected the sap flow through the peduncle such as xylem development stage.

Near the end of Stage Two (three weeks post-flowering), when peduncle growth had slowed, night peduncle sap flow rate tended to be slightly higher than day peduncle sap flow rate. This pattern was consistent throughout Stage Two for all treatments. There also appeared to be a difference in night peduncle sap flow rates between the two RH treatments, but no difference for day peduncle sap flow for vines that retained their laterals (Figure 63). The low RH treatment appeared to have higher night peduncle sap flow compared to the high RH treatment (noting that the comparable vapour pressure deficits were 0.79 and 0.27 kPa, respectively). Although day peduncle sap flow rates appeared to be the same for the two RH treatments, the difference between night and day peduncle sap flow rates in the low RH treatment was larger (approx. 0.2 g hr\(^{-1}\)) than that of the high RH treatment (approx. 0 g hr\(^{-1}\)) (Figure 63). The difference between day and night VPD values for the two RH treatments was also greater in the low RH treatment.
Figure 63: Twenty-four hour cycle of sap flow through the peduncle of bunches from low (40\%) and high (80\%) RH treatments with similar leaf areas and with laterals retained, and the temperature change in the CE rooms during the late stages of Stage Two (approx. three weeks post-flowering). VPD changes follow an identical pattern to that of temperature change. Green arrow indicates lights on, black arrow indicates lights off.

For vines with laterals removed, night peduncle sap flow rate was again higher in the low RH treatment compared to the high humidity treatment, but day peduncle sap flow rates were also different (Figure 64). This may be in part due to the difference in leaf area of the canes. As occurred in the vines that retained their laterals, the difference between night and day peduncle sap flows was larger in the low RH treatment (approx. 0.55 g hr\(^{-1}\)) compared to the high RH CE room (0.1 g hr\(^{-1}\)).
Figure 64: Twenty-four hour cycle of sap flow through the peduncle of bunches from low (40\%) and high (80\%) RH treatments and with laterals removed, and the temperature change in the CE rooms during the late stages of Stage Two (approx. three weeks post-flowering). VPD changes follow an identical pattern to that of temperature change. Green arrow indicates lights on, black arrow indicates lights off.

During Stage Three the 24-hour pattern for vines with laterals was different from that recorded in Stage Two. Night flow was now lower than day flow in both RH treatments. However, the difference between day and night flows still followed the same pattern as that in Stage Two, with there being a greater difference between day and night flows in the low RH conditions compared to the high RH conditions (Figure 65). This difference was two to three fold greater in the low RH treatment compared to the high RH treatment, again noting that the difference between day and night VPD values for the two RH treatments was three fold greater in the low RH treatment. Day sap flows were approximately the same for the low RH conditions as in Stage Two although the peduncle sap flow during the day in the low humidity treatment was now approximately 30\% more than in the high RH treatment.

Due to there being few suitable peduncles for sap flow measurements, vines with laterals removed were unable to be compared during this stage.
Figure 65: Twenty-four hour cycle of sap flow through the peduncle of bunches from low (40%) and high (80%) RH treatments with similar leaf areas and laterals retained, and the temperature change in the CE rooms during Stage Three (approx. four weeks post-flowering to one week pre-veraison). VPD changes follow an identical pattern to that of temperature change. Green arrow indicates lights on, black arrow indicates lights off.

**Lateral manipulation.** During Stage Two when the peduncle sap flow rate was compared between canes with laterals retained and canes without laterals in the low RH treatment, it appeared that the night peduncle sap flow was the same between the canes with and without laterals. However, the difference between day and night peduncle sap flow was greater for canes with laterals removed compared to the canes with laterals (Figure 66).
Figure 66: Twenty-four hour cycle of sap flow through the peduncle on vines in the low RH (40%) treatment for canes with laterals and canes with laterals removed, and the temperature change in the CE rooms during the later stages of Stage Two (approx. three weeks post-flowering). VPD changes follow an identical pattern to that of temperature change. Leaf area of cane with laterals was 1200 cm², leaf area of cane without laterals was 1500 cm². Green arrow indicates lights on, black arrow indicates lights off.

As in the low RH treatment, the night peduncle sap flow in the high RH treatment was the same between vines with and without laterals (Figure 67). However, in contrast there was very little difference in the peduncle sap flow during the day between the two lateral manipulation treatments.
Chapter Five - Controlled environment studies: Results

Figure 67: Twenty-four hour cycle of sap flow through the peduncle on vines in the high RH (80%) treatments for canes with laterals and canes with laterals removed, and the temperature change in the CE rooms during the later stages of Stage Two (approx. three weeks post-flowering). VPD changes follow an identical pattern to that of temperature change. Leaf area of cane with laterals was 1200 cm², leaf area of cane without laterals was 1700 cm². Green arrow indicates lights on, black arrow indicates lights off.

Due to the lack of suitably sized peduncles, sensors were unable to be placed on canes to compare the lateral manipulation treatment during Stage Three.

Phloem or xylem flow. A peduncle was girdled to determine whether or not phloem sap flow was being measured as part of the peduncle sap flow. This girdling took place at the end of Stage Three. It appears that at this time, phloem flow was a major part of the sap flow being measured as, immediately after girdling, day sap flow through the peduncle was dramatically reduced and night sap flow was non-existent (Figure 68).
5.3.4.2 Cane sap flow

**Low vs High relative humidity.** Sap flow through the cane was measurable during daylight hours within the CE treatments, but unlike the results for the peduncle there was no measurable cane sap flow during the night (Figure 69). In both the low and high RH conditions cane sap flow increased from zero from the time the lights were turned on to reach a peak around 12:00 h. Then there was a gradual decline in cane sap flow until temperature and VPD changes began, and then cane sap flow decreased rapidly to reach zero again soon after the time the lights were turned off. When comparing two canes that had similar leaf areas and had laterals, the canes in the low RH treatment had approximately 30 percent higher sap flow than the canes in the high RH treatment (Figure 69).
Figure 69: Twenty-four hour cycle of sap flow through canes with laterals from low (40%) and high (80%) RH treatments with similar leaf areas and the temperature change in the CE rooms during Stage Two (approx. three weeks post-flowering). VPD changes follow an identical pattern to that of temperature change. Green arrow indicates lights on, black arrow indicates lights off.

The leaf area for vines with the laterals removed was different and therefore sap flow was adjusted for this difference. Once adjusted the sap flow for these vines appeared to be identical between the two RH treatments (Figure 70) and therefore it appeared that VPD did not influence the cane sap flow for vines with laterals removed as it did for vines that retained their laterals. In comparison to vines with laterals retained, vines with laterals removed had a higher absolute cane sap flow with a maximum cane sap flow before adjustment of between 12 – 13.5 g hr⁻¹ while vines with laterals retained ranged between 5 – 7 g hr⁻¹ (Figure 69).
Figure 70: Twenty-four hour cycle of leaf area adjusted sap flow through canes with laterals removed from low (40%) and high (80%) RH treatments and the temperature change in the CE rooms during Stage Two (approx three weeks post-flowering). VPD changes follow an identical pattern to that of temperature change. Green arrow indicates lights on, black arrow indicates lights off.

Interestingly, the difference in cane sap flow between the two RH treatments was reduced during Stage Three, and the vines in the low RH conditions now had approximately 20% lower cane sap flow compared to the vines in the high RH conditions (Figure 71). This is in comparison to the 30% higher cane sap flow in the low RH vines in Stage Two. Although absolute cane sap flow appeared to have increased in Stage Three, the leaf area had also increased. When adjusted for leaf area the maximum cane sap flow for the high RH treatment was 0.0067 g hr⁻¹ cm⁻² during Stage Three, while during Stage Two it was 0.0042 g hr⁻¹ cm⁻² and had therefore increased over time. While for the low RH treatment adjusted cane sap flow was 0.0051 g hr⁻¹ cm⁻² during Stage Three, while during Stage Two it was 0.0067 g hr⁻¹ cm⁻² and had therefore decreased.
Chapter Five - Controlled environment studies: Results

Figure 71: Twenty-four hour cycle of sap flow through canes from low (40%) and high (80%) RH treatments with similar leaf areas and the temperature change in the CE rooms during Stage Three (approx. four weeks post-flowering to one week pre-veraison). VPD changes follow an identical pattern to that of temperature change. Green arrow indicates lights on, black arrow indicates lights off.

Again, due to the lack of suitably sized peduncles, sensors were unable to be placed on canes to compare the lateral manipulation treatment during Stage Three.

**Lateral manipulation.** When vines with and without laterals in the same RH treatment were compared during Stage Two (approx. three weeks post-flowering), vines without laterals appeared to have higher cane sap flow than those with laterals retained even when cane sap flow was adjusted for leaf area (Figure 72). This increase in cane sap flow in vines with laterals removed occurred in both RH treatments. It therefore appears as though the removal of laterals increases the sap flow per cm² of leaf area.
Chapter Five - Controlled environment studies: Results

Figure 72: An example of a twenty-four hour cycle of sap flow through canes with laterals intact and canes with laterals removed, and the temperature change in the CE rooms. VPD changes follow an identical pattern to that of temperature change. Green arrow indicates lights on, black arrow indicates lights off.

The load cell measurements of water use followed a very similar pattern to that of the sap flow measurements. Both indicated an increase in water use/sap flow from when the lights were turned on until a maximum was reached around the middle of the day. There was then a decline in water use/sap flow with the minimum being reached during the hours of darkness (Figure 73). Soil temperature lagged behind air temperature by approximately three hours and never reached a plateau but cycled in a wave-like pattern. However, the rate of increase in soil temperature did decline during the day as soil temperature approached air temperature (Figure 73).
5.3.5 Bunch stem necrosis

Due to the nature of the data for the first assessment, assumptions of normality and heterogeneity of variance were not met and none of the transformations accessed were able to adjust the raw data to meet these assumptions. Normal ANOVA analysis was therefore not possible. Also, there are no suitable non-parametric tests for split-split-plot, therefore, no statistical analyses could be performed on the data. Trends of means can therefore only be discussed for this assessment. The second and third assessment did meet the assumptions of normality and heterogeneity of variance and therefore normal ANOVA analysis was able to be performed.

There were no overall significant differences in BSN incidence due to the main treatments of RH or lateral removal at any of the assessment dates (Table 38 and Table 39). There was however, a significant difference below the 10% level for the RH and lateral removal interaction for assessments two \((P=0.0684)\) and three \((P=0.0769)\) (Table 40).
Chapter Five - Controlled environment studies: Results

Table 38: Mean BSN incidence (%) for bunches placed in the high (80%) and low (40%) RH treatments. Values are means of vines with laterals either retained or removed.

<table>
<thead>
<tr>
<th>Relative Humidity</th>
<th>5 November 2004</th>
<th>5 December 2004</th>
<th>5 January 2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>16.0</td>
<td>35.5</td>
<td>40.4</td>
</tr>
<tr>
<td>Low</td>
<td>20.8</td>
<td>35.8</td>
<td>45.0</td>
</tr>
</tbody>
</table>

*ns* – not significantly different at $P=0.05$

Table 39: Mean BSN incidence (%) for bunches from canes with either laterals retained or removed. Values are means for vines in high and low RH treatments.

<table>
<thead>
<tr>
<th>Laterals removed</th>
<th>5 November 2004</th>
<th>5 December 2004</th>
<th>5 January 2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>18.1</td>
<td>35.6</td>
<td>44.4</td>
</tr>
<tr>
<td>Yes</td>
<td>18.8</td>
<td>36.9</td>
<td>41.2</td>
</tr>
</tbody>
</table>

*ns* – not significantly different at $P=0.05$

Table 40: Overall mean BSN incidence (%) for bunches in high (80%) and low (40%) RH CE rooms and from canes with either laterals retained or with laterals removed.

<table>
<thead>
<tr>
<th>Relative Humidity (%)</th>
<th>Laterals removed</th>
<th>5 November 2004</th>
<th>5 December 2004</th>
<th>5 January 2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>No</td>
<td>17.0</td>
<td>39.6 a</td>
<td>44.4 a</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>15.0</td>
<td>30.1 b</td>
<td>36.6 b</td>
</tr>
<tr>
<td>Low</td>
<td>No</td>
<td>19.1</td>
<td>31.7 b</td>
<td>44.5 a</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>23.0</td>
<td>43.0 a</td>
<td>45.7 a</td>
</tr>
</tbody>
</table>

Means within a column with a different letter are significantly different from each other at $P \leq 0.08$ (LSMeans, SAS).

There was no interaction of the stage treatment with either the RH treatment or the lateral manipulation treatment. The stage at which the vines were placed in the CE rooms significantly affected the incidence of BSN (second assessment $P=0.0007$, third assessment $P=0.0006$). For the last two assessments, vines that were placed in the CE rooms during Stage Two had a significantly higher BSN incidence than vines placed in the CE rooms either during Stage One (second assessment $P=0.0010$, third assessment $P=0.0005$) or Stage Three (first assessment $P=0.0005$, third assessment $P=0.0008$) (Figure 42). There was a similar trend for assessment one.
Table 41: Mean BSN incidence (%) for bunches from vines placed in the CE rooms at any one of three stages. Stage One – pre-flowering, Stage Two – post-flowering, Stage Three – pre-veraison. Means for vines not placed in the CE rooms also included (control).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>One</td>
<td>8.3</td>
<td>24.5 b</td>
<td>26.5 b</td>
</tr>
<tr>
<td>Two</td>
<td>38.8</td>
<td>69.4 a</td>
<td>76.0 a</td>
</tr>
<tr>
<td>Three</td>
<td>6.9</td>
<td>15.6 b</td>
<td>27.0 b</td>
</tr>
<tr>
<td>Control</td>
<td>4.5</td>
<td>14.3 7</td>
<td>24.2 7</td>
</tr>
</tbody>
</table>

Means within a column with a different letter are significantly different from each other at P ≤ 0.05 (LSMeans, SAS).

*Control means not used in statistical analysis

Means from vines that were not placed in the CE rooms (control) have been presented (Table 41) but have not been included in the statistical analyses. This was due to the low vine number and therefore the inability to produce a balanced statistical design involving vines maintained throughout in the field. However, means suggest that control vines that were maintained in the field are unlikely to be different from either Stage One or Three vines.

Because of the highly significant difference among stages, and because only vines placed in the CE rooms during Stage Two appeared to be different from the outside controls, only RH, lateral removal and interaction effects for Stage Two will be discussed. However, RH and lateral removal interaction BSN values for the last assessment for both Stages One and Three are presented in Appendix 12 and 13.

For vines placed in CE rooms during Stage Two, there were no significant differences in BSN incidence due to the treatments of RH, removal of laterals, or the interaction between the RH treatments and lateral removal treatments, although there were large differences in actual values. The lack of significant difference may be due to the variability in the data. It did appear that the low RH treatment tended to reduce BSN incidence compared to the high RH treatment when the laterals were retained and this difference increased as time went on (Figure 74). When laterals were removed it
appeared that BSN incidence tended to increase in the low RH treatment compared to the high RH treatment (Figure 74).

Figure 74: Relative humidity and lateral manipulation interaction effect on BSN incidence at the first, second and third assessments for vines placed in CE rooms during Stage Two (post-flowering).
5.4 Discussion

5.4.1 Canopy development

Lettuces grown in high relative humidity (RH) conditions (85%) have been shown to have significantly faster growth rates than lettuces grown in lower RH (50%) (Tibbitts and Bottenberg, 1976). However, there were no significant differences in vegetative growth due to humidity treatments in the current study. This may be due to the length of time that vines were in the CE conditions as it may not have been long enough to influence the growth rate to a degree that was measurable.

However, vine growth was affected by the growth stage or time of placement into the CE rooms. Although at the second assessment (early Stage Two - post-flowering), leaf area measurements for the three treatment stages were not significantly different from each other, the rate of growth for vines placed in the CE conditions during Stage One was significantly faster for vines with laterals, but not for those without laterals, compared to vines placed in the CE rooms at any other time (Figure 60). Therefore the CE conditions influenced the growth of vines with laterals retained. This was probably due to the increase in growing point number that increased dramatically during Stage One for vines in the CE conditions during that time (Figure 61). As growing points were removed from the other vines, leaf area did not increase to such an extent and the influence of the CE conditions on growth is likely to have been reduced and may have been hidden due to the removal of leaf area during the removal of laterals. When vines placed in the CE conditions during Stage One were removed, they demonstrated slower subsequent growth rates for both the lateral removed and lateral retained treatments. The slight decline in growing point number at the last assessment was probably due to the trimming of the vines and little further growth occurring for those vines.

The growth of vines was also influenced by the CE conditions when placed in the CE rooms during Stage Two (Figure 60). Vines without laterals had a significantly faster growth rate while in the CE conditions compared to vines not in the CE conditions. Vines with laterals also appeared to be influenced by their time in the CE conditions, but there was only a difference when compared to vines that had been in the CE
conditions during Stage One, but not when compared to vines that would go into the CE conditions during Stage Three. As Stage Three vines had not been placed in the CE conditions during the time of leaf area assessments, the increase in the rate of growth for vines with laterals was probably due to the development period rather than the CE conditions. However, vines with laterals in the CE conditions during Stage Two had dramatically more laterals and a higher rate of lateral increase than the vines with laterals that were to be placed in the CE conditions during Stage Three (Figure 61). Therefore the CE conditions appear to have influenced the rate of growing point emergence rather than leaf area for these vines.

The increase in growing points for Stage Two vines could also be seen on the vines with laterals removed. Due to lateral removal there should have been no difference in growing point number between the three treatment stages. However, further growth of the vines, and lateral removal occurring only weekly, resulted in some growing points being present during the assessment. Therefore a similar pattern for the rate of increase of growing points can be seen in the vines with laterals removed compared to vines with laterals retained.

5.4.2 Photosynthesis measurements

Stomatal conductance in the low RH conditions was lower in both assessments compared to the high RH conditions but only significantly different for the second assessment (Table 36). The slight decrease in stomatal conductance in the low RH conditions may have been due to a slight water stress of the vines as one of the earliest responses to water stress is a decrease in stomatal conductance (Lawlor, 2001). However, reduced growth is one of the mechanisms that plants use to respond to water stress (Hsiao, 1993; Lovisolo et al., 2000) and this was not evident in vines placed in the CE rooms during Stage Two (Appendix 12 and 13) and could not be determined for vines placed in the CE rooms during Stage Three as lateral number and leaf area assessments were not carried out at the end of this stage.
Chapter Five - Controlled environment studies: Discussion

The stomata may have responded directly to the RH rather than the evaporation from the leaf. Although in some species stomatal conductance is correlated to VPD rather than RH (Lloyd, 1991), Schultz et al. (1999) found that grapevines were one of the species that fitted examples given by Ball (referenced in Schultz et al., 1999) and Leuning (1990). These studies showed that stomata respond directly to RH, and not the transpirational flux through stomata due to VPD. Although the equation \((A \times (RH/CO))\) (Leuning, 1990; Schultz et al., 1999), used in these studies was correlated with stomatal conductance, and also included the photosynthesis rate \((A)\) and external \(CO_2\) concentration \((CO)\), basically if \(A\) and \(CO\) remained constant, as RH increased so did the stomatal conductance.

Gas exchange measurements using the CIRAS showed that generally vines in the high RH conditions had a lower transpiration rate (first assessment) and higher photosynthetic rate (Table 36). It has been noted in some studies that as water stress increases, photosynthetic rates decrease (Hsiao, 1993) suggesting that the vines in the low RH treatment may have been water stressed. The reduction in photosynthesis is also directly linked to the closure of the stomata (Hsiao, 1993), i.e., the reduction in stomatal conductance. It has been noted that if the reduction in photosynthesis is caused mainly by the closure of stomata, then the internal \(CO_2\) concentration would also be decreased (Hsiao, 1993). As this occurred in the low RH treated vines it can be suggested that the decrease in photosynthesis in vines in the low RH treatment was due to stomatal closure.

Even though stomatal conductance was lower in the low RH treatment compared to the high RH treatment, total conductance from the leaf includes stomatal conductance, cuticular conductance and boundary layer conductance (Lawlor, 2001). The increase in transpiration rate for the vines in the low RH treatment is therefore due to the larger VPD. Therefore although stomatal conductance is lower in the low RH treatment, it appears not to have been low enough at the first assessment to negate the difference in humidity.
5.4.3 Sap flow

Some studies have found that the heat balance method for measuring sap flow on vines thicker than 35 mm diameter may in fact overestimate the sap flow due to significant conduction in very old xylem vessels (Braun and Schmid, 1999). However, as the canes that were measured in this study had a maximum diameter of 10 mm, and were the previous season’s growth, it is not expected that this problem occurred. Other studies using Dynamax® stem flow gauges on vines with a stem diameter of 15 – 18 mm found that the gauges were accurate to within 5 to 10% of the daily value of transpiration as measured gravimetrically (Lascano et al., 1992).

Cane sap flow followed the diurnal pattern described in studies on xylem sap flow in plants (Steinberg et al., 1990; Schmid and Bettner, 1999). There appeared to be little or no sap flow during the ‘night’ hours and once the lights were turned on, sap flow increased to a maximum around ‘noon’ and then declined throughout the ‘afternoon’. This pattern was also confirmed by the water usage data (Figure 73). During the day PPF values did not fluctuate as seen in the field and temperature and VPD were constant except for during the two hours of ramping at the beginning and end of the ‘day’, so therefore the steady increase and then decrease in sap flow can be attributed to the circadian rhythm of the plant. The vine continued to follow its original cycle even though the CE conditions were stable (Salisbury and Ross, 1992). The maximum water use per unit of leaf area was approximately 0.46 – 0.6 l m⁻² day⁻¹ for vines with laterals and 0.9 l m⁻² day⁻¹ for vines without laterals. These findings appear to be reasonable as using the Granier method Schmid and Braun (1997) found field grown ‘Riesling’ vines to have a maximum water use of 0.8 – 1.0 l m⁻² day⁻¹.

The lower transpiration rate in the high RH treatment during Stage Two is also evident in the cane sap flow measurements. The sap flow for the cane with laterals in the high RH treatment was also reduced during this stage compared to the cane with laterals in the low RH treatment (Figure 69). This correlation between transpiration rate and sap flow has been found in other studies (Escalona et al., 2000; Lovisolo et al., 2000). As sap flow has been found to be directly related to leaf area (Schmid and Bettner, 1999) vines with laterals removed where adjusted for leaf area in order to compare sap flows.
Cane diameter was not used to adjust sap flow measurements as the trunk diameter has been found not to affect transpiration, and consequently sap flow (Schmid and Bettner, 1999). Therefore it appears that the differences in cane diameter can be largely discounted. When adjusted for leaf area the cane sap flows, for canes with laterals removed, become the same within each of the two different RH treatments (Figure 70). It may be possible that the vines with laterals removed in the low RH treatment were under more stress than those vines in the high RH treatment, and therefore their transpiration rate, and consequent sap flow, would have been affected. Ginestar et al., (1998) found that vines under water stress had lower transpiration rates as measured by sap flow sensors. It is difficult to confirm this as sap flow assessments and CIRAS measurements were carried out on different days. This is due to the fact that a) on the day of CIRAS measurements a complete 24 hr cycle of sap flow assessments were not carried out and b) although CIRAS assessments could be carried out on vines in both RH treatments on the same day, sap flow assessments could not. However, the CIRAS measurements that were taken may indicate some degree of stress in the vines in the low RH treatment (Appendix 11). Water stress may also account for the different pattern in cane sap flow in vines with laterals between Stages Two and Three. Again, although not significantly different, CIRAS measurements indicate that vines in the low RH treatment during Stage Three with laterals retained, may have experienced more stress than vines in the high RH treatment (Appendix 11). CIRAS measurements also indicate an increase in stress during Stage Three for low RH treatment vines compared to Stage Two.

The results of cane sap flow measured using the dynagauges generally indicate little or no flow during the ‘night’ period. However, using the Granier method Schmid and Braun (1997) did find high ‘night’ flows (up to 30% of the peak flow during the ‘day’). Lysimeter assessments indicate that there may in fact have been some very minor sap flow during the ‘night’ in the current study (Figure 73). However, this would appear to be much less than found by Schmid and Braun (1997), as the maximum ‘night’ weight change was only 10% of the maximum ‘day’ weight change. ‘Night’ flow appeared to be more evident during the early hours of the ‘night’ period, becoming zero near ‘dawn’. This may indicate that during the ‘day’ sap flow was unable to provide
adequate sap to the vine and therefore during the ‘evening’ vines re-hydrated causing sap flow to continue.

A lack of measurable ‘night’ sap flow during Stage Two may be due to the way in which the Dynamax program, Flow32, filters data. For the SGA5 sensors the minimum $dT$ is 0.75 °C and therefore for values less than this flow rates are screened out and reported as zero (van Bavel, 2000). Also, if the Ksh setting is not accurate this may result in a negative $Q_f$ value that is filtered by the program and the flow becomes zero (van Bavel, 2000). Even though the Ksh value was recalibrated before ‘dawn’ every day, at times it was difficult to ensure that the Ksh was correct and a ‘best guess’ scenario had to be implemented. It is noted in the Dynamax manual that a true zero flow rate is rarely noticed on large plants, trees, or crop plants in a natural, growing condition (van Bavel, 2000). It is therefore possible that the zero flow rates recorded are in fact just very low flow rates that were unable to be measured.

Water stress, resulting in a reduction of cane sap flow, has been found to decrease leaf area and lateral number (Ginestar et al., 1998) and leaf area development has been correlated with water use (Gómez-del-Campo et al., 2000). This may be due to a reduction in photosynthesis due to a reduction in RuBP supply (Tezara et al., 1999) or a loss of turgor. It is therefore interesting that the RH treatments did not significantly affect leaf area or lateral development considering the differences in cane sap flow between the RH treatments. As already stated, the three week period in which vines were placed in the CE rooms may not have been an adequate period of time to significantly affect the growth rate of vines.

During Stage Two the ‘night’ peduncle sap flow into bunches was higher in the low RH treatment compared to the high RH treatment (Figure 63 and 64). It did not appear to matter what the leaf area was, if the laterals had been retained or removed, or even what the bunch size was (Appendix 14), the ‘night’ peduncle sap flow was the same within each RH treatment i.e. approximately 0.8 g hr$^{-1}$ in the high RH treatment and 1.0 g hr$^{-1}$ in the low RH treatment (Figure 66 and 67). This would indicate that ‘night’ peduncle sap flow is driven directly by the VPD of the atmospheric environment, and not the leaf
area or lateral number of the cane or the size of the bunch. However, it may have been expected to see a larger difference in ‘night’ peduncle sap flow between the two RH treatments as there was almost a three fold difference in night VPD.

At this stage in berry development the stomata are still active (Blanke and Leyhe, 1987). However, Blanke and Leyhe (1988) and others assumed that all stomata would be closed during the night period and therefore any transpiration during the night was due to cuticular transpiration. Cuticular transpiration is considered to be high in grape berries, even at early stages in berry development (Blanke and Leyhe, 1987). However, as stated by Blanke and Leyhe (1988), in grapes possibly about 20% of the stomata stay fully or partially open at night (Royle and Thomas, 1973). Through their work, Blanke and Leyhe (1988) suggested that in grapes, cuticular transpiration may be higher in the light and lower in the dark than previously estimated.

This was not the case for ‘day’ peduncle sap flow that was the same for bunches on canes with the same leaf area and that also had the laterals retained, whether they were in the high or low RH conditions (0.8 g hr$^{-1}$) (Figure 63). However, for bunches that were on canes with the laterals removed, and a slightly different leaf area, the high RH treatment resulted in a higher ‘day’ peduncle sap flow compared to the low RH treatment (Figure 64). If VPD was acting directly on the bunches, it would be expected that the bunches in the low RH treatment would have the higher peduncle sap flow (perhaps up to three times as high due to there being a three fold difference in day VPD values). If the difference was solely due to competition with cane sap flow there should be no difference as the cane sap flow was the same for both RH treatments for canes with laterals removed when adjusted for leaf area. Also, for canes with laterals retained, there should have been a difference in ‘day’ peduncle sap flow if this was dependant solely on competition with cane sap as the low RH treatment vines had a higher sap flow rate. As none of these scenarios occurred, it appears that there is another variable which is responsible for the rate of ‘day’ peduncle sap flow, such as bunch size.

To give a general indication of bunch size, assessments were carried out on the bunches (Appendix 14). For bunches on canes that had laterals removed, the bunch in the high
RH treatment was generally much larger than that in the low RH treatment. This would indicate that bunch/berry size influences the rate of peduncle sap flow during the day as the larger bunch had the faster rate of peduncle sap flow.

It appears that in the early stages of berry development regardless of the type of cultivar, there are 1-2 stomata per mm$^2$ of berry. This declines to 1 stomata per mm$^2$ of berry as the berry grows (Blanke and Leyhe, 1987). The highest transpiration rate per berry surface area occurs one week after full bloom and then decreases thereafter (Blanke and Leyhe, 1987). However, on a per berry basis the transpiration rate increased from one week after full bloom until harvest (Blanke and Leyhe, 1987). Early in the berry’s development the stomata are active and the berry is able to regulate some transpiration through the stomata. However, there is also cuticular transpiration from the berry that causes an increase in the transpiration rate per berry, because as the berry develops cuticular transpiration increases (Blanke and Leyhe, 1987). Early in berry development transpiration may be more dependent on stomatal activity and the total surface area of the berries, while later in berry development, the transpiration rate may depend more on cuticular transpiration and the number of berries. Therefore, during Stage Two of the CE trial, bunches with more berries or larger berries would have had a higher number of active stomata and therefore a higher possible transpiration rate resulting in a higher potential sap flow through the peduncle during the ‘day’.

However, although in the high RH treatment, bunches on canes with laterals removed had the largest bunch/berry size, they did not have the highest ‘day’ peduncle sap flow compared to the bunches on canes with laterals retained. Also, the two bunches on canes with laterals retained and with the same leaf area, had similar sized bunches, but the berries in the low RH treatment were much larger and therefore it might have been expected that this bunch would have had a faster rate of peduncle sap flow during the ‘day’.

Other studies have found that pre-veraison, when the flow into the berry is predominantly through the xylem (Ollat and Gaudillere, 1996), that by reducing cane transpiration, daytime berry contraction was reduced (Greenspan et al., 1994). Also
'night' sap flow tended to be either greater than, or similar to, 'day' peduncle sap flow in the current study. However Blanke and Leyhe (Blanke and Leyhe, 1987; 1988) found that daytime berry transpiration was greater than nighttime transpiration. This would therefore support the idea that for bunches where day peduncle sap flow was less than night peduncle sap flow, that there was competition with the cane for sap resulting in the reduction in day peduncle sap flow.

It is therefore possible that, not only is it the actual bunch that influences day peduncle sap flow, but also the competition between the bunch and cane for sap flow. The competitive ability of the bunch during the day is therefore determined not only by bunch characteristics, but vine characteristics as well. Therefore, when comparing the two bunches in the high RH treatment on different canopy manipulation treatments, although the bunch with the strongest potential sink strength (due to bunch/berry size) should have a higher peduncle sap flow, because this vine also had the largest leaf area and had its laterals removed (resulting in the higher cane sap flow and greatest competition between bunch and cane) day peduncle sap flow was lower.

Equally, comparing the two bunches from vines with laterals retained, the bunch in the low RH treatment with the largest berries may have had the largest potential sink strength, but due to also having the most competition with the cane because of the higher VPD, any increase in strength may have been negated. Additionally, in the low RH treatment the bunch from the vine with laterals removed, which possibly had the smallest potential sink strength overall due to small bunch/berry size, also had a high rate of competition due to the high VPD, and therefore had the lowest day peduncle sap flow overall. This bunch had the largest difference between day and night peduncle sap flow indicating the large competition between cane and peduncle for sap flow.

Surprisingly during Stage Three, the vines in the low RH conditions appeared to have lower cane sap flow than those in high RH conditions although leaf area and cane diameter were very similar (Figure 65). This change in sap flow from Stage Two is also indicated by the fact that there was no longer any difference in transpiration rate between the two RH treatment conditions. Vines in the low RH conditions may have
been under more water stress than during Stage Two. The initial leaf area during Stage Three was larger than in Stage Two, yet the maximum root volume and water holding capacity of the pot was the same as in Stage Two. The lack of potential increase in water uptake may therefore have resulted in the root system being unable to supply the increased leaf area with sufficient water in the high VPD conditions of the low RH treatment, resulting in water stress and a decrease in sap flow (Ginestar et al., 1998). As leaf area and lateral number assessments were not carried out on vines that were in the CE conditions during Stage Three at the end of their time in the rooms, it is difficult to determine if water stress had occurred resulting in a reduction in growth.

In the low RH conditions for the final assessment during Stage Three, stomatal conductance, photosynthesis and internal CO$_2$ were even lower than in Stage Two (Appendix 11). These measurements all indicate that any water stress was greater during Stage Three compared to Stage Two.

Also, the gas exchange measurements in the second assessment were carried out in the morning around 10:00 – 11:00 am. The cane sap flow graphs show that sap flow around that time is in fact very similar between the two RH treatments. In field grown vines under water stress, as the day progresses the intensity of the stress increases due to an increase in VPD and a drying of the soil (Hsiao, 1993). This results in a reduction of photosynthesis as the day progresses, which corresponds with a decrease in the stomatal conductance (Hsiao, 1993). It would therefore be expected that sap flow would also decrease. Between these responses and the circadian rhythm that vines appear to follow in the CE conditions, cane sap flow levels off at a lower rate for the vines in the low RH treatment compared to the high RH treatment (Figure 71).

Day peduncle sap flow during Stage Three was higher in the vines in the low RH treatment compared to vines in the high RH treatment (Figure 65). As cane sap flow was lower in the low RH treatment compared to the high RH treatment during Stage Three, the results are consistent with the idea that the competitive ability of the bunch during the day is influenced by cane sap flow. However, it may also be due to the bunch reacting directly to its environment during Stage Three of the CE trial. Studies
indicate that post-veraison, when sap flow into the berry is predominantly due to the phloem, daytime contraction was reduced by reducing berry transpiration, and not cane transpiration as happens pre-veraison (Greenspan et al., 1994). As this stage of the trial was pre-veraison it is possible that both mechanisms were at work, resulting in daytime peduncle flow being higher in the low RH treatment compared to the high RH treatment.

Night peduncle sap flow was the same for the peduncles measured in both the high and low RH treatments during Stage Three. As already discussed, the increase in berry transpiration per berry is due to the increase in cuticular transpiration as the stomata lose their regulatory ability (Blanke and Leyhe, 1987). As stomata are unable to regulate transpiration the transpiration rate is cuticular driven and is therefore solely dependant on the VPD. However, night peduncle flow during Stage Three was the same for both high and low RH treatments that may suggest otherwise. In contrast, the bunch measured in the high RH treatment was also the larger of two, with more berries and a higher surface area than the bunch in the low RH treatment. This would have increased bunch transpiration rate in comparison to the smaller bunch resulting in night peduncle sap flows being the same.

Night peduncle sap flow had decreased when compared to Stage Two and this may be due to a reduction in both xylem and phloem flow into the bunch. During the first stage of berry development both xylem and phloem flow increase, reaching a peak around 20 days after flowering, and then steadily decreasing to a minimum at the lag stage of berry development. Post-veraison, phloem flow increases again (Figure 2. from Dry and Coombe, 2004).

Near the end of Stage Three a peduncle was girdled to determine if the measured peduncle sap flow was xylem or phloem sap flow. Immediately after the peduncle was girdled, sap flow reduced dramatically indicating that some of the peduncle sap flow was in fact phloem sap flow (Figure 68). However, as there was still some measurable sap flow during the day it appears that the sap flow still present was within the xylem. After girdling had taken place no night sap flow was determined. This indicates that at
this stage in berry development there was no or immeasurable amounts of xylem flow during the night. Initially, water flows predominantly in the peripheral xylem system and later in the axial system that is less conductive (During et al., 1987), so it is possible that at the time of girdling all peripheral xylem movement had ceased and that axial xylem flow occurred only during the day. Blanke and Leyhe (1987) also found that the difference between day and night transpiration decreased as berries developed, resulting in similar transpiration rates post-veraison. It is suggested that there may be more xylem sap flow than phloem sap flow during the day, especially in the early stages of berry development, and this would result in the larger difference between day and night transpiration early on.

At the time of girdling, veraison was expected to begin soon after, and the hypothesised reduction of xylem sap flow is supported by literature on the decrease in xylem function around the time of veraison (Lang and During, 1991; Creasy and Lombard, 1993; Greenspan et al., 1996; Ollat and Gaudillere, 1996; Dry and Coombe, 2004). However, this suggested reduction in sap flow occurred in the current study before the rapid increase in berry growth and therefore supports the work of Creasy et al., (1993) that found that dye uptake through the xylem was reduced around the time of softening that occurs before rapid berry growth and colouration.

It was not possible in the current study to determine whether flow into the bunch during the night consisted of xylem flow at any time during bunch development. It would be interesting to repeat the sap flow measurements, and to girdle different bunches throughout bunch development from fruit set to veraison to see how xylem and phloem flows change over that time. This information would also help researchers understand the differences in day and night sap flow due to the bunch/vine competition, sink strength and VPD differences.

5.4.4 BSN

Determining any trends in BSN incidence in the current study due to the RH and lateral removal treatments was limited due to bird and fungal damage to the bunches after
veraison. Estimations of BSN may have been over or under estimated resulting in some significant differences among treatments being obscured.

Unlike Jordan (1985), who found that high RH conditions significantly increased BSN incidence, we found no effect of RH on BSN. However, the development stage treatment had a highly significant effect on BSN incidence. It appeared that overall, only Stage Two significantly affected the incidence of BSN when compared to vines that were never placed in the CE conditions (Figure 42). Although not significantly different, when comparing vines that retained laterals, vines placed in the low RH treatment during Stage Two had 10% less BSN than vines placed in the high RH treatment. This is in accordance with Jordan’s (1985) work although in that study, vines in the 80% RH CE conditions had twice as much BSN than the vines in the 40% RH CE conditions. The difference in magnitude between the two studies may have been due to a variety of reasons including the damage caused by birds and fungal infections. Also, the vines in the current study did not increase in canopy size as much as Jordan’s (unpublished data) vines did. This may have been due to the length of time in which the vines were in the CE rooms, or the fact that the temperature of the CE conditions was not as high as it was in Jordan’s study (unpublished data). As the temperature was not as high, the VPD values in the current study would not have been as high as those in Jordan’s (1985) study, thus possibly reducing the effect of VPD on BSN. Heat stress may also have been a factor in Jordan’s (1985) study. Also, vines placed in the CE rooms may have reacted to an increase in vigour in a similar way to vines headed back in Season One. Increasing the vigour of vines in the field by heading back of the canes only increased BSN incidence in the already high vigour season of Season One (Chapter Three). Vines placed in the CE rooms were not very vigorous initially and therefore high RH CE room conditions, which may increase the vigour of the vines compared to the low RH CE room conditions, may not have increased vigour to the degree where a large increase in BSN incidence would be exhibited.

The finding in the current study that the period immediately after flowering is directly related to the incidence of BSN supports the finding from the field where shade post-FB increased BSN incidence (Chapter Three). Other studies have also found that certain
environmental conditions around flowering or immediately after, may influence the incidence of BSN (Theiler, 1983; Theiler and Muller, 1986; Boselli et al., 1987; Baldacchino-Reynaud, 2000). Theiler and Coombe (1985) suggested that a deficiency in GAs around this time may be critical to BSN incidence due to their role in peduncle development. However, during this period other processes that may influence BSN incidence, such as cell division, also occur in berry development. This finding is very important as future work can now concentrate on a much more defined time frame for determining how environmental factors affect BSN. As Stage Two of the trial was the only stage where conditions affected BSN incidence, the discussion will focus on the influences during this time that may have affected the incidence of BSN.

In the high RH treatment the removal of laterals appeared to reduce the incidence of BSN (Figure 74). This supported the original hypothesis that a reduction in apical bud number will result in a reduction of the competition between the reproductive and vegetative sinks. However in the low RH treatment, removing laterals tended to increase the incidence of BSN (Figure 74). This is in agreement with the results obtained from the field trial work with plant growth regulators (see Chapter Four). Although the removal of laterals was an attempt to reduce the strength of vegetative sinks by removing apical buds, this treatment may have in fact have had the opposite effect in both the field and in the low RH treatment and increased the vegetative competitive ability due to the subsequent increase in vegetative growth (See Chapter 4 for a detailed discussion). When assessing the trends of lateral numbers and BSN incidence in the low RH treatment, the increase in the number of laterals from the second to the third assessment was greater for vines with laterals removed compared to vines that had laterals retained (Appendix 13). In the high RH treatment it was the vines with laterals retained that had the higher rate of lateral increase between assessments two and three and these vines had a higher BSN incidence compared to vines with laterals removed (Appendix 13).

However, the rate of lateral increase after flowering cannot explain all the variations in the data. For the vines that were placed in the CE rooms during Stage Three, vines placed in the high RH treatment and with laterals retained had a similar rate of lateral
increase after flowering as some of the vines placed in the CE conditions during Stage Two. However, the BSN incidence for the Stage Three vines was much lower than for vines placed in the CE conditions during Stage Two. Therefore, factors that influence BSN incidence such as physiological and/or morphological changes to the vines, other than lateral increase, must have occurred in vines placed in the CE conditions during Stage Two.

There does not appear to be a consistent relationship between night and day peduncle sap flows and BSN incidence among the RH and lateral manipulation treatments (Table 42). This may be due to the bird and fungal damage already mentioned and therefore a repeat of this work is required before any conclusions can be made concerning this. However, it has been shown in this study that many factors interact to influence the competitive ability of the bunch for sap flow. Jordan’s (1985) study clearly demonstrated that BSN incidence was increased by high RH. The high RH treatment in the current study had a lower peduncle sap flow during the ‘night’ and also a higher BSN incidence compared to the low RH treatment (Table 42). This would indicate that the amount of ‘night’ sap flow into the bunches after anthesis may be important in reducing the incidence of BSN. Therefore bunches may be predisposed to BSN by a deficiency in some substances entering the bunch at night during the first few weeks of bunch development.

A difference in substance concentrations between day and night peduncle sap flow and between vines with laterals retained and vines with laterals removed is possible. If the sap moving through the peduncle is all xylem sap, and there being no further cane sap flow during the night hours, any xylem sap moving through the peduncle must already be present in the xylem system in the cane before cane sap flow rates reach zero. Soil temperature almost reaches its peak just prior to the time during which cane sap flow declines and by the time cane sap flow resumes soil temperature has reached its minimum (Figure 73). Soil temperature has been found to affect the uptake of elements such as potassium and calcium (Marschner, 1995) with the concentration of potassium increasing and the concentration of calcium decreasing as the temperature increases. The sap stored in the cane, which then may move into the bunch during the night, may
Table 42: Approximate BSN incidence overall, only for Stage Two and only for vines assessed with sap flow sensors, night peduncle sap flow rate, day peduncle sap flow rate and difference between night and day peduncle sap flow rates, for vines in the CE conditions during Stage Two.

<table>
<thead>
<tr>
<th>Relative humidity</th>
<th>Lateral removed</th>
<th>Stage Two BSN incidence (%)</th>
<th>Individual BSN incidence (%)</th>
<th>Night peduncle sap flow (g hr⁻¹)</th>
<th>Day peduncle sap flow (g hr⁻¹)</th>
<th>Total peduncle sap flow (g day⁻¹)</th>
<th>Maximum cane sap flow (g hr⁻¹)</th>
<th>Difference between day/night peduncle sap flow (g hr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>No</td>
<td>81</td>
<td>90</td>
<td>0.80</td>
<td>0.80</td>
<td>19</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>High</td>
<td>Yes</td>
<td>68</td>
<td>80</td>
<td>0.80</td>
<td>0.72</td>
<td>18</td>
<td>13.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Low</td>
<td>No</td>
<td>71</td>
<td>50</td>
<td>1.00</td>
<td>0.80</td>
<td>21</td>
<td>6.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Low</td>
<td>Yes</td>
<td>82</td>
<td>70</td>
<td>1.00</td>
<td>0.45</td>
<td>15</td>
<td>16.5</td>
<td>0.55</td>
</tr>
</tbody>
</table>
be higher in potassium and lower in calcium than day sap. However, it is unlikely that all of the peduncle night sap flow is xylem sap flow.

Xylem and phloem sap carry different amounts of substances, for example, potassium, which is carried in far greater quantities in the phloem than the xylem (Mengel and Kirkby, 1987; Marschner, 1995). Therefore if certain conditions affect one transport system more than the other it may affect nutrients moving into the developing bunch.

The rate of sap flow can also change the concentration of substances in the xylem sap (Marschner, 1995). Therefore in the vines with laterals removed, where the transpiration rate was higher, the concentration of the xylem sap may have been different (i.e. lower). However, if this was the case it might be assumed that there would be a trend between the concentration of a substance in the rachis or berry and BSN incidence. Although many authors have found correlations between nutrient ions such as potassium, magnesium and calcium and BSN incidence (Lauber and Koblet, 1967; Hartmair, 1975), many other authors have either found no correlation (Claus, 1965; Redl, 1983) or have found an opposite relationship (Redl, 1983; Ruiz and Moyano, 1994). In our field trial no correlations between treatments that affected BSN incidence and potassium, magnesium and calcium were found (see Chapter Three). Also, During and Oggionni (1986) found a positive relationship between calcium accumulation and transpiration rate, and yet Boşelli and Vaio did not (1996). Further work using techniques that demonstrate localised distribution of these nutrients early in the predisposition of BSN is required before any conclusions can be made concerning the incidence of BSN and nutrient concentrations.

The use of the sap flow sensors has demonstrated how varying factors can change the competitive ability of the bunch. However, further work is required before any conclusions can be reached about how this may also affect the incidence of BSN and therefore begin to explain the results obtained in both the field and laboratory.
6 General Discussion and Conclusions

Research in the present study focused on two key hypotheses, that being, that there is a critical time in which grape bunches become predisposed to the disorder of BSN, and that the vigour of the vine influences the severity of that disorder. Shade and plant growth regulator applications, as well as controlled environment studies, have shown that the critical time in which bunches become predisposed is immediately post-FB (Chapters Three, Four and Five). Following on from this research, it is suggested that further events, near veraison, must occur for symptoms to be manifested in predisposed bunches (the manifestation stage). Treatments that affect the vigour of grape vines immediately post-FB, have also shown that there is a strong positive correlation between the vigour of the vines and the severity of the disorder (Chapter Three). Therefore, factors that influence the vigour of a vine around this critical time will also influence the incidence of BSN. It is hypothesised that this is due to competition between vegetative growth and the developing young bunch for either assimilates and/or nutrients. The competitive ability of a bunch can be influenced by plant growth regulators and therefore endogenous concentrations of plant growth regulators in the vine and/or bunch will influence the incidence of BSN. As endogenous plant growth regulator concentrations can be influenced by the environment, management practices and cultivar type, these factors will all influence the severity of BSN.

6.1 Critical timing

Many studies on BSN have indicated that environmental conditions around a certain time may predispose vines to BSN development. However, the literature is not consistent as to when that time may be. Pre-flowering (Holzapfel and Coombe, 1995), flowering (Theiler, 1983; Theiler and Muller, 1986), post-flowering (Boselli et al., 1987) and around veraison (Brechbuhler, 1987; Redl, 1987) are all times that the literature indicates may be important stages in the development of the grapevine when berries may be predisposed to BSN. Nonetheless, it has been clearly demonstrated in this thesis that the period immediately following flowering is the most critical development time. Shade and placement of vines in CE conditions during the early stages of the first phase of berry development significantly increased the incidence of
BSN, while application of specific plant growth regulators during this time could reduce the incidence. Shade and placement of vines in CE conditions prior to flowering either reduced BSN incidence or had no effect. Placement of vines in CE rooms prior to veraison also had no effect on the subsequent incidence of BSN.

The berry’s final size, shape and texture is strongly influenced by the number, size and shape of cells in the flesh and skin and by cell wall properties (Dry and Coombe, 2004) and these are determined during the first phase of berry development. During the first two weeks after flowering, berry growth is mainly determined by cell division with cell expansion occurring in the subsequent four weeks (Dry and Coombe, 2004). However, cell division ceases during anthesis and then begins again with most cell division in the pericarp occurring between the fifth and tenth day after anthesis (Coombe, 1960). Although xylem cell development in the peduncle and rachis has ceased by the end of bloom, there is further development of the phloem, parenchyma and collenchyma during this first phase of berry development (Theiler and Coombe, 1985). Most of the seed development also occurs during this phase although the endosperm and the embryo do not fully develop until after veraison (Dry and Coombe, 2004). It is therefore clear that factors adversely affecting cell division and expansion in the peduncle, rachis, berry and/or seed, during this early stage, may have detrimental effects on final berry quality and the incidence of BSN.

Factors that may affect cell division within the peduncle, rachis and berry, either directly or indirectly, include a reduction in available nutrients or assimilates, temperature and plant growth regulator concentrations. Shade may decrease cell division as a result of the reduction in available assimilates through a decrease in photosynthesis, a direct reduction in plant growth regulator activity or synthesis through a change in temperature or nutrient supply (Marschner, 1995), or a reduction in cell division itself due to a change in temperature or nutrient supply. While there appears to be no literature on how root pruning affects cell numbers in fruit of any crop species, it has been shown to not alter the cell number per leaf, although leaf size is reduced (Milligan and Dale, 1988). However, a difference in fruit size was obvious in apples at the end of the cell division stage (and at harvest) after root pruning at bloom (Ferree and
Chapter Six - General discussion and conclusions

Knee, 1997a). As root pruning in that study was carried out at bloom, it may have had a greater influence on cell division than root pruning in the current study that was carried out during the dormant stage. Nonetheless, in the current study, berry size was markedly reduced as a consequence of the root pruning.

In studies, including the shade pre-FB treatment in the current study, where conditions pre-bloom influenced the incidence of BSN, it may have been the difference in conditions during pre-bloom and during the early stages of berry development that influenced the predisposition to BSN. For example, rain pre-bloom may have resulted in higher vine vigour post-flowering and it is this growth, not the rain event, which led to the increased incidence of BSN. Vines may acclimate to high temperatures or light pre-bloom resulting in a perceived reduction in temperature or light intensity post-bloom, resulting in an increase in BSN incidence, and vice versa. Conversely, high temperatures pre-bloom may also mean vines may be under more stress post-bloom, therefore reducing overall vegetative growth and reducing BSN incidence. Redl (1987) found an increase in BSN incidence due to high temperatures during the early stage of berry growth that may appear to contradict the above explanations. However if soil moisture was also high, the increase in temperature may have increased vine growth, by either altering the competition between vegetative and reproductive sinks, or by increasing canopy shade.

Although it is suggested in this study that the early stage of berry growth post-flowering is the critical time during which bunches are predisposed to BSN, another event is required for the actual development and expression of BSN (manifestation stage). Before and during veraison is a time in development where many processes within the grape berry are dramatically changing. Due to the dysfunction of the xylem, sap flow that is predominately xylem in origin pre-veraison, changes to being phloem in origin post-veraison. This in turn results in a change in the accumulation of nutrients, soluble solids and various acids in the berry after veraison (Dry and Coombe, 2004). Plant growth regulator concentrations in the berry flesh dramatically change at this time with IAA decreasing to a minimum and ABA beginning to increase (Zhang et al., 2003). During the lag stage of berry development, the IAA concentration in the seeds increases
to a peak and then decreases again at veraison. These changes may impact on factors associated with berry maturation that are occurring within the peduncle, rachis or berry (including the breakdown of the middle lamella in the berry flesh and xylem dysfunction in the peduncle resulting in increased phloem sap flow into the berry). In a bunch that is predisposed to BSN, if these events are disrupted, then there will be subsequent development of visible symptoms of the disorder. Conversely, in a bunch that is predisposed to BSN, these changes may either remove a factor that suppresses BSN symptom development, or begin a process that does not have the essential elements required for the normal progression of the maturation process.

If the hypotheses concerning the manifestation stage are correct, environmental factors, such as rain around veraison, may enhance vegetative growth, which may increase the overall development of BSN (i.e., it may cause an increase in the development of detrimental symptoms so that they are obvious to the eye in contrast to the initial symptoms of BSN (non-girdling spots) that are not as obvious and are not damaging to berry quality). Therefore, if conditions cause bunches that have the initial symptoms to progress into detrimental symptoms, BSN incidence (as measured by these detrimental symptoms) will be assessed as to have increased. This may, therefore, explain why some literature reports that there is an increase in BSN incidence due to rain events around veraison, while in other studies rain has no effect on BSN incidence because bunches had no prior predisposition to it due to favourable conditions immediately post-flowering.

### 6.2 Nutrient

Many studies have investigated nutrient concentrations within the petiole, rachis and/or berry around veraison to try and find correlations with BSN incidence. It is suggested that by this time, although not physically obvious, BSN may already have begun to affect nutrient concentrations in the bunch, and therefore any correlations found are most likely to be a consequence and not a cause of BSN. This would possibly explain the contradictory results in the literature and also why no correlation between BSN and nutrient concentrations were found in the current study. A possible nutrient imbalance during the time after flowering, which our studies have indicated to be a critical time in
the predisposition of BSN, may be the cause of BSN, and therefore measurement of nutrient concentrations and ratios around this time are likely to provide a better understanding of what may be occurring. However, these tests would need to be specific to certain zones of tissues within the bunch as bulk sampling may mask any possible deficiencies.

BSN has often been considered to be a calcium disorder similar to that of bitter pit and watercore in apples, and blossom-end rot in tomatoes and peppers (Boselli and Fregoni, 1986; Haub, 1986). Calcium is important in a number of metabolic and developmental processes including the integrity of cellular membranes and the formation of new middle lamella. It is also essential for normal membrane functions in all cells (Salisbury and Ross, 1992). A deficiency in calcium results in tissue becoming soft due to the dissolution of the cell walls. Growing tips and young leaves become deformed and chlorotic, and at advanced stages, necrosis occurs in the leaf margins. Also, cellular breakdown can occur resulting in many calcium-related disorders in a wide range of fruits and vegetables (Mengel and Kirkby, 1987). Blossom-end rot in tomatoes has also been associated with the disintegration and increased ion permeability of cell membranes resulting in loss of turgor and cell fluids invading the intercellular air space (Saure, 2001 and references therein).

Calcium deficiency in the vine (Kadam et al., 1995) and low calcium concentration or high K/Ca ratios in the bunch have, on occasion been correlated with BSN incidence. Also, as calcium is important to cell integrity, cell function and the integrity of the middle lamella, it has often been considered the nutrient that, if deficient, causes BSN incidence. However, only a few studies have found calcium sprays to suppress BSN (Lauber and Koblet, 1967) with many more studies finding no difference in BSN incidence when calcium sprays are applied (Christensen and Boggero, 1985; Jordan, 1985; Cline, 1987; Capps and Wolf, 2000).

The middle lamella is broken down by enzymes during the normal ripening processes of fruits and this is why fruit become soft upon ripening as cells are now able to slide past one another. It appears that the middle lamella is the first part of the cell wall to be
affected by BSN (Jahnl, 1975). The first types of cells to exhibit BSN symptoms are the stomata, epidermis and hypodermis of the peduncle or rachis (Delas et al., 1976; Theiler, 1976; Brendel et al., 1983). As BSN development begins around the time of ripening and when berries begin to soften, it is possible that as well as breaking down the middle lamella in the berry mesocarp for the ripening process, the middle lamella between the cells within the peduncle and rachis are also broken down, resulting in BSN symptom development.

As application of magnesium sprays or NAA directly to the bunch or rachis prior to veraison can often reduce the symptoms of BSN (Lauber and Koblet, 1967; Koblet et al., 1969; Schaller, 1977; Haub, 1983; Cline, 1987; Brechbuhler, 1991), it is suggested that these applications either strengthen the middle lamella so that it is more likely to remain intact, or that they reduce or prevent the production of the enzymes involved in the breakdown of the middle lamella. This may be a direct result of an increase in the concentration of magnesium or auxin in the bunch, or from the production of secondary metabolites. Magnesium also plays a major role in enzyme activity, especially the enzymes involved in photosynthesis, respiration, and the formation of DNA and RNA (Mengel and Kirkby, 1987; Salisbury and Ross, 1992). RNA synthesis immediately stops in response to magnesium deficiency and generally, 5-10% of the total magnesium in leaves is firmly bound to pectate in the cell walls or is sparingly soluble in the vacuole (Marschner, 1995). Therefore, magnesium plays an important role in cell division and cell wall formation.

The uptake and translocation of magnesium is restricted by other cations, including K⁺, NH₄⁺ and Ca²⁺, although increasing potassium supply can increase the magnesium concentration of fruit, while reducing it in leaves (Mengel and Kirkby, 1987). Unlike calcium, magnesium is very mobile in the phloem (Mengel and Kirkby, 1987). In the current study it was found that sap flow into the bunch at night was higher in vines placed in low RH conditions compared to those placed in high RH conditions (section 5.3.4). BSN incidence has also been found to be lower in vines placed in low RH conditions compared to high RH conditions (Jordan, 1985). Although the current study did not achieve such a dramatic difference between RH treatments for BSN incidence as
Jordan's study (Jordan, 1985), the results for vines with laterals retained were consistent with his findings. Girdling of the peduncle in the current study also demonstrated that there was phloem flow both during the day and night periods, but it was difficult to determine how much, if any, xylem flow may also have occurred in the night period, especially early in bunch development. It may be possible that phloem flow is higher than xylem flow during the night period that would result in an increase of phloem transported ions into the low RH treated bunches. Allweigt and Hifny (1972) also found that calcium concentration in the rachis was higher in the affected bunches during the early stages of BSN development. The high concentration of calcium may have reduced magnesium concentration in the rachis resulting in enzyme activity, RNA synthesis, cell division and cell wall formation being affected. This hypothesis lends more support to magnesium playing an important role in the reduction of BSN incidence.

The use of sap flow sensors in the current study demonstrated that peduncle sap flow at night was solely affected by the VPD around the bunch, whereas peduncle sap flow during the day can be influenced by many variables. These include the potential sink activity and the rate of cane sap flow (section 5.3.4). If BSN incidence is influenced by a nutrient flow into the bunch, it is important to determine the varying amounts of xylem and phloem flow into the bunch at different stages in bunch development and also at different times during the day. This may then help to indicate which nutrient or nutrients are influencing BSN development.

6.3 Plant growth regulators

There is a lot of evidence to suggest that blossom-end rot in tomatoes may be related to a calcium deficiency and, as with BSN, there is a lot of contradictory evidence that suggests that calcium may not be the primary factor in that disorder (Saure, 2001). However, unlike blossom-end rot, BSN incidence has been shown to be decreased by the application of exogenous gibberellins (Allweigt and Hifny, 1972; Beetz and Bauer, 1983; Haub, 1983; Theiler and Coombe, 1985) and this was confirmed in the current study (section 4.3.6). This suggests that BSN may not be a calcium disorder like blossom-end rot or bitter pit in apples, as although similar symptoms may occur, the
mechanism behind the development of the symptoms is likely to be different. Fruit drop in apples, known as “June drop” in the Northern Hemisphere, is also reduced by the application of gibberellins (Wertheim, 1973). “June drop” occurs about the time of the onset of rapid growth of the embryo (Wertheim, 1973). At the end of the lag stage of grape development the embryo is incompletely developed and therefore further embryo growth must occur during the final stage of berry development, after veraison, which is when BSN symptoms first begin to appear. The concept behind “June drop” is that the developing embryo digests the surrounding endosperm tissue faster than it is replaced, and as the endosperm is the principal hormone source, this reduction in hormone concentration leads to fruit abscission (Luckwill, 1949, 1953). In grapes, the endosperm is also not fully developed at the time of veraison, and it is therefore possible that BSN is a disorder more like “June drop” rather than blossom-end rot, and that hormones play an important role in not only the predisposition of bunches to BSN, but the actual development of it as well.

As calcium is an antagonist to magnesium transport, and gibberellic acid is an antagonist to calcium transport (Saure, 2005 and references therein), this would suggest that the application of gibberellins to a bunch may also reduce the calcium concentration in the bunch, therefore allowing increased transport of magnesium into the bunch overall or into specific cells within the bunch. Interestingly, it has been suggested that root pruning reduces gibberellin concentration (Saure, 2005), and yet the current study found that root pruning reduced BSN incidence (section 3.3.8). Research into the effect of root pruning on gibberellin concentrations in the berry early after anthesis is therefore required.

Gibberellins increase cell division and expansion but it has been reported that seeded berries do not respond to applications of gibberellins, as it is thought that seeded cultivars have sufficient endogenous gibberellin for full development (Coombe, 1973). However in the current study, ‘Cabernet Sauvignon’, a seeded cultivar, did respond to the application of GA₃, which possibly suggests that this cultivar may have suboptimal concentrations of gibberellins after anthesis. Gibberellin concentration has been found to decrease during anthesis and then increase again until the beginning of the lag stage.
Lack of endogenous gibberellins may lead to a reduction in cell division in the young fruit that results in low potential sink activity and the inability to compete effectively against vegetative sinks. Insufficient concentrations of endogenous gibberellins during xylem development may also result in a reduction in xylem development. Therefore the restriction in xylem that Lang et al. (1994) found in susceptible cultivars and suggested lead to the development of BSN through reducing nutrient flow into the bunch, may be purely another product of the low gibberellin concentration, and not actually directly related to BSN development.

It has been noted that immature leaves and shoot apices reduce fruit set proportional to their growth rate, and that their removal increases fruit set (Coombe, 1973; Vasconcelos and Castagnoli, 2000). In the very early stages of berry development, after anthesis and before cell division has resumed or increased to such a degree that the berry achieves sufficiently strong sink activity, this competition between vegetative sinks may lead to a reduction in assimilate or nutrient supply to the berry. This would be more obvious in vines that had very weak unhealthy growth and a limited amount of assimilate and nutrient supply. It has been noted in a few studies that BSN can be more prevalent in vines that have a very weak growth habit. However, BSN incidence is also prevalent in very vigorous vines where there is an ample supply of both nutrients and assimilates. In these cases primigenic dominance may be responsible for an insufficient amount of assimilates or nutrients reaching the developing bunch. In vigorous vines, if vegetative sinks are relatively stronger sinks than the developing bunch they will dominate over the bunch and therefore prevent or limit the transport of assimilates and nutrients to that bunch.

Root pruning, which reduced BSN incidence (section 3.3.8), may reduce the competition between vegetative and reproductive growth even though nutrient concentrations into the bunch were reduced and berries were smaller (which suggests a smaller sink activity in comparison to the bunches on the control vines). The observed smaller sink ability is in contradiction to the hypothesis of the predisposition of BSN being due to an inadequate supply of assimilates or nutrients after anthesis. However, in our study, root pruning dramatically reduced vegetative growth (section 3.3.1), so the
vegetative sinks had comparatively lower sink activity compared to the bunches and were, therefore, unable to dominate those bunches. IAA is thought to be the main plant growth regulator involved in both competition and dominance. Endogenous IAA concentrations may therefore play an important role in the reduction of BSN due to root pruning. It is possible that in the case of root pruned vines, the bunches then became dominant over the vegetative sinks and continued to suppress vegetative growth.

Therefore to summarise, it is believed that the incidence of BSN is due to a predisposition to the disorder occurring immediately after flowering. This is hypothesised to be usually due to vegetative growth being dominant over the young developing berries on the bunch. It is also possible that earlier developed bunches also dominate over later developed bunches that often have a higher incidence of BSN (personal observation). Environmental and cultural practices will influence this dominance effect on bunches, thus resulting in some years and with some management techniques having higher incidences of BSN. Cultivars that are more susceptible to BSN may have naturally low concentrations of important plant growth regulators ensuring that bunches of those cultivars are more likely to be dominated by vegetative sinks.

Internal changes, which are essential for the ripening of the berry, such as berry IAA and ABA concentration changes, and middle lamella break down, may be the cause of BSN symptoms developing after veraison. Extensive research in this area is required. However, it is speculated that due to a possible deficiency during the development of the middle lamella in peduncle and rachis tissue that this tissue looses its integrity. This may be caused by the production of enzymes in the peduncle and rachis that dissolve the middle lamella as they do during the berry ripening process. In healthy bunches this may not occur due to the high concentration of a nutrient or plant growth regulator in the peduncle or the rachis that possibly prevents the production of these enzymes. Application of auxin or magnesium before this event may therefore increase the concentration of the substance that restricts that enzyme activity.
Changes in berry, peduncle and rachis composition occur due to reduced phloem sap flow into the bunch as the necrosis that rings the peduncle or rachis essentially girdles the bunch. It is expected that cellular changes caused by BSN occur in the bunch before symptoms become visible, so therefore nutrient analyses of the bunch immediately prior to and during symptom development are unlikely to help with the understanding of the causes of BSN.

### 6.4 Future research

Root pruning has been shown to effectively reduce BSN incidence for at least three years after the root pruning has been carried out. It would be interesting to assess when exactly this effect dissipates totally and when BSN incidence returns to commercially significant levels. Not only did root pruning decrease BSN incidence but many wine quality aspects were also greatly enhanced indicating that root pruning, where feasible, is an effective tool to use in vineyard management to produce quality grapes and quality wines.

However, root pruning is not always a feasible option due to site access, type of soil and/or cost. As a reduction in vegetative growth, especially around flowering, has been correlated with a reduction in BSN incidence it would be interesting to assess the effectiveness of growth retardants on BSN incidence. However, as some growth retardants are also gibberellin inhibitors, and gibberellins can be transported from the roots and the leaves, and the application of GA₃ to the bunch has already been shown to decrease BSN incidence, the use of a non-gibberellin inhibitor growth retardant would be required. A combination of applying a gibberellin inhibitor and a non-gibberellin inhibitor growth retardant to various parts of the vine would also help with determining from where endogenous gibberellins, which may reduce the incidence of BSN, are derived.

Investigating the concentration and source of gibberellins and other plant growth regulators in berries on root pruned vines after flowering would also help researchers to understand the mechanism behind the reduction in BSN incidence due to root pruning. If plant growth regulator concentrations are not affected by root pruning it may be
assumed that the reduction in BSN incidence was purely caused by a reduction in the dominance of the vegetative sinks.

Application of plant growth regulators or their inhibitors solely to the peduncle, rachis or the berry will also help to determine the role of plant growth regulators in BSN incidence. It may also result in an understanding of whether or not it is the peduncle, rachis or berry itself that is affected by the dominance of other sinks.

Girdling above and/or below the bunch would also help determine whether or not it was purely apical buds and stems that were dominating the bunch or if the rest of the vine may also influence this. A repeat of the experiments carried out in Chapter Four, which would also include girdling below the bunch, would help to determine if the results obtained from removing laterals was due to an increase in reproductive sink activity of the hormone treated bunches, or whether it was solely a result of lateral removal.

The question of whether or not calcium or magnesium affects the incidence of BSN could also be determined by girdling the peduncle early in bunch development. It would be important that the girdle was very small, for example a shallow scalpel cut, or that a spiral girdle was used to ensure bunch survival. If girdling did not increase BSN incidence it could be assumed that xylem flow is important in the reduction of BSN, while an increase in BSN incidence due to girdling might indicate that it is phloem flow that is important. As calcium is transported mainly in the xylem, and only in the phloem in very small quantities, it may be assumed that if BSN does develop with girdling that it may not be related to a deficiency in calcium.

Girdling the peduncle and measuring the subsequent sap flow at the early stage of development would also lead to an understanding of how much sap flow can be attributed to either the xylem or phloem at different stages of berry development and at different times in the day. This, along with subsequent assessments of BSN, would help researchers determine which conditions may affect BSN incidence and therefore help
with the control of this disorder. Again, a girdle that ensured the survival of the bunch would be important, in order for subsequent BSN assessments to be carried out.

Repeating the controlled environment experiments with older and more established vines and improving vine management to reduce disease and bird damage might prove beneficial in understanding how sap flow into the bunch may affect BSN incidence. Peduncle sap flow may indicate how dominance over the bunches may be affected by humidity and the removal of laterals. Including carbon-14 analyses in such an experiment may also help with determining if BSN is a disorder to do with nutrients or assimilates. Application of carbon-14 to source leaves at the critical time that has been identified, and determining where the assimilated carbon was transported to, would help indicate how the different treatments affect the competitive ability for assimilates at this time in berry development. Application of plant growth regulators and measuring the subsequent peduncle sap flow would help to improve the understanding of how hormones may influence the incidence of BSN.

Further analysis of nutrient concentrations in the peduncle, rachis and berries early in the development of the bunch would be beneficial in determining whether BSN is a nutrient disorder. However, these assessments would need to be carried out at a cellular level within the peduncle and rachis, not a bulk sample, in case the deficiency is localised within certain tissues or cells. Such assessments will be complicated in that BSN symptoms do not develop until after veraison and it will therefore be difficult to determine the difference between healthy bunches and bunches that are predisposed to BSN. However, this study has demonstrated that certain conditions at a critical time in bunch development will increase the incidence of BSN. Therefore, by placing vines in optimum growing conditions that increase vine vigour dramatically immediately after flowering, it should now be possible to produce bunches with a very high probability of presenting BSN symptoms.

The many ways in which vegetative sinks may become dominant over reproductive sinks may explain much of the variation in the literature on BSN incidence. Whether or not predisposition to BSN is due to assimilates, nutrients or both, is yet to be confirmed.
and there is still a lot of research to be carried out before a clear understanding of BSN and how it develops can be attained. However, future research can now focus on a defined development stage, which is around the three weeks immediately after anthesis. This will result in such research becoming more focused and this time frame may also be reduced even further as the mechanisms behind BSN are more fully understood.
Appendices


A)

<table>
<thead>
<tr>
<th>Level 1</th>
<th>Level 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: Nothing</td>
<td>Nothing</td>
</tr>
<tr>
<td>2: Root pruned</td>
<td>Nothing</td>
</tr>
<tr>
<td>3: Root pruned</td>
<td>Extenday</td>
</tr>
<tr>
<td>4: Root pruned</td>
<td>Shade pre-FB</td>
</tr>
<tr>
<td>5: Root pruned</td>
<td>Shade post-FB</td>
</tr>
<tr>
<td>6: Root pruned</td>
<td>Heading back</td>
</tr>
<tr>
<td>7: Heading back</td>
<td>Nothing</td>
</tr>
<tr>
<td>8: Heading back</td>
<td>Extenday</td>
</tr>
<tr>
<td>9: Heading back</td>
<td>Shade pre-FB</td>
</tr>
<tr>
<td>10: Heading back</td>
<td>Shade post-FB</td>
</tr>
<tr>
<td>11: Nothing</td>
<td>Extenday</td>
</tr>
<tr>
<td>12: Nothing</td>
<td>Shade pre-FB</td>
</tr>
<tr>
<td>13: Nothing</td>
<td>Shade post-FB</td>
</tr>
</tbody>
</table>

Each cell represents 1 Bay consisting of 5 vines.
End two vines (of each bay) were used as guard vines.
B)

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>Laterals retained</td>
</tr>
<tr>
<td>NL</td>
<td>Laterals removed</td>
</tr>
</tbody>
</table>

Guard rows/bays
Unused bays

Each cell represents one vine
End two vines (within each block) were used as guard vines and out of the three remaining, only two vines were chosen as treatment vines based on their uniformity.
Appendices.

Appendix 2: Spray Schedule

<table>
<thead>
<tr>
<th>Disease</th>
<th>Pre Budburst</th>
<th>Shoots 2-3cms</th>
<th>Interval</th>
<th>Interval</th>
<th>Capital 5%</th>
<th>Capital 80%</th>
<th>Post Flowering</th>
<th>post Christmas</th>
<th>Pre Bunch</th>
<th>Period to Harvest</th>
<th>Post Harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dowry</td>
<td></td>
<td></td>
<td>+10 days</td>
<td>+14 days</td>
<td></td>
<td>Mancozeb or</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mildew</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mancozeb or</td>
<td>Copper</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dead Arm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mancozeb or</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black Spot</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Copper</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Powdery Mildew</td>
<td>Please Consult</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mancozeb or</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VSM. Especially if you had a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Liquid Sulphur</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>problem last season</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broadly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Captan or Euparen Multi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(if weather dictates)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wettable or Liquid Sulphur</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st DMI</td>
<td>Topas or</td>
<td></td>
<td></td>
<td></td>
<td>Wettable or</td>
<td>Liquid Sulphur</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Switch</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Topas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>or Scythes or Atto</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Scythes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Option 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Switch or Topas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>or Scythes or Atto</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Option 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Switch or Topas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>or Scythes or Atto</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Option 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Switch or Scythes or Atto</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>or Scythes or Atto</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Option 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Switch or Scythes or Atto</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>or Scythes or Atto</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf roller Caterpillar</td>
<td>BT (only if required)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mealy Bug</td>
<td>Tokuthion/Oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Applied/Pean + Oil</td>
<td>(NO OIL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grass grub</td>
<td>Korean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Page 377</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendices.

Appendix 3: Weather data for Seasons One and Two.

Figure 75: Maximum and minimum daily temperature (°C), rain fall and irrigation (mm) applied in A) Season One and B) Season Two at Moteo, Taradale, New Zealand. No irrigation was applied in Season Two. F – approximate time of flowering, V – approximate time of veraison.
Appendix 4: Correlations between point quadrat assessments and BSN.

Other dates of vigour measurements and other assessment types were also investigated for a correlation with BSN. One example is of LLN two months after FB. This assessment date did give a slightly better correlation than LLN three weeks after FB (Figure 76). However, this was possibly due to an increase in treatments that were measured. Also, the increase was small indicating that treatment differences in vigour are already apparent three weeks after FB and that this does not change as the season progresses, and that either measurement time may be a good indicator of BSN incidence later in the season.

Figure 76: Correlation between leaf layer number two months after FB and BSN incidence (%) across all three seasons. \( Y = 3.2x + 0.64 \), \( R^2 = 0.75 \), \( p < 0.0001 \).

A second example is using PG rather than LLN. The correlation between PG and BSN was not as good as LLN (Figure 77), possibly due to the fact that once the vine has filled the canopy area, further canopy growth does not decrease PG values.
Figure 77: Correlation between percentage gaps approximately three weeks after FB and BSN incidence (%) across all three seasons. \( Y = -0.46x + 15.5, \ R^2 = -0.59, \ p=0.1856. \)
Appendices.

Appendix 5: Harvest juice quality measurements carried out on juice from vines with canopy manipulation and plant growth regulator applications to the bunches.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Brix (*)</th>
<th>TA (g/l)</th>
<th>B/TA (ratio)</th>
<th>pH</th>
<th>Malic acid (g/l)</th>
<th>Tartaric acid (g/l)</th>
<th>Calcium (mg L⁻¹)</th>
<th>Magnesium (mg L⁻¹)</th>
<th>Potassium (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Laterals retained</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>19.0</td>
<td>11.7</td>
<td>1.6</td>
<td>3.30</td>
<td>4.0</td>
<td>6.2</td>
<td>29</td>
<td>56</td>
<td>2225</td>
</tr>
<tr>
<td>GA₃</td>
<td>18.5</td>
<td>14.4</td>
<td>1.3</td>
<td>3.23</td>
<td>6.7</td>
<td>6.9</td>
<td>30</td>
<td>54</td>
<td>2479</td>
</tr>
<tr>
<td>IAA</td>
<td>18.7</td>
<td>13.3</td>
<td>1.4</td>
<td>3.28</td>
<td>3.6</td>
<td>6.5</td>
<td>29</td>
<td>55</td>
<td>2369</td>
</tr>
<tr>
<td>NPA</td>
<td>19.1</td>
<td>11.6</td>
<td>1.7</td>
<td>3.29</td>
<td>3.3</td>
<td>6.4</td>
<td>28</td>
<td>54</td>
<td>2362</td>
</tr>
<tr>
<td>(b) Laterals removed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>17.9</td>
<td>13.0</td>
<td>1.4</td>
<td>3.20</td>
<td>0.9</td>
<td>6.3</td>
<td>29</td>
<td>53</td>
<td>1872</td>
</tr>
<tr>
<td>GA₃</td>
<td>16.3</td>
<td>13.5</td>
<td>1.2</td>
<td>3.24</td>
<td>6.6</td>
<td>7.9</td>
<td>37</td>
<td>57</td>
<td>2450</td>
</tr>
<tr>
<td>IAA</td>
<td>16.7</td>
<td>14.2</td>
<td>1.2</td>
<td>3.17</td>
<td>4.1</td>
<td>8.3</td>
<td>41</td>
<td>64</td>
<td>2385</td>
</tr>
<tr>
<td>NPA</td>
<td>16.5</td>
<td>12.1</td>
<td>1.4</td>
<td>3.23</td>
<td>1.7</td>
<td>7.6</td>
<td>33</td>
<td>53</td>
<td>2486</td>
</tr>
</tbody>
</table>
Appendices.

Appendix 6: Modified Hoagland’s Nutrient Solution (1/2 strength)

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Formula</th>
<th>M</th>
<th>Grams/100 L</th>
<th>Element</th>
<th>Mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid stock solution:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitric acid</td>
<td>HNO₃</td>
<td>63</td>
<td>400</td>
<td>NO₃</td>
<td>6.35</td>
</tr>
<tr>
<td>Phosphoric acid</td>
<td>H₃PO₄</td>
<td>98</td>
<td>100</td>
<td>H₃PO₄</td>
<td>1.02</td>
</tr>
<tr>
<td>A Stock Solution:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium nitrate</td>
<td>Ca(NO₃)₂·4H₂O</td>
<td>236</td>
<td>14760</td>
<td>Ca</td>
<td>62.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NO₃</td>
<td>125.08</td>
</tr>
<tr>
<td>EDTA</td>
<td>[CH₂N(CH₂COOH)₂] · FeNa</td>
<td>430</td>
<td>400</td>
<td>Fe</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Na</td>
<td>0.93</td>
</tr>
<tr>
<td>B Stock Solution:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium nitrate</td>
<td>KNO₃</td>
<td>101</td>
<td>6320</td>
<td>K</td>
<td>62.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NO₃</td>
<td>62.57</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>MgSO₄·7H₂O</td>
<td>246</td>
<td>6162</td>
<td>Mg</td>
<td>25.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SO₄</td>
<td>25.05</td>
</tr>
<tr>
<td>Mono-potassium phosphate</td>
<td>KH₂PO₄</td>
<td>136</td>
<td>1700</td>
<td>K</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H₃PO₄</td>
<td>12.5</td>
</tr>
<tr>
<td>Micro-elements:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manganese chloride</td>
<td>MnCl₂·4H₂O</td>
<td>198</td>
<td>22.6</td>
<td>Mn</td>
<td>114.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cl</td>
<td>228.28</td>
</tr>
<tr>
<td>Boric acid</td>
<td>H₃BO₃</td>
<td>62</td>
<td>36</td>
<td>B</td>
<td>580.65</td>
</tr>
<tr>
<td>Copper sulphate</td>
<td>CuSO₄·5H₂O</td>
<td>250</td>
<td>4</td>
<td>Cu</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SO₄</td>
<td>4</td>
</tr>
<tr>
<td>Sodium molybdate</td>
<td>Na₂MoO₄·2H₂O</td>
<td>242</td>
<td>0.335</td>
<td>Mo</td>
<td>1.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Na</td>
<td>2.77</td>
</tr>
<tr>
<td>Zinc sulphate</td>
<td>ZnSO₄·7H₂O</td>
<td>288</td>
<td>2.75</td>
<td>Zn</td>
<td>9.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SO₄</td>
<td>9.55</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>KCL</td>
<td>74.6</td>
<td>78</td>
<td>K</td>
<td>1045.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cl</td>
<td>1045.58</td>
</tr>
</tbody>
</table>

The final nutrient solution contains equal proportions of each stock solution and has been diluted 220 fold.

pH of final solution = 5.5 – 6.5
Appendices.

Appendix 7: Inflorescence distribution

<table>
<thead>
<tr>
<th>Block One</th>
<th>Block Two</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vine number</td>
<td>Inflorescence number</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

Appendix 8: Leaf diameter/leaf area relationship

Figure 78: Relationship to determine leaf area from leaf diameter, $y = 0.6339x^2 + 2.177x - 10.644$. $R^2 = 0.96$
Appendix 9: Gauge specifications

<table>
<thead>
<tr>
<th>Model No.</th>
<th>Gauge height (mm)</th>
<th>Shield height (mm)</th>
<th>Stem diameter (mm)</th>
<th>Input voltage (volts)</th>
<th>Input power (Watts)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Min</td>
<td>Typical</td>
<td>Max</td>
</tr>
<tr>
<td>SGA2</td>
<td>35</td>
<td>70</td>
<td>2.1</td>
<td>2.5</td>
<td>3.5</td>
</tr>
<tr>
<td>SGA5</td>
<td>35</td>
<td>70</td>
<td>5</td>
<td>5.5</td>
<td>7</td>
</tr>
<tr>
<td>SGA9</td>
<td>70</td>
<td>180</td>
<td>8</td>
<td>9</td>
<td>10</td>
</tr>
</tbody>
</table>
Appendix 10: Stem heat balance theory: Extracted directly from van Bavel (2000) therefore references to sections and figure numbering applies to the Flow32™ manual and not this thesis.

STEM HEAT BALANCE THEORY
This section of the manual familiarizes one with the fundamental energy balance method to measure sap flow and sap heat flux. The basic equations, the thermodynamics, and the calculation of the sap flow are all the same for this type of sensor, even though the construction details may vary slightly. As noted in Section 3.1, the Trunk gages use multiple pairs of differentially wired TCs, and since the signals are averaged together electronically, we treat them all as one dT measurement when making the sap flow calculation. Microsensors SGA2 and SGA3 have variations where only a single pair of TC measure the dT, and for compatibility the Ah and Bh cable wires are connected redundantly to the same TC. The general theory is the same and there are only a few considerations required when calculating the Qv for these two sensors. Please see SEC. 3.3.

HOW DYNAGAGE WORKS

The SHE method requires a steady state and a constant energy input from the heater strip inside the gauge body. Therefore the stem section must be insulated from changes in the environment. For the same reason, the gauge time constant is limited from five minutes to an hour, depending on the flow rate and the stem size. The Dynamax loggers have a power down mode so that power is saved at night and the stem is preserved from overheating. During the power down mode and at the transitions to power on, the sap flow is not computed to maintain the accumulated flow accurately during this unbalanced transition.

Figure 8 - Stem Gauge Schematic

Figure 8 shows a stem section and the possible components of heat flux, assuming no heat storage. The heater surrounds the stem under test and is powered by a DC supply with a fixed amount of heat, Qh. Qh is the equivalent to the power input to the stem from the heater, Pin. Qr is the radial heat conducted through the gauge to the ambient. Qv, the vertical, or axial heat conduction through the stem has two components, Qu and Qd. By measuring Pin, Qu, Qd, and Qr, the remainder, Qf can be calculated. Qf is the heat convection carried by the sap. After dividing by the specific heat of water and the sap temperature increase, the heat flux is converted directly to mass flow rate.

ENERGY BALANCE EQUATIONS
The energy balance is expressed as:

\[ Pin = Q_r + Q_v + Q_f (W) \]  
\[ Pin = V^2/R \]  

from Ohms Law.
Fourier’s Law describes the vertical conduction components:

\[ Q_v = Q_u + Q_d \]

Where

\[ Q_u = K_{st} A \frac{dT_u}{dX} \]
\[ Q_d = K_{st} A \frac{dT_d}{dX} \]

where \( K_{st} \) is the thermal conductivity of the stem (W/m*K); \( A \) is the stem cross-sectional area (m²); the temperature gradients are \( dT_u/dX \) (K/m) and \( dT_d/dX \); \( dX \) is the spacing between thermocouple junctions (m). One pair of thermocouples is above the heater and one pair is below the heater as shown on the schematic in Figure 9.

There are two differentially wired thermocouples both measuring the rise in sap temperature. Channel AH measures the difference in temperature A-Ha (mV). Channel BH measures the difference in temperature B-Hb (mV). By subtraction of these two signals:

\[ BH - AH = (B - Hb) - (A - Ha) = (B - A) + (Ha - Hb) \] (mV)

The result yields the two components of axial heat conduction out of the stem section, \( Q_u \) and \( Q_d \) (See Fig. 9). Since the distances, \( dX \), separating the upper TC pair and lower TC pair are fixed by design for each particular gauge to the same value, the components of \( Q_v \) are combined with a common denominator:

\[ Q_v = \frac{K_{st} A (BH - AH)}{dX} \times 0.040 \text{ mV/C} \]

The factor 0.040 mV/C converts the thermocouple differential signals to degrees C. \( K_{st} \) values are given for varying stem conductivity: 0.42 W/m K (woody stem), 0.54 (herbaceous), and 0.28 (hollow).

Figure 9 - Dynagage Schematic
Appendices.

Appendix I1: Means of gas exchange measurements carried out on potted vines in Season Three in the high and low RH CE rooms and for canes with or without laterals removed. Assessment times are 1) Stage Two – post-flowering, and 2) Stage Three – pre veraison.

<table>
<thead>
<tr>
<th>Relative Humidity</th>
<th>Lateral removal</th>
<th>Transpiration rate (Tr) (μmol m⁻² s⁻¹)</th>
<th>Stomatal conductance (Gs) (μmol m⁻² s⁻¹)</th>
<th>Photosynthetic rate (Pn) (μmol m⁻² s⁻¹)</th>
<th>Internal CO₂ concentration (Ci) (μmol mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Assessment</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>High</td>
<td>No</td>
<td>0.7</td>
<td>0.9</td>
<td>126</td>
<td>108</td>
</tr>
<tr>
<td>High</td>
<td>Yes</td>
<td>0.7</td>
<td>0.8</td>
<td>109</td>
<td>88</td>
</tr>
<tr>
<td>Low</td>
<td>No</td>
<td>1.1</td>
<td>0.8</td>
<td>85</td>
<td>50</td>
</tr>
<tr>
<td>Low</td>
<td>Yes</td>
<td>1.0</td>
<td>0.9</td>
<td>70</td>
<td>54</td>
</tr>
</tbody>
</table>

*ns* - not significantly different at 10%

Means within a column with a different letter are significantly different from each other at $P \leq 0.10$ (LSMeans, SAS).

Page 287
Appendix 12: Mean values for all treatment combinations of leaf area, increase in leaf area between assessments two and three and BSN incidence for the last assessment.

<table>
<thead>
<tr>
<th>Relative humidity</th>
<th>Laterals removed</th>
<th>Leaf area Assessment 2</th>
<th>Leaf area Assessment 3</th>
<th>Leaf area increase*</th>
<th>BSN incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stage One</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>N</td>
<td>1253</td>
<td>1525</td>
<td>272</td>
<td>25</td>
</tr>
<tr>
<td>H</td>
<td>Y</td>
<td>1271</td>
<td>1295</td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td>L</td>
<td>N</td>
<td>1330</td>
<td>1498</td>
<td>168</td>
<td>29</td>
</tr>
<tr>
<td>L</td>
<td>Y</td>
<td>1059</td>
<td>1193</td>
<td>134</td>
<td>29</td>
</tr>
<tr>
<td><strong>Stage Two</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>N</td>
<td>967</td>
<td>1475</td>
<td>508</td>
<td>81</td>
</tr>
<tr>
<td>H</td>
<td>Y</td>
<td>1323</td>
<td>2012</td>
<td>689</td>
<td>68</td>
</tr>
<tr>
<td>L</td>
<td>N</td>
<td>1188</td>
<td>1743</td>
<td>555</td>
<td>71</td>
</tr>
<tr>
<td>L</td>
<td>Y</td>
<td>1388</td>
<td>1540</td>
<td>152</td>
<td>81</td>
</tr>
<tr>
<td><strong>Stage Three</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>N</td>
<td>925</td>
<td>1618</td>
<td>693</td>
<td>30</td>
</tr>
<tr>
<td>H</td>
<td>Y</td>
<td>1076</td>
<td>1316</td>
<td>240</td>
<td>25</td>
</tr>
<tr>
<td>L</td>
<td>N</td>
<td>1006</td>
<td>1604</td>
<td>598</td>
<td>24</td>
</tr>
<tr>
<td>L</td>
<td>Y</td>
<td>1062</td>
<td>1451</td>
<td>389</td>
<td>29</td>
</tr>
</tbody>
</table>

*Means within a stage and within a column with a different letter are significantly different from each other at P≤0.15 (Lsmeans, SAS).

* No statistical analyses carried out on lateral increase
Appendices.

Appendix 13: Mean values for all treatment combinations of lateral number, increase in lateral number between assessments two and three and BSN incidence for the last assessment.

<table>
<thead>
<tr>
<th>Relative humidity</th>
<th>Lateral number</th>
<th>Lateral increase</th>
<th>BSN incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Assessment 2</td>
<td>Assessment 3</td>
<td></td>
</tr>
<tr>
<td><strong>Stage One</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H N</td>
<td>7.5 a</td>
<td>6.9 a</td>
<td>-0.7</td>
</tr>
<tr>
<td>H Y</td>
<td>1.3 b</td>
<td>0.8 b</td>
<td>-0.6</td>
</tr>
<tr>
<td>L N</td>
<td>7.2 a</td>
<td>6.3 a</td>
<td>-1.0</td>
</tr>
<tr>
<td>L Y</td>
<td>1.3 b</td>
<td>1.0 b</td>
<td>-0.3</td>
</tr>
<tr>
<td><strong>Stage Two</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H N</td>
<td>4.2 b</td>
<td>7.3 b</td>
<td>3.1</td>
</tr>
<tr>
<td>H Y</td>
<td>1.9 b</td>
<td>4.1 b</td>
<td>2.2</td>
</tr>
<tr>
<td>L N</td>
<td>3.8 b</td>
<td>7.3 b</td>
<td>3.5</td>
</tr>
<tr>
<td>L Y</td>
<td>1.6 b</td>
<td>6.2 b</td>
<td>4.6</td>
</tr>
<tr>
<td><strong>Stage Three</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H N</td>
<td>1.5 b</td>
<td>4.3 b</td>
<td>2.8</td>
</tr>
<tr>
<td>H Y</td>
<td>2.8 b</td>
<td>4.1 b</td>
<td>1.5</td>
</tr>
<tr>
<td>L N</td>
<td>2.8 b</td>
<td>3.1 b</td>
<td>0.5</td>
</tr>
<tr>
<td>L Y</td>
<td>2.7 b</td>
<td>4.2 b</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Means within a stage and within a column with a different letter are significantly different from each other at \( P \leq 0.15 \) (Lsmeans, SAS).

* No statistical analyses carried out on lateral increase
Appendices.

Appendix 14: Bunch measurements for bunches with sensors during Stage Two and Three. Assessments carried out on 17/12/04 and 20/01/05, respectively

<table>
<thead>
<tr>
<th>Relative humidity</th>
<th>Laterals removed</th>
<th>Bunch width (mm)</th>
<th>Bunch length (mm)</th>
<th>Mean berry diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage Two</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High No</td>
<td>80</td>
<td>100</td>
<td>4.07</td>
<td></td>
</tr>
<tr>
<td>High Yes</td>
<td>90</td>
<td>160</td>
<td>5.55*</td>
<td></td>
</tr>
<tr>
<td>Low No</td>
<td>80</td>
<td>90</td>
<td>5.69</td>
<td></td>
</tr>
<tr>
<td>Low Yes</td>
<td>70</td>
<td>75</td>
<td>4.39</td>
<td></td>
</tr>
<tr>
<td>Stage Three</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High No</td>
<td>70</td>
<td>90</td>
<td>8.42 **</td>
<td></td>
</tr>
<tr>
<td>High Yes</td>
<td>40</td>
<td>60</td>
<td>8.14</td>
<td></td>
</tr>
<tr>
<td>Low No</td>
<td>55</td>
<td>60</td>
<td>8.27**</td>
<td></td>
</tr>
<tr>
<td>Low No</td>
<td>75</td>
<td>90</td>
<td>9.26</td>
<td></td>
</tr>
</tbody>
</table>

* It was noted that there were some very large berries on this bunch, diameter 8.25 mm

** Bunches used for sap flow comparisons
8 References


References.


References.


References.


References.


References.


References.


References.


References.


References.


Hinkel, R. F. (1992). The effects of pruning, greening and nitrogen on grapevine yield and on the soil. *Results of two field experiments with the varieties Müller-Thurgau and Riesling in the*
References.


References.


References.


References.


References.


References.


References.


References.


References.


References.


References.


References.

Yakushiji, H., Morinaga, K. and Kobayashi, S. (2001). Promotion of berry ripening by 2, 3, 5-
Triiodobenzoic acid in 'Kyoho' grapes. *Journal of the Japanese Society for Horticultural Science*
*70*(2): 185-190.

elements by polar auxin transport inhibitors through intracellular auxin depletion. *Plant and Cell
Physiology* *46*(12): 2019-2028.

Young, E. (1989). Cytokinin and soluble carbohydrate concentrations in xylem sap of apple during
dormancy and budbreak. *Journal of the American Society for Horticultural Science* *114*: 297-
300.

responses of seeded and seedless grape berries to shoot girdling. *Journal of the American Society
for Horticultural Science* *128*(3): 316-323.