Vigour Control in Apple (*Malus domestica*) and Kiwifruit (*Actinidia deliciosa* and *Actinidia chinensis)*

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

Doctor of Philosophy

in

Plant Physiology

at

Massey University, Palmerston North, New Zealand

Noveline Mercy Rose Gali Vattiprolu

2012

Abstract

Recent knowledge suggests the physiological basis of scion vigour control by dwarfing apple rootstocks involves shoot-root-shoot hormonal signalling. An aim of the present study was to further explore the role of gibberellin in control of apple (*Malus domestica*) scion vigour on a dwarfing rootstock and also an attempt was made to elucidate whether a generalised signalling mechanism exists in kiwifruit vines. For commercially grown kiwifruit (*Actinidia deliciosa* and *Actinidia chinensis),* there have been no studies on whether gibberellins stimulate the vigorous shoot growth that normally occurs.

For apple, dwarfing rootstocks of Malling Nine ('M.9') and vigorous rootstocks 'Royal Gala' ('RG') were grafted with scions of 'M.9' or 'RG' using a reciprocal grafting treatment structure. The 'M.9' rootstock increased the proportion of 'RG' primary shoots that terminated early with reduced growth rate and plastochron while exogenous gibberellins partially reversed the effect by stimulating both apical and sub-apical meristems and prolonging shoot extension, thus suggesting that 'RG' scions on 'M.9' rootstocks were deficient in bioactive gibberellins. The gibberellin foliar sprays increased the internode length by acting on cell division primarily and on cell elongation secondarily. The 'M.9' shoots of apple are short with reduced node number and early termination compared to vigorous 'RG' plants. This low vigour phenotype was maintained even on the vigorous rootstock, 'RG'. Foliar sprays of GA_3+GA_{4+7} to the scion did not reverse the primary or sylleptic shoot growth of 'M9' scion, which suggests that 'M.9' may be unable to convert exogenous gibberellins to bioactive GA_1.

For kiwifruit, the auxin transport inhibitor 1-N-naphthylphthalamic acid (NPA) applied to the stem of young rooted stem cuttings of *Actinidia chinensis* 'Hort16A' significantly reduced primary shoot length but the architectural changes imposed were different from those of NPA applied to apple. For apple, both NPA and a dwarfing rootstock reduced number and length of sylleptic axillary shoots (SAS) on the primary shoot and caused early termination of both SAS and primary shoots, whereas for kiwifruit NPA did not have any effect on total shoot growth compared with control. Given these dissimilarities, it was proposed the reduction of indole-3-acetic acid (IAA) to the root system of kiwifruit may not affect shoot growth through an effect on root-produced hormones. However, more work is needed using different auxin transport inhibitors to evaluate the effect of auxin restriction to the root system on root-produced hormones.

Foliar sprays of 1-naphthaleneacetic acid (NAA) and NPA reduced total shoot length of kiwifruit 'Hayward', possibly due to supra-optimal synthetic auxin levels for NAA and low levels of natural auxin levels for NPA in kiwifruit stems respectively. Foliar sprays of 500-1000 mg L^{-1} gibberellins (GA_3+GA_{4+7}) applied to mature 'Hayward' vines was found to be optimum for stimulating vigorous shoot growth. Since the anti-gibberellin prohexadione-Ca, decreased kiwifruit shoot growth, it may be possible to use this compound commercially to control excessive vegetative growth. As this anti-gibberellin inhibits the action of enzyme GA_{20} oxidase, which promotes the ultimate step of converting GA_{20} and GA_{19} to GA_1 in GA-biosynthesis pathway, it may also be possible to suppress the expression of gene encoding GA_{20} oxidase with the help of molecular biology techniques or produce better rootstock by conventional breeding.

Gibberellins activity in stimulating apical and sub-apical meristem is the most important event involved in shoot extension growth of kiwifruit and apple. For both kiwifruit and apples exogenous gibberellins inhibited flowering. For composite apple tree rather surprisingly, the 'RG' rootstock promoted flowering for 'RG' scion whereas 'M.9' rootstock reduced flowering. For kiwifruit, BAP promoted a high percentage of synchronised bud breaks. For both apple and kiwifruit gibberellins GA_3+GA_{4+7} appeared to stimulate sylleptic axillary shoot formation. For kiwifruit the possibility exists to select low vigour seedling, increase their vigour with gibberellins during the establishment phase, and then decrease vigour by withholding gibberellins once the canopy is established.

Acknowledgements

A journey travelling together becomes easier, as interdependence is more valuable than independence. This thesis is the achievement at the end of this journey and a result of work accompanied and supported by many people. It is a pleasant aspect that I have the opportunity to express my gratitude for all of them.

The first person I would like to thank is my chief supervisor Dr. David. J. Woolley. Thank you very much sir, for your integral view on research and mission for providing 'only-quality work and not less', which has made a deep impression on me. Your passion for science is mostly appreciable and compassion for candidate enabled me to continue this journey.

To my co-supervisor Dr. Ben van Hooijdonk - a great inspiration behind this study and a great source of encouragement. Thank you very much for co-supervising this thesis and for your valuable advice. Your conversance with apple rootstock physiology cleared my mind from confusion.

I would also like to thank the technical staff of the Plant Growth Unit for their help and technical assistance and, the staff at Institute of Agriculture and environment: Kay Sinclaire, James Slater for their whole-hearted assistance and added humor and, Chris Rawlingson for teaching me HPLC techniques, and Denise Stewart for her pleasant gestures to offer help whenever required. I thank Dr. Dimitry Sokolov from Manawatu microscopy and imaging centre (MMIC) for teaching confocal microscopy. I also would like to thank Gustavo Hernandez, a visiting scholar from Spain with whom I worked during the initial period of apple experiment. A special thanks to Dr. Xiong Zhao He, from IAE for statistical help.

A special thanks to Uttara for her cordial friendship, encouragement and constant company during lunch-breaks.

My deepest gratitude I must reserve for my wonderful husband, Samuel Vattiprolu for being patient, supportive and playing roles of both mother and father to our sons. I can never thank you enough. Thank you, Francis and Jonathan for taking care of your studies during this period of my life. I do not do justice if failed to thank Dr. Fredrick Christopher and John Peter Vattiprolu, without your unseen help it would have become

impossible to start this journey at the first instant. You deserve my sincere expression of thanks for providing the initial resources.

My heartfelt gratitude to my parents, late Devasahayam Gali and Dr. Mary Sarojini Gali, for providing education through their dedication and commitment, which enabled me to approach this level of study at this point of my life. Huge thanks to my brother Santhi Kiran Gali, for motivating and helping our family during the initial days to settle in New Zealand. Help from my sister Santhosh Vijaya Kumari Gali for going through endnote list, while visiting New Zealand, is greatly appreciable. I am greatly indebted to my parents-in-law Emmanuel Vattiprolu and Lilly Pushpam Vattiprolu for their constant moral and prayer support during this study.

Many thanks to my friends Zelda Anderson and Liz Cross for proof reading the final version of my thesis.

I am greatly indebted to the financial assistance from Massey Doctoral scholarship, doctoral hardship bursaries and many times Helen E Akers scholarships and turners and growers research grant. My heartfelt thanks to scholarship committee for taking decisions that gave me peaceful mind to concentrate on my studies.

Above all, I give all my praise and honour to my saviour Lord Jesus Christ for enabling me to go through this journey peacefully and cheerfully.

Table of Contents

Abstract i

Acknowledgements iii

Table of Contents v

List of Figures xvii

List of Tables xxxvi

List of abbreviations xli

Chapter 1 General introduction 1

1.1 Apple and kiwifruit as horticultural crops 1

1.2 Fruit trees – rootstocks 1

1.3 Rootstocks - advantages 3

1.3.1 Apple rootstocks 4

1.3.2 Apple rootstocks and their utilization in New Zealand 5

1.3.3 Focus of the present study 6

1.3.4 Rootstock breeding – constraints and further developments 7

1.3.5 Apple dwarfing rootstock – effect on scion architecture 8

1.4 Suggested mechanism of vigour control by dwarfing apple rootstocks 10

1.4.1 Nutrients 10

1.4.2 Photosynthesis 11

1.4.3 Hydraulic capacity - graft union 12

1.4.4 Hormonal regulation of scion vigour 14

1.4.4.1 Auxin transport and shoot-root-shoot signalling 15

1.4.4.2 Shoot-root-shoot signalling via cytokinins and gibberellins 18

1.5 Apple tree shoot apical meristem (SAM) 21

1.5.1 Role of auxin and cytokinin in apical dominance / correlative inhibition - apple tree 22
1.5.1.1 Apical dominance / correlative inhibition 22
1.5.1.2 Role of auxin in apical dominance 23
1.5.1.3 Role of cytokinin in promoting axillary bud outgrowth 24
1.5.1.4 Role of auxin - cytokinin interaction in promoting axillary bud outgrowth 25
1.5.1.5 A third plant hormone – shoot branching 26
1.5.1.6 Branching in apple tree and crotch angle 27
1.6 Apple tree shoot extension and termination – involvement of gibberellins 28
1.7 Kiwifruit vines 32
1.7.1 Introduction 32
1.7.2 Kiwifruit Rootstocks - vegetative growth 33
1.7.3 Techniques to control vigour 37
1.7.3.1 Canopy management- Crop load 37
1.7.3.2 Orchard manipulations to reduce vegetative vigour 38
1.7.3.2.1 High density planting 39
1.7.3.2.2 Pruning 40
1.7.3.2.3 Girdling 41
1.7.3.2.3.1 Girdling in kiwifruit 42
1.7.3.2.3.2 Physiology of girdling 43
1.7.4 Role of hormones in kiwifruit vine vigour 44
1.7.4.1 In vitro studies an insight into the role of hormones in vine growth 44
1.7.4.2 Use of growth retardants to reduce kiwifruit vigour 45
1.8 Summary, rationale and thesis objectives 47
1.8.1 Thesis rationale and major objectives 48

Chapter 2 An improved understanding of shoot-root-shoot signalling and vigour control by rootstock and scion using reciprocal grafting and gibberellins foliar sprays to apple trees 51

2.1 Introduction 51

2.2 Aim: To test hypothesis-1 and hypothesis-2 56

2.3 Materials and methods 56

2.3.1 Experimental plant material 56

2.3.2 Maintenance of plant material 57

2.3.3 Foliar sprays of the gibberellins GA_3 and GA_{4+7} 58

2.3.4 Measurements of scion growth 58

2.3.5 Statistical analysis 59

2.4 Results 60

2.4.1 Treatment effects on primary shoot growth 60

2.4.1.1 Treatment effects on the 'early growth' of the primary shoot 61

2.4.1.1.1 Shoot length, node number and internode length 61

2.4.1.2 Treatment effects on the 'late growth' of the primary shoot 66

2.4.1.2.1 Shoot length, node number and internode length 66

2.4.1.3 Treatment effect on termination of the shoot apical meristem on the primary shoot 78

2.4.1.4 Final shoot cross-sectional area (SCA) of the primary shoot 80

2.4.2 Treatment effects on the formation of sylleptic axillary shoot formation on the primary shoot 81

2.4.2.1 Treatment effects on the number of sylleptic axillary shoots formed per scion 82

2.4.2.2 Treatment effects on the termination of sylleptic shoots 84

2.4.2.3 Treatment effects on the final mean total length of sylleptic axillary shoots (spurs + Sylleptic shoots) 85

2.4.2.4 Treatment effects on the length and node number of sylleptic shoots for 'RG' scions 87

2.4.2.5 Treatment effects on the total length and node number of sylleptic axillary shoots (spurs + sylleptic shoots) for 'M.9' scions 89

2.4.3 Treatment effect on the total scion growth 91

2.4.3.1 Treatment effect on the mean total shoot length of scion (Primary shoot + SAS) 91

2.4.4 Overview of growth response 93

2.4.4.1 Overview of 'RG' scion growth in response to rootstock and GA foliar sprays (Rootstock effect: Hypothesis I) 93

2.4.4.2 Overview of 'M.9'scion growth in response to rootstock and GA foliar sprays (Scion effect: Hypothesis II) 94

2.4.5 Treatment effects on the mean root dry weight at the end of the first growing season 96

2.4.5.1 Treatment effects on the total dry weight 96

2.4.5.2 Treatment effects on dry weight of hard roots 99

2.4.5.3 Treatment effects on dry weight of medium roots 99

2.4.5.4 Treatment effects on dry weight of soft roots 100

2.4.6 Treatment effects on the first occurrence of flowering in the second spring after tree grafting 100

2.5 Discussion 102

2.5.1 Early growth of the primary shoot and effect of gibberellin foliar sprays 103

2.5.2 Final growth of the primary shoot 104

2.5.3 Gibberellin effect on the final growth of primary shoot 106

2.5.4 Growth of sylleptic axillary shoots 112

2.5.5 Gibberellin effect -growth of sylleptic shoots 113

2.5.6 Treatment effects on the first occurrence of flowering in the second spring after tree grafting 114

2.6 Conclusion 115

Chapter 3 Role of gibberellins in cell division and/or cell elongation of shoot internodes for composite 'Royal Gala' apple scions grown on a dwarfing and a vigorous rootstock 117

3.1 Introduction and Aims 117

3.1.1 Shoot apical meristem (SAM) 118

3.1.1.1 SAM - Structure 118

3.1.1.2 Shoot apical meristem – activity 121

3.1.1.3 Shoot apical meristem-hormonal regulation 122

3.1.2 Internode elongation 127

3.1.2.1 Role of cellular activity cell division and/or cell elongation in internode elongation 127

3.1.2.2 Role of gibberellins on cellular activity cell division and/or cell elongation in internode elongation 128

3.1.3 Pattern of Pith development 132

3.2 Materials and methods 135

3.2.1 Selection of material 135

3.2.2 Sample preparation 136

3.2.3 Confocal microscope 137

3.2.4 Confocal imaging and image analysis 138

3.2.5 Measurements and calculations 139

3.2.6 Data analysis 140

3.3 Results 141

3.3.1 Comparing mean cell number, length and width between samples at different positions within the pith of an internode 141

3.3.2 Effect of rootstocks and gibberellins treatment on internode length, cell expansion and cell division 141

3.3.2.1 Mean internode length 141

3.3.2.2 Mean cell length and number 143

3.3.2.3 Estimating total cell number for each internode: contribution of cell elongation and cell division for internode length increase 148

3.3.3 Effect of rootstocks and gibberellin treatment on different attributes of internode across the width 150

3.3.3.1 Mean width of internode, pith and cell 150

3.3.3.2 Estimating the total number of cells across the pith and its correlation with internode width 152

3.4 Discussion 154

3.4.1 Rootstock effect on internode elongation in terms of cell division and/or cell elongation 154

3.4.2 Gibberellin effect in the internode elongation in terms of cellular activity cell division and/or cell elongation 155

3.4.3 Rootstock and gibberellins effect on the internode width 157

3.5 Summary 159

Chapter 4 Is the hormonal signalling in kiwifruit similar to apple? 161

4.1 Introduction 161

4.1.1 Objectives 164

4.2 Materials and methods 165

4.2.1 Establishment of experimental plant material 165

4.2.1.1 Propagation of self-rooted kiwifruit stem cuttings - background 165

4.2.1.2 Soft and semi hardwood cuttings 166

4.2.1.3 Hardwood cuttings 168
4.2.1.4 Experimental material for experiment two 170
4.2.2 Synthesis of the auxin transport inhibitor 1-N-naphthylphthalamic acid (NPA) 170
4.2.2.1 Determination of suitable physiological concentration of NPA to restrict auxin transport 171
4.2.3 Treatments 172
4.2.3.1 Treatments for experiment one 172
4.2.3.2 Treatments for experiment two 173
4.2.3.3 Application of treatments 173
4.2.3.3.1 Control, NPA, NPA+IAA and IAA 173
4.2.3.3.2 Girdling ± NPA and ± Root restriction 174
4.2.3.4 Growing medium and irrigation 174
4.2.3.5 Measurements of shoot growth 175
4.3 Results 176
4.3.1 Experiment one 177
4.3.1.1 Treatment effect on primary shoot length 177
4.3.1.2 Treatment effect on the mean node number and internode length of the primary shoot 179
4.3.1.3 Treatment effect on the formation of sylleptic axillary shoots (SAS) 181
4.3.1.4 Treatment effect on the mean total secondary shoot length 182
4.3.1.5 Treatment effect on the total shoot growth per vine 183
4.3.1.6 NPA effect on the dry weight of the root system 184
4.3.1.7 Treatment effect on pruning weight 185
4.3.2 Experiment two 186
4.3.2.1 Treatment effect on primary shoot length 186

4.3.2.2 Treatment effect on the mean node number and internode length of the primary shoot 187

4.3.2.3 Treatment effect on mean leaf area 188

4.3.2.4 Treatment effect on growth attributes of sylleptic (secondary and tertiary) shoot 190

4.4 Discussion 191

4.4.1 Treatment effect on the growth of the primary shoot 191

4.4.2 Treatment effect on the growth of sylleptic axillary shoots 193

4.4.3 Treatment effect on the growth of root system 198

4.5 Summary 198

Chapter 5 Effect of foliar sprays of auxin, gibberellins, cytokinins, auxin transport inhibitor (NPA) and anti-gibberellin (PBZ) on the vegetative growth of 'Hort16A' and 'Hayward' 201

5.1 Introduction 201

5.2 Materials and methods 203

5.2.1 Experimental plant material 203

5.2.1.1 Aerial layering 203

5.2.2 Growing medium and irrigation 205

5.2.3 Treatments and concentrations 205

5.2.3.1 Solution preparations 206

5.2.3.2 Application of treatments 206

5.2.4 Measurement of vine growth 206

5.2.5 Experimental design and statistical analysis 207

5.3 Results 207

5.3.1 Treatment effects on the length of primary shoot 207

5.3.1.1 Treatment effects (GA, BAP and NAA) on the length of primary shoot of 'Hort16A' and 'Hayward' 207

5.3.1.2 Treatment effects (GA, BAP and NAA, PBZ and NPA) on the length of the primary shoot of 'Hayward' 211

5.3.1.3 Treatment effects on the termination of the primary shoot 211

5.3.2 Treatment effects on mean node number and internode length of primary shoot 213

5.3.2.1 Treatment effects (GA, BAP and NAA) on mean node number and internode length of primary shoot of 'Hort16A' and 'Hayward' 213

5.3.2.2 Treatment effects (GA, BAP and NAA, PBZ and NPA) on mean node number and internode length of primary shoot of 'Hayward' 213

5.3.3 Treatment effect on sylleptic axillary shoot formation 215

5.3.3.1 Treatment effects (GA, BAP and NAA) on mean number and total length of sylleptic axillary shoots (SAS) of 'Hort16A' and 'Hayward' 215

5.3.3.2 Treatment effects (GA, BAP and NAA, PBZ and NPA) on mean number and total length of sylleptic axillary shoots (SAS) of 'Hayward' 215

5.3.4 Treatment effect on the total shoot length (primary plus sylleptic secondary shoots) 216

5.3.4.1 Treatment effects (GA, BAP and NAA) on the total shoot length (primary plus sylleptic axillary shoot) of 'Hort16A' and 'Hayward' 216

5.3.4.2 Treatment effects (GA, BAP, NAA, PBZ and NPA) on the total shoot length (primary plus sylleptic secondary shoots) 216

5.3.5 Treatment effect on the spread of bud break and flowering for 'Hort16A' and 'Hayward' in the following spring 217

5.3.5.1 Treatment effect (GA, BAP and NAA) on the final percentage of bud break for 'Hort16A' and 'Hayward' in the following spring (Oct, 2009) 219

5.3.5.2 Treatment effect (GA, BAP, NAA PBZ and NPA) on the final percentage of bud break for 'Hayward' in the following spring 220

5.3.6 Treatment effects on the first occurrence of vine flowering 220

5.3.6.1 Effect of GA, BAP and NAA for 'Hort16A' and 'Hayward' on total number of flowers produced in the following spring 220

5.3.6.2 Effect of GA, BAP, NAA, PBZ and NPA for 'Hayward' on the number of flowers produced in the following spring 221

5.4 Discussion 222

5.4.1 Hormone treatment effect (GA_3+GA_{4+7} and BAP) on vegetative growth of 'Hort16A' and 'Hayward' kiwifruit vines 223

5.4.1.1 Primary shoot 223

5.4.1.2 Sylleptic axillary shoots 224

5.4.1.3 Spring bud break and flower formation 227

5.4.1.4 Flower formation for both cultivars 228

5.4.2 NAA treatment effect on the growth of 'Hort16A' and 'Hayward' kiwifruit vines 231

5.4.3 NPA treatment effect on the growth of 'Hayward' kiwifruit vines 232

5.4.4 Paclobutrazol treatment effect on 'Hayward' 233

5.5 Summary 235

Chapter 6 Vegetative growth of 'Hayward' kiwifruit vines in response to gibberellins and anti-gibberellins 239

6.1 Introduction 239

6.2 Materials and methods 243

6.2.1 Experimental plant material 243

6.2.1.1 Experimental treatments 243

6.2.1.2 Experimental design and statistical analysis 244

6.2.1.3 Measurements of shoot growth 245
6.3 Results 246
6.3.1 Treatment effect on shoot length 246
6.3.2 Treatment effect on shoot type / termination 247
6.3.3 Dose/response growth curve for length of lateral shoots for different concentrations of GA_3+GA_{4+7} 251
6.3.4 Treatment effect on shoot cross-sectional area (SCA) 252
6.3.5 Dry weight of shoot 253
6.3.6 Treatment effect on node formation 253
6.3.7 Treatment effects on mean internode length 254
6.3.8 Treatment effect on leaf specific weight (SLW) 257
6.3.9 Treatment effect on the number and length of sylleptic axillary shoots formed on lateral shoots 258
6.4 Discussion 259
6.4.1 Effect of gibberellins on vegetative growth 259
6.4.1.1 Growth of lateral shoots 259
6.4.1.2 Sylleptic axillary shoots (SAS) 261
6.4.2 Effect of Anti-gibberellins 262
6.4.2.1 Effect of paclobutrazol (PBZ) on vegetative growth 262
6.4.2.2 Effect of Prohexadione-Calcium (Ca-Pro) 264
6.5 Summary 265
Chapter 7 General discussion and conclusions 267
7.1 The role of gibberellins in vigour control in apple 267
7.1.1 Introduction 267
7.1.2 Dwarfing rootstock and gibberellin effect 268

7.1.2.1 Primary shoot 268
7.1.2.2 Primary shoot termination 272
7.1.2.3 Sylleptic axillary shoots (SAS) 273
7.1.2.4 Termination of SAS 274
7.1.3 Growth of 'M.9' as a scion - gibberellin effect 275
7.1.3.1 Primary shoot 275
7.1.3.2 'M.9' Scion and gibberellin effect on primary shoot termination 276
7.1.3.3 'M.9' Scion and gibberellin effect on formation of SAS 278
7.1.3.4 'M.9' Scion and gibberellin effect on termination of SAS 278
7.1.4 Rootstock and gibberellin effect on formation of flower buds 279
7.2 Comparison of hormonal signalling of apple and kiwifruit 281
7.2.1 Auxin –shoot growth 281
7.2.2 Gibberellins - shoot growth 284
7.2.3 Flowering in apple and kiwifruit 288
7.2.4 Cytokinin - spring bud break for kiwifruit vines 289
7.3 Directions for future research-Summary 289
7.4 Final comment 292
8. **Appendix** 293
9. References 297

List of Figures

Figure 1.1. Effect of 'M.9' (dwarfing), 'MM.106' (semi-vigorous), 'M.793' (vigorous) and 'Royal Gala' (very vigorous control) rootstocks (from left to right, respectively) on the architecture of 'Royal Gala' apple scions by the end of the first growing season from grafting. Yellow rule is 1 m. (source: van Hooijdonk, 2009). The final length of the primary shoot and number and length of sylleptic axillary shoots were reduced for scion on 'M.9' rootstock by promoting early termination. 10

Figure 1.2. Effect of the auxin transport inhibitor 1-N-naphthylphthalamic acid (NPA) applied to the stem of 'M.9', 'MM.106', 'M.793', and 'Royal Gala' from left to right, respectively) on scion architecture of 'Royal Gala' apple trees by the end of their first growing season from grafting (source: van Hooijdonk et al., 2009). Yellow rule is 1 m. Observe reduced secondary shoot formation and early termination similar to the effects of dwarfing rootstock, thereby indicating the basipetal transport of auxin is an important physiological signal regulating rootstock-induced scion dwarfing. 17

Figure 1.3. Interaction of NPA and GA_{4+7} on the growth of 'Royal Gala' scion grafted on 'M.9', 'MM.106', 'M.793' and 'Royal Gala' rootstocks by the end of their first growing season from grafting. The early termination imposed by NPA was prevented by GA_{4+7} thus alleviating the effects of probable impaired auxin transport. Yellow rule is 1 m. (van Hooijdonk et al., 2010). 20

Figure 1.4. Interaction of NPA and BAP on the growth of 'Royal Gala' scion grafted on 'M.9', 'MM.106', 'M.793' and 'Royal Gala' rootstocks by the end of their first growing season from grafting. The decreased secondary axes formation with NPA could be reinstated by applying cytokinin. Yellow rule is 1 m (van Hooijdonk et al., 2010). 20

Figure 1.5. A. Schematic diagram representing a junction of basipolar IAA from apical bud and axillary bud. The wavy lines mark the area of auto-

transport inhibition (ATA) from where strong inhibition signal extents acropetally into axillary bud [source: Bangerth et al., (2000)] and strigolactone (SL) signal from roots to inhibit branching (Shimizu-Sato et al., 2009) B. Model showing the interaction between auxin and cytokinin in shoot branching through *PIN* and *IPT* [source: Shimizu-Sato et al., (2009); Müller and Leyser, (2011)] after decapitation. PIN: IAA efflux proteins and IPT: Isopentenyl transferase. 25

Figure 1.6. Scheme of gibberellin biosynthetic pathway showing the last reactions catalysed by GA deoxygenases source:(Fagoaga et al., 2007). *GA20ox* and *GA3ox* are the activating enzymes and *GA2ox* is the deactivating enzyme. GA_1 and GA_4 are bioactive enzymes. 30

Figure 1.7 Excessive vegetative growth that kiwifruit 'Hayward' normally exhibits in an orchard system. 35

Figure 2.1. Reciprocal grafts of dwarfing rootstock 'M.9' and vigorous rootstock 'Royal Gala' ('RG') with 'M.9' and 'RG' scions. The combinations are always written with rootstock/scion: M9M9 (A), RGM9 (B), M9RG (C), and RGRG (D). 58

Figure 2.2. Rootstock × scion interactions on the mean length of primary shoots of 'RG' and 'M.9' scions during the spring to early summer growing period: A (early growth: from 16th Oct-12th Jan) and B; during the full growth period (early + late) following grafting onto 'M.9' and 'RG' rootstocks in August, 2007. ***, * represent the significance level at $P \leq 0.001$, 0.04 on 10th December and 12th January respectively. The arrows on 'A' are pointing to the dates of significant differences Arrow on 'B' separates the early growth from late growth. Means are averaged over GA treatments. Bars represent the standard error. 62

Figure 2.3. Rootstock × GA interaction on the mean length of the primary shoot of 'RG' and 'M.9' scions on 'M.9' and 'RG' rootstocks during early growth (16th Oct, 2007-12th Jan, 2008). -GA and GA (Nov)

represent no GA or fortnightly GA foliar spray from November onward until growth cessation. Means sharing the same letter are not different significantly at *P=0.05* using the Duncan's Multiple Range test. Data are averaged over scions. 63

Figure 2.4. Rootstock × scion interaction on the mean node number (black columns) and mean internode length (red columns) of primary shoot on the 12th of January, 2008 during the spring and early summer of 'RG' and 'M.9' scions on 'M.9' and 'RG' rootstocks. Columns sharing the same letter for each attribute (mean node number and mean internode length) are not significantly different at *P=0.05* using Duncan's Multiple Range test. Data are pooled over GA treatments. 64

Figure 2.5. Rootstock × scion interaction on the mean internode length of 'RG' (A) and 'M.9' scions (B) on 'M.9' and 'RG' rootstocks over the entire growing season. The dotted arrow in 'A' indicates the date on which there was a significant (P=0.006) rootstock effect on the internode length. Bars represent standard errors. The vertical solid arrow in both A and B separates the early growth from late growth. Data are pooled over GA treatments..... 65

Figure 2.6. Rootstock × GA interaction on the mean node number (black columns) and internode length (red columns) of the primary shoot of 'M.9' and 'RG' scions grafted onto 'M.9' and 'RG' rootstocks. Means sharing the same letter for nodes number and internode length are not significantly different at *P=0.05* using Duncan's Multiple Range test. Data are averaged over scions. –GA, GA (Nov) and GA (Feb) represents no GA, GA (GA_3+GA_{4+7}) foliar sprays from November and February, respectively. Data are pooled over scions..... 68

Figure 2.7. The relationship between the node number and length of the primary shoot of 'RG' (A) and 'M.9' (B) scions on 'M9' and 'RG' rootstocks with GA_3+GA_{4+7} (+GA Nov) and without foliar sprays. The internode lengths can be compared from the regression line of data on each graph by dividing a y-value by its corresponding value of x

in the graph. In figure B the dotted line from x-axis to y-axis from node number 62 shows the internode lengths - a (shorter) and - b (longer) for the same node number. 71

Figure 2.8. Effect of 'M.9' and 'RG' rootstock on 'RG' scion (A) and on 'M.9' scion (B) on the rate of shoot growth (mm day^{-1}) throughout the growing season; -GA, GA (Nov), GA (Feb) represent no GA and GA (GA_3+GA_{4+7}) foliar sprays from November and February respectively that continued till the end of the growing season. Bars represent the standard error. 72

Figure 2.9. Effect of 'M.9' and 'RG' rootstock on 'RG' scion (A) and on 'M.9' scion (B) on the rate of node production (nodes day^{-1}); -GA, GA (Nov), GA (Feb) represent no GA and GA(GA_3+GA_{4+7}) foliar sprays from November and February respectively that continued till the end of the growing season. Bars represent the standard error. 73

Figure 2.10. Rootstock × GA interactions on primary shoot length during the first growing season from grafting for 'Royal Gala' (A) and M.9 (B) scions grafted onto M.9 and 'Royal Gala' rootstocks. Bars represent standard errors; GA (Nov) and GA (Feb) represent the foliar spray of GA_3+GA_{4+7} from November and February, respectively, that continued until the end of the growing season. Vertical dotted line separates early growth from late growth. The early growth represent the growth during spring and early summer (from 14th Nov to 12th Jan on this graph) and late growth represents the growth from mid-summer to the end of the growth season (from 12th Jan to 5th June). The solid vertical line along the x-axis denotes the time after which shoot termination first began for all treatments (15th April, 2008). Bars represent the standard error. 75

Figure 2.11. Rootstock × scion × GA interactions on primary shoot length during the first growing season from grafting for 'Royal Gala' and M.9 scions grafted onto M.9 and 'Royal Gala' rootstocks. Bars represent standard errors; GA (Nov) and GA (Feb) represent the foliar spray of GA_3+GA_{4+7} from November and February respectively, that

continued till the end of the growing season. Vertical dotted line separates early growth from late growth. The early growth represents the growth during spring and early summer from 14th Nov to 12th Jan. on this graph and late growth represents the growth from mid-summer to the end of the growth season (from 12th Jan to 5th June). The vertical line along the x-axis denotes the time after which shoot termination first began for 'M.9' rootstock and scion without GA. Bars represent the standard error. 76

Figure 2.12. Rootstock × scion × GA interactions on mean node number of primary shoot during the first growing season from grafting for 'Royal Gala' and M.9 scions grafted onto M.9 and 'Royal Gala' rootstocks. Bars represent standard errors; GA (Nov) and GA (Feb) represent the foliar spray of GA_3+GA_{4+7} from November and February, respectively, that continued till the end of the growing season. Vertical dotted line separates early growth from late growth. The early growth represent the growth during the spring and early summer from 14th Nov to 12th Jan. on this graph and late growth represents the growth from mid-summer to the end of the growth season from 12th Jan to 5th June. Bars represent the standard error. 77

Figure 2.13. Rootstock × scion × GA interactions on the final mean shoot cross-sectional area (SCA) of primary shoot of 'RG' and 'M9' scions on 'M.9' and 'RG' rootstocks at growth cessation in June, 2008. –GA, GA (Nov) and GA (Feb) represent no GA (GA_3+GA_{4+7}), GA foliar sprays from November and February respectively and continued until the end of the growing season. Means sharing the same letter are not significant at *P=0.05*..... 81

Figure 2.14. Rootstock × scion interaction on mean total length of sylleptic axillary shoots of composite trees 'RGRG' and 'M9RG' with 'RG' scion; 'RGM9' and 'M9M9' with 'M.9' scions (rootstock/scion combination). Means sharing the same letter are not different at *P=0.05* using Duncan's Multiple Range test. Means are averaged over GA treatment. 86

Figure 2.15. Effect of ‘M9’ and ‘RG’ rootstocks (‘M9RG’ and ‘RGRG’) , GA (Nov) foliar sprays (‘M9RG (Nov)’ and ‘RGRG’ (Nov) and GA (Feb) (‘M9RG’ (Feb) and ‘RGRG’ (Feb) foliar sprays on the relationship between SS length and node number for ‘RG’ scion on ‘M.9’ (left) and ‘RG’ (right) rootstocks. GA (Nov) and GA (Feb) represent the GA (GA_3+GA_{4+7}) foliar sprays from November and February, respectively and continued till the end of the growing season (May, 2008). 88

Figure 2.16. Effect of rootstock ‘M.9’ (A) and ‘RG’ (B) each without GA (GA_3+GA_{4+7}) and with GA (Nov) and GA (Feb) foliar sprays on the relationship between mean total node number and mean total length of SAS of individual tree replicates of ‘M.9’ scions at the end of the growing season (June,2008). GA (Nov) and GA (Feb.) are the gibberellin foliar sprays from November and February continued till the end of the growing season. 90

Figure 2.17. Effect of rootstock scion interaction on mean total shoot length (mm) of ‘M9’ or ‘RG’ scions on ‘M9’ or ‘RG’ rootstocks at the end of the growing season. The composite trees ‘RGRG’ and ‘M9RG’ are with ‘RG’ scion; ‘RGM9’ and ‘M9M9’ are with ‘M.9’ scion. Means sharing the same letter are not different at *P=0.05* using the Duncan’s Multiple Range test. Means are averaged over GA treatments. 91

Figure 2.18. Rootstock × scion interactions on the mean dry weight of soft, medium and hard roots for composite trees of ‘M9RG’, ‘RGRG’; M9M9, RGM9 (rootstock/scion combination). Vertical bars are the standard error. For the same category of roots, columns with the same letter are not significantly different at *P=0.05* using Duncan’s Multiple Range test. Data are averaged over GA treatment. Bars represent the standard error. 98

Figure 2.19. Effect of scion × GA interactions, on the mean dry weight of the total root system of ‘M.9’ and ‘RG’ rootstocks with (+GA) and without GA (-GA) foliar sprays (GA_3+GA_{4+7}). The vertical bars

sharing the same letter are not significantly different at *P=0.05* using Duncan's Multiple Range test. Means are averaged over rootstocks. 99

Figure 2.20. Mean number of flower cluster per scion for the composite trees RGRG, M9RG (with 'RG' scion); RGM9, M9M9 (with 'M.9' scion) during the second spring (October, 2008) from grafting (August, 2007) values are means with standard error bars. GA treatments are excluded because no flowers were present. 101

Figure 2.21. The mean proportion of total buds per scion that were floral for the composite trees RGRG, M9RG (with 'RG' scion); RGM9, M9M9 (with 'M.9' scion) during the second spring (October, 2008) from grafting (August, 2007) values are means with standard error bars. GA treatments are excluded because no flowers were present. 101

Figure 3.1. Comparison of shoot apex (A) and shoot apical meristem (B & C). A: The entire shoot apex with apical dome at the centre with leaf primordia, leaf axillary buds, immature nodes. B: Diagrammatic representation of the shoot apical meristem C: Tissue organisation of the shoot apical meristem. In shoot apical meristem (C) the central zone is shown in red, the peripheral zone in green, and the rib meristem in blue In B peripheral zone L1, L2 and L3 correspond to the genetically defined three cell layers from exterior of the meristem to the interior, respectively. Distance between two consecutive matured nodes is an internode as represented in A and B, below the central rib zone is the sub-apical meristem. (modified from http://biology.kenyon.edu/course/biol114/Chapter 12A.html;and (Medford, 1992). 120

Figure 3.2. A model for apple SAM shoot development: nodes, internodes, involvement of apical, rib and sub-apical meristems. Effect of auxin, gibberellin, cytokinins, cell elongation and/or cell division on internode elongation of a plant body. The solid black column beside dotted column in internode indicates the increased elongation due to exogenous gibberellins Dotted lines on left from root indicate the supply of endogenous GAs and cytokinin through xylem sap. The

black arrow from node on left to PAT indicates basipetal auxin transport to root. The black arrow on left from the youngest node (P0) to the apical meristem indicates acropetal auxin supply to the PZ and CZ of the SAM. The region between rib meristem and node P1 indicate the extension of sub-apical meristem and below this region is the region of maturation. On the right side the exogenous gibberellin supply to sub-apical and apical meristem, which increased internode length and node number by cell division and cell elongation. 131

Figure 3.3. Schematic diagram of confocal microscope. The lens focuses the light beam at a spot within the specimen. The fluorescent light, because of its longer wavelength, comes to a focus at the plane of the detector pinhole. Only that fluorescence emission from the in-focus spot is able to pass through the pinhole detector to the computer screen (Modified after http://rpd.oxfordjournals.org/content/119/1-4/357/F2.large.jpg) 137

Figure 3.4. The boundary was drawn manually along the cell wall of each cell using the computer mouse and the corresponding measurements were given by 'ImageJ' software. For each cell, 'ab' denotes the length and 'cd' the width of the cells measured. 138

Figure 3.5. Image showing the cells that were counted: number of cell files in rows, columns and the total number of cells. The boundary was drawn manually along the cell wall of each cell using the computer mouse and the corresponding measurements were given by 'ImageJ' software..... 139

Figure 3.6. Internode lengths from shoot apex until the 10th node on the primary shoot of 'M9RG' and 'RGRG' without GA and with GA (+GA). 'M9RG' and 'RGRG' represent 'M.9' and 'RG' rootstock with 'RG' scion, respectively, without GA and, 'M9RG' +GA' and 'RGRG' +GA' represent GA_3+GA_{4+7} treatment applied from November(Nov) continued until the end of the growing season. Notice the gradual

decrease in the internode elongation towards the end of the growing season (June, 2008). 142

Figure 3.7. Effect of rootstock and gibberellin interaction on mean internode length and cell number and GA effect on mean cell length of sub-sampled internodes of 'RG' scions on 'M.9' and 'RG' rootstocks 'M9RG' and 'RGRG' represent 'M.9' and 'RG' rootstock with 'RG' scion, respectively, without GA and, 'M9RG' +GA' and 'RGRG' +GA' represent GA_3+GA_{4+7} treatment applied from November (Nov) continued until the end of the growing season. 143

Figure 3.8. Rootstock and gibberellin treatment effect on range and distribution of individual cells with similar mean cell length (µm) of middle portion of the sub-sampled zone of the internode of 'RG' scions on 'M.9' and 'RG' rootstocks that was photographed 'M9RG' and 'RGRG' represent 'M.9' and 'RG' rootstock with 'RG' scion respectively, without GA and, M9RG +GA' and 'RGRG +GA' represent GA_3+GA_{4+7} treatment applied from November (Nov) continued until the end of the growing season (June, 2008). 145

Figure 3.9. Representative images of longitudinal sections through middle portion of the pith region of internodes of 'RG' scions on 'M.9' without GA (A) and with GA (B) observed under a confocal microscope. 'M9RG' represent 'RG' scion with 'M.9' rootstock. The +GA represents the GA_3+GA_{4+7} treatment from November continued until the end of the growing season. Observe the longer cells of 'M9RG' with GA in B. 146

Figure 3.10. Images of longitudinal sections through middle portion of the pith region of internodes of 'RG' scions on 'RG' rootstocks without GA (A) and with GA (B) observed under confocal microscope 'RGRG' represent 'RG' scion on 'RG' rootstock. The 'RGRG' +GA represents 'RGRG' with GA_3+GA_{4+7} treatment from November continued until the end of the growing season. 147

Figure 3.11. Relationship between internode length (mm) and number of cells in a column along the length of internode of 'RG' scions on 'M.9' and 'RG' rootstocks with and without GA treatment. 'M9RG' and 'RGRG' represent 'M.9' and 'RG' rootstock with 'RG' scion respectively without GA and, 'M9RG +GA' and 'RGRG +GA' represent GA_3+GA_{4+7} treatment applied from November (Nov) continued until the end of the growing season. 149

Figure 3.12. Effect of rootstock and gibberellin treatment on mean internode and pith width (mm) and mean cell width (µm) of 'RG' scions on 'M.9' and 'RG' rootstocks 'M9RG' and 'RGRG' represent 'M.9' and 'RG' rootstock with 'RG' scion respectively, without GA and, M9RG +GA' and 'RGRG +GA' represent GA_3+GA_{4+7} treatment applied from November (Nov) continued until the end of the growing season. Columns sharing the same letters for a single variable are not significantly different at P=0.05 using multiple t-Test (LSD) LSD. Bars represent the standard error. 151

Figure 3.13 Rootstock and gibberellin treatment effect on range and distribution of individual measurements with similar mean cell width (µm) 'M9RG' and 'RGRG' represent 'M.9' and 'RG' rootstock with 'RG' scion respectively, without GA and, M9RG +GA' and 'RGRG' +GA' represent GA_3+GA_{4+7} treatment applied from November (Nov) continued until the end of the growing season. Bars represent the standard error. 152

Figure 3.14. Relationship between pith width and total number of cells acroos the pith in mature internodes of 'RG' scions on 'M.9' and 'RG' rootstock with and without gibberellin.. 'M9RG' and 'RGRG' represent 'M.9' and 'RG' rootstock with 'RG' scion, respectively, without GA and, M9RG +GA' and 'RGRG +GA' represent GA_3+GA_{4+7}treatment applied from November (Nov) continued until the end of the growing season. 153

Figure 4.1. Misting unit used for soft and semi hardwood cuttings of kiwifruit 'Hort16A' and 'Hayward' for rooting with misting arranged at regular intervals for 10 seconds at frequency of 10 minutes. 167

Figure 4.2. Formation of roots for IBA treated (500 mg ml^{-1}) soft and semi-hard wood cuttings of kiwifruit 'Hort16A' and 'Hayward'. Root balls formed after 6-8 weeks of misting (June, 2007). 167

Figure 4.3. Hardwood cuttings of kiwifruit 'Hort16A' and 'Hayward' arranged for rooting in the cold room maintained at an air temperature of 7ºC and bench heated to maintain the medium at 20-22ºC during winter (August, 2007) 168

Figure 4.4. Formation of roots for IBA (500 mg ml^{-1} and 1000 mg ml^{-1}) treated hardwood cuttings of kiwifruit cultivars 'Hort16A' and 'Hayward'. Long and healthy roots were formed by October, 2007. 169

Figure 4.5. Rooted hardwood stem cuttings of *Actinidia chinensis* 'Hort16A' (gold kiwifruit) transferred into space-saver pots after spring bud break..... 170

Figure 4.6. Effect of different concentrations of 1-N-naphthyphthalamic acid (NPA) on the mean length of lettuce roots expressed as a percentage of mean root length for control seedlings. The two arrows indicate the concentration of NPA at which roots lost their geotropism..... 171

Figure 4.7. Effect of single application of NPA at 0 (control), 1, 10 and 25 mg/ml^{-1} per plant applied to the stem portion below the first node of the primary shoots of *A. chinensis* 'Hort16A' on primary shoot length (A) and node number (B). NPA in lanolin paste was applied on 21st December, 2007. 172

Figure 4.8. Diagrammatic representation of stem portion with two three cm long regions separated by one cm gap on the primary shoot below the first internode where only lanolin applied on both regions for untreated control vines;NPA and lanolin to the upper and lower regions respectively for NPA treatment; lanolin and IAA to the upper and

lower regions respectively for IAA treatment; NPA and IAA to the upper and lower regions respectively for NPA+IAA treatment during experiment two from October to March (2009-2010). 174

Figure 4.9. Kiwifruit vines of *A. chinensis* 'Hort16A' exhibiting epinasty three days after application of NPA to the potted vines in the tunnel house.... 176

Figure 4.10. The yellow circle shows the activation of axillary buds below NPA application for kiwifruit vines 'Hort16A' where lanolin applied on the upper and NPA on the lower portions covered with aluminum foil. 177

Figure 4.11. Mean primary shoot length (cm) of kiwifruit vines A.chinensis 'Hort16A' for untreated control vines, auxin inhibitor naphthylphthalamic acid (NPA) applied below first node of the stem, indole-3-acetic acid applied below NPA (NPA+IAA), root restriction (RR: plants grown in 1 L pots), root restriction with NPA (RR+NPA). The red arrows indicate the dates of NPA application and the black arrows indicate the dates of IAA application to the stem. Bars represent standard error. 179

Figure 4.12. Treatment effect on the relationship between primary shoot length and node number for kiwifruit vines *A.chinensis* 'Hort16A' for untreated control vines, auxin inhibitor Naphthylphthalamic acid (NPA) applied below the first node on the primary shoot, indole-3-acetic acid applied below NPA (NPA+IAA) root restriction (RR: plants grown in 1 L pots), root restriction with NPA (RR+NPA). 180

Figure 4.13. Mean number of SAS formed for kiwifruit vines *A.chinensis* 'Hort16A' by the end of the growing season (10th March, 2008) for untreated control vines, auxin inhibitor naphthylphthalamic acid (NPA) applied below first node of the stem, indole-3-acetic acid applied below NPA (NPA+IAA), root restriction (RR: plants grown in 1 L pots), root restriction with NPA (RR+NPA). Bars represent standard error. Columns sharing the same letter are not significantly different using Duncan's Multiple Range Test at *P=0.05.* 182

Figure 4.14. Treatment effect on the mean total SAS length formed on kiwifruit vines *A.chinensis* 'Hort16A' for untreated control vines, auxin inhibitor naphthylphthalamic acid (NPA) applied below first node of the stem, indole-3-acetic acid applied below NPA (NPA+IAA), root restriction (RR: plants grown in 1 L pots), root restriction with NPA (RR+NPA). Bars represent standard error. Means sharing the same letter are not significantly different using Duncan's Multiple Range Test at *P=0.05*. Mean number of SAS formed in response to each treatment are shown on the right..... 183

Figure 4.15. The effect of treatments on the total shoot length (primary + sylleptic shoots) of kiwifruit vines of *A. chinensis* 'Hort16A' for untreated control vines; the auxin transport inhibitor naphthylphthalamic acid (NPA) applied below first node of the stem; indole-3-acetic acid applied below NPA (NPA+IAA); root restriction (RR: plants grown in 1 L pots); root restriction with NPA (RR+NPA) treatment of the stem. Bars represent standard error..... 184

Figure 4.16. Mean dry weight of very soft, soft (feeder roots); medium (med) and hard roots (structural roots) and of the total root system for untreated control vines and NPA (auxin transport inhibitor naphthylphthalamic acid) applied below first node of the stem of *A.chinensis* 'Hort16A'. Bars represent standard error. 185

Figure 4.17. Summer pruning fresh weights of kiwifruit vines 'Hort16A' in response to vigour modifying treatments; the auxin transport inhibitor naphthylphthalamic acid applied below the first node of the stem (NPA),; indole-3-acetic acid applied to the stem below NPA (NPA+IAA); root restriction (RR: plants grown in 1 L pots); root restriction with NPA (RR+NPA) and untreated control. Bars represent standard error. Means sharing same letters are not significant at *P=0.05* using Duncan's Multiple Range Test..... 186

Figure 4.18. Mean primary shoot length (cm) of kiwifruit vines *A.chinensis* 'Hort16A' for untreated control vines; auxin inhibitor naphthylphthalamic acid (NPA); indole-3-acetic acid (IAA), indole-

3-acetic acid applied below NPA (NPA+IAA) applied below the first node on the stem. The red arrows indicate the date of NPA and black arrows IAA application to the vine stem. Bars represent standard error..... 187

Figure 4.19. Treatment effect on the relationship between primary shoot length and node number for kiwifruit vines *A.chinensis* 'Hort16A' for untreated control vines, auxin inhibitor naphthylphthalamic acid (NPA) and indole-3-acetic acid (IAA) applied below the first node on the primary shoot, indole-3-acetic acid applied below NPA (NPA+IAA). 188

Figure 4.20 Treatment effect on mean leaf area (cm^2) of kiwifruit vines *A.chinensis* 'Hort16A'at the end of the growing season (April, 2010) for untreated control vines, auxin inhibitor naphthylphthalamic acid (NPA) and indole-3-acetic acid (IAA) applied below the first node on the primary shoot, indole-3-acetic acid applied below NPA (NPA+IAA) Columns sharing the same letters are not significantly different at *P=0.05* using the Duncan's Multiple Range Test. Bars represent the standard error..... 189

Figure 5.1. Portion of *Actinidia deliciosa* 'Hayward' shoot after aerial layering (Jan, 2008) showing callus with a few roots produced by the end of the growing period (May, 2008). 204

Figure 5.2. Effect of foliar sprays of auxin (NAA), benzylaminopurine (BAP) gibberellins (GA_3+GA_{4+7}), and untreated control on mean primary shoot length of both cultivars pooled together. Figure A with n=4 for GA (i.e., broken shoots removed) and n=8 for all other treatments; Figure B with n=8 for all treatments (i.e., broken shoots included). The black arrows represent the dates of gibberellin and BAP spray and red the NAA sprays. There was no spraying during early March due to bad weather. Means sharing the same letter shown on the right are not significantly different using LSD at *P=0.05*. Vertical bar on the top of the last measurement date represent LSD at *P=0.05*. Black

dotted inverted arrow indicates the time when sylleptic axillary shoots were first observed in the gibberellin treated plants. 210

Figure 5.3. Effect of exogenous auxin (NAA), benzylaminopurine (BAP) gibberellins (GA_3+GA_{4+7}), auxin transport inhibitor (NPA) and gibberellin biosynthesis inhibitor (PBZ) on the mean primary shoot length of 'Hayward'. The black solid arrows represent the dates of gibberellins and BAP sprays and red the NAA, and black dotted arrows NPA and PBZ. During early March due to bad weather there was no spraying. Foliar sprays of NAA were given three times (Jan 17th, Feb 17th and March 17th); PBZ and NPA sprays were given three time (Jan 22nd, Feb17th and March 17th). Means sharing the same letter shown on the right are not significantly different using LSD at *P=0.05* (n=2 for GA treatment (i.e., broken shoots removed) and n=4 for all other treatments). Vertical bar on the top of the last measurement date represents LSD at P=0.05. 212

Figure 5.4. Effect of exogenous auxin (NAA), benzylaminopurine (BAP) gibberellins (GA_3+GA_{4+7}), on correlation between node number and primary shoot length of 'Hort16A' (n=2 for GA treatment (i.e., broken shoots removed) and n=4 for all other treatments). The internode lengths can be compared from the regression line of data on each graph by dividing a y-value by its corresponding value of x in the graph. Trendline was not given for GA treatment as only two data points were available. 214

Figure 5.5. Effect of exogenous auxin (NAA), benzylaminopurine (BAP) gibberellins (GA_3+GA_{4+7}), auxin transport inhibitor (NPA) and an anti-gibberellin (PBZ) on correlation between node number and primary shoot length of 'Hayward' (n=2 for GA treatment (i.e., broken shoots removed) and n=4 for all other treatments). The internode lengths can be compared from the regression line of data on each graph by dividing a y-value by its corresponding value of x in the graph. Trendline was not given for GA treatment as only two data points were available. 214

Figure 5.6. Effect of foliar sprays of auxin (NAA), benzylaminopurine (BAP) gibberellins (GA_3+GA_{4+7}) and untreated control vines of 'Hort16A' on the mean bud break percentage during spring of the second growing season (2009). Percentage values were square root transformed before analysis. Means sharing the same letter at the top of the last bar column are not significantly different using LSD at *P=0.05*. Bars represent the standard error. 218

Figure 5.7. Effect of exogenous auxin (NAA), benzylaminopurine (BAP) gibberellins (GA_3+GA_{4+7}), auxin transport inhibitor (NPA) and gibberellin biosynthesis inhibitor (PBZ) and untreated control vines of 'Hayward' during spring of the second growing season (2009). Percentage values were square root transformed before analysis. Means sharing the same letter for the final date are not significantly different using LSD at *P=0.05*. Bars represent the standard error. 219

Figure 6.1. Scheme of gibberellin biosynthetic pathway showing the steps at which anti-gibberellin PBZ and Ca-Pro inhibit gibberellin biosynthesis. PBZ inhibiting early step of GA biosynthesis and Ca-Pro the last reactions catalysed by GA deoxygenases source :(Fagoaga et al., 2007). GA20ox and GA3ox are the activating enzymes and GA2ox is the deactivating enzyme. GA_1 and GA_4 are bioactive enzymes. The inhibition by PBZ and Ca-Pro are pointed with an 'X' mark. 242

Figure 6.2. Selection of experimental kiwifruit material of Actinidia deliciosa 'Hayward' in the Massey University experimental orchard during spring Five emerging shoots per cane and nine canes per vine were selected immediately after spring bud break for application of treatments. Canes were labelled distributing the treatments randomly (other emerging shoots and flowers on the selected canes were removed). 244

Figure 6.3. Diagrammatic representation of proleptic bud (A), proleptic shoot (lateral shoot) (B), and sylleptic axillary shoot (C) on a cane of kiwifruit vine. 245

Figure 6.4. Effect of gibberellin treatment at 0, 500, 1000, 2000, 4000 mg L^{-1} and anti-gibberellins PBZ and Ca-Pro at 0, 400, 600 and 0, 250, 350 mg L^{-1} respectively, on mean length of lateral shoots of Actinidia deliciosa 'Hayward'. The black vertical dotted lines represent the dates of treatment gibberellin application (seven applications) and red solid lines represent foliar sprays of anti-gibberellins. Means sharing the same letter shown on the right are not significantly different using LSD at P=0.05. Vertical bars represent the standard error. Black solid line separates the periods before and after initiation of shoot termination. 247

Figure 6.5. Effect of gibberellin treatment at 0, 500, 1000, mg L^{-1} and anti-gibberellins PBZ at 0, 400, 600 and Ca-Pro at 0, 250, 350 mg L^{-1} on the number of shoots (A): short, medium and long proleptic shoots developed from the emerging shoot selected at the onset of spring bud break (October, 2009) for *Actinidia deliciosa* 'Hayward'. 'B' represents the number of long shoots of all treatments. Values within the bar column in 'A' and across bar columns in 'B' sharing the same letter are not significantly different at P=0.05 using χ^2 test..... 250

Figure 6.6. Effect of anti-gibberellins Ca-Pro at 0 (control), 250, 350 mg L^{-1} and GA_3+GA_{4+7} 500 mg L^{-1} respectively on the number of shoot types: short, medium and long proleptic shoots developed from the emerging shoot selected at the onset of spring bud break (October, 2009) for *Actinidia deliciosa* 'Hayward'. Values within a bar column sharing the same letter are not significantly different at P=0.05 using χ^2 test. 251

Figure 6.7. Dose/response growth curve for GA_3 + GA_{4+7} applied fortnightly at 0 (control), 500, 1000, 2000, and 4000 mg L^{-1} on mean lateral shoot length of *Actinidia deliciosa* 'Hayward'. Bars represent the standard error. There was a significant response at 500 and 1000 mg L^{-1} GA_3 + GA_{4+7} on the length of lateral shoots. 252

Figure 6.8. Effect of gibberellins GA_3 + GA_{4+7} at 0, 500, 1000, 2000, 4000 mg L^{-1} and anti-gibberellin PBZ at 0, 400, 600 and Ca-Pro at 0, 250, 350

mg L^{-1}, on the mean shoot cross-sectional area (SCA) measured after harvesting (Feb 8th, 2010) shoots of *Actinidia deliciosa* 'Hayward'. Bars represent the standard error. 253

Figure 6.9. Relationship between node number and lateral shoot length of *Actinidia deliciosa* 'Hayward' vines in response to foliar sprays of gibberellin (GA_3 + GA_{4+7}) at 0 (A), 500 (B), 1000 mg L^{-1} (C) or anti-gibberellins PBZ 400 (D), 600 (E) and Ca-Pro 250 and 350 mg L^{-1} (F) applied fortnightly from October, 2009 to February, 2010. 255

Figure 6.10. Internode length of the control, GA (GA_3 + GA_{4+7}) 500 mg L^{-1} and Ca-pro 250 mg L^{-1} treated shoots of *Actinidia deliciosa* 'Hayward' measured on the destructive shoot harvest (8th Feb, 2010). Values are means ± SE, n=5 shoots for each treatment. Internodes of shoots treated with Ca-pro 250 mg L^{-1} and untreated control are completely elongated (growth was fully terminated); for GA 500 mg L^{-1} shoots still exhibiting elongation. 256

Figure 6.11. Shoots of *Actinidia deliciosa* 'Hayward' treated with Ca-pro 250 mg L^{-1} (A) and a shoot treated with GA_3+GA_{4+7} 500 mg L^{-1} (B) on the final harvest day (8th Feb, 2010) displayed to measure internodes. Internodes of shoots treated with Ca-pro 250 mg L^{-1} are fully elongated (growth terminated); for GA 500 mg L^{-1} shoots still exhibiting elongation. 256

Figure 6.12. Effect of gibberellins (GA_3 + GA_{4+7}) at 0 (control), 500, 1000, 2000 and 4000 mg L^{-1}; anti-gibberellins: PBZ and Ca-Pro 0, 400, 600 and 0, 250, 350 mg L^{-1}, applied fortnightly from October, 2009 to February, 2010 on mean specific leaf weight of *Actinidia deliciosa* 'Hayward'. Vertical bars are the standard error. Columns with same letter are not significantly different at $P \leq 0.05$ using the multiple LSD test. 257

Figure 6.13. Effect of gibberellins (GA_3 + GA_{4+7}) at 0, 500, 1000, 2000, 4000 mg L^{-1}, anti-gibberellins; PBZ at 0, 400, 600 and Ca-pro at 0, 250, 350 mg L^{-1}, on number and total length of sylleptic axillary shoots (SAS)

formed on the proleptic shoots of *Actinidia deliciosa* 'Hayward'. Columns with the same colour and the same letter are not significantly different at P≤0.05 using the multiple LSD test. 258

Figure 6.14. Effect of gibberellins (GA_3 + GA_{4+7}) at 0, 500, 1000, 2000, and 4000 mg L^{-1}, anti-gibberellins 0, 400, 600 PBZ and 0, 250, 350 mg L^{-1} Ca-pro, respectively, on the average length of sylleptic axillary shoots formed on the treated lateral shoots of *Actinidia deliciosa* 'Hayward' during 2009-2010. 259

Figure 7.1 Diagrammatic representation of dwarfing rootstock 'M.9' in reducing vigour of composite apple tree with 'RG' scion (Minus GA) (A), the attributes increased by foliar sprays of GA_3+GA_{4+7} (Plus GA) (B), at the end of the first year of growth after grafting (June, 2008). C represents gibberellins role in shoot extension growth via its effects on the apical (node) and sub-apical meristems (internode length) and cellular activity in internode elongation.SAS denotes sylleptic axillary shoots. Mean values with letters in brackets denote they are significantly different at *P=0.05* using the Duncan's Multiple range test. PAT: Polar auxin transport. Yellow and red arrows represent PAT to root system and GA&Cytokinin to shoot respectively. 270

Figure 7.2 Diagrammatic representation of the effect of root restriction and restricting basipetal transport of IAA by applying NPA to base of stem for potted one-year-old rooted stem cuttings of kiwifruit 'Hort16A' (A); effect of foliar sprays of NAA, NPA PBZ and gibberellins (GA_3+GA_{4+7}) on potted rooted kiwifruit stem cuttings (B); gibberellins (GA_3+GA_{4+7}) and Ca-Pro on length of lateral shoots of matured kiwifruit vines in the orchard 'C'. SAS denotes sylleptic axillary shoot. 283

Figure 7.3 Diagrammatic representations of different stages in the shoot development passing through different stages of dormancy. 287

List of Tables

Table 2.1. Main effects of scion and rootstock on the mean length, node number and internode length of primary shoots of 'M.9' and 'RG' scions on 'M.9' and 'RG' rootstocks during the spring and early summer (from October, 2007 to January 2nd week) growing period following grafting (August, 2007). Means for scion main effect are averaged over rootstock and GA treatments; means for rootstock are averaged over scion and GA treatments. 61

Table 2.2. Main effect of rootstock, scion and gibberellin (GA_3+GA_{4+7}) foliar spray on the final mean length, node number, internode length and shoot cross-sectional area (SCA) of the primary shoot at the end of the first growing season (June, 2008) after grafting (August, 2007). –GA, GA (Nov) and GA (Feb) represents no GA, GA sprays from November and February, respectively. 67

Table 2.3. Rootstock × GA treatment interactions for the final mean primary shoot length, node number and internode length for the scions at the end of their first growing season (June, 2008) from grafting. –GA, GA (Nov) and GA (Feb) represents no GA, GA (GA_3+GA_{4+7}) sprays from November and February, respectively Data are pooled over scions. 68

Table 2.4. Interactions of rootstock, scion and gibberellins (GA_3+GA_{4+7}) foliar spray on the final mean primary shoot length of 'M.9' and 'RG' scions on 'M.9' and 'RG' rootstocks at the end of the growing period (June, 2008) after grafting. –GA, GA (Nov) and GA (Feb) represents no GA, GA sprays from November and February, respectively. 69

Table 2.5. Interactions of rootstock, scion and gibberellins (GA_3+GA_{4+7}) foliar spray on the final mean node number (*P=0.09*) of 'M.9' and 'RG' scions on 'M.9' and 'RG' rootstocks at the end of the growing period (June, 2008) after grafting. –GA, GA (Nov) and GA (Feb) represents no GA, GA sprays from November and February, respectively. 78

Table 2.6. Interactions of rootstock, scion and gibberellins (GA_3+GA_{4+7}) foliar spray on the final mean internode length (*P=0.07*) of 'M.9' and 'RG'

scions on ’M.9’ and ‘RG’ rootstocks at the end of the growing period (June, 2008) after grafting. –GA, GA (Nov) and GA (Feb) represents no GA, GA sprays from November and February, respectively. 78

Table 2.7. Effect of rootstock and gibberellins (GA_3+GA_{4+7}) foliar sprays on the percentage of primary shoots that were fully terminated in April, May and June for ‘Royal Gala’ and ‘M.9’ scions during their first growing season after grafting (August, 2007). 80

Table 2.8. Main effect of scions (‘M.9’ or ‘R.G’), gibberellin (GA_3+ GA_{4+7}) foliar sprays to the scion commencing in November GA (Nov) or February GA(Feb) and rootstocks (‘M.9’ or ‘R.G’) on the mean total number, length of SAS [spurs + sylleptic shoots (SS)] and total shoot length of the scion at the end of the first growing season (June, 2008) after grafting (August, 2007). SS represent sylleptic shoots. 83

Table 2.9. Rootstock × scion × GA interactions on the mean number of sylleptic shoots formed on primary shoots of ‘M.9’ and ‘RG’ scions on ‘M.9’ or ‘R.G’ rootstocks at the end of the first growing season (June, 2008) after tree grafting (August, 2007). Gibberellin (GA_3+ GA_{4+7}) foliar sprays applied to the scion commencing in November GA (Nov) or February GA (Feb),) and –GA represents without GA foliar sprays. 83

Table 2.10. Effect of rootstock and GA (GA_3+GA_{4+7}) foliar sprays on the mean proportion (%) of sylleptic shoots that were fully terminated for ‘M.9’ and ‘RG’ scions during their first growing season after grafting. No GA, Nov and Feb indicate no GA sprays and sprays from November, 2007 and February, 2008 until the end of the growing season (June, 2008)..... 85

Table 2.11. Scion × gibberellin interaction on the final mean total length of sylleptic axillary shoot (SAS) (*P=0.02*) and mean total shoot length (primary shoot plus SAS length) of ‘M.9’ and ‘RG’ scions (*P=0.02*) at the end of their first growing season (June, 2008) from grafting (August, 2007). –GA, GA (Nov) and GA (Feb) represent no gibberellin (GA_3+GA_{4+7}) foliar sprays and foliar sprays from

November and February and continued till the end of the growing period. Means are averaged over rootstocks. 87

Table 2.12. Rootstock and GA treatment effects on the final length of primary shoot, number and length of sylleptic shoots, total shoot growth, termination dates and % of shoots terminated on given dates of 'RG' scions (Hypothesis I). 95

Table 2.13 Rootstock and GA treatment effects on the final length of primary shoot, number and length of sylleptic shoots, total shoot growth (A+B), termination dates and % of shoots terminated on given dates of 'M.9' scions (Hypothesis II). 96

Table 2.14 Main effects of rootstock ('M.9' and 'RG'), scion ('M.9' and 'RG') and gibberellin (±GA) foliar sprays on the dry weight of hard, medium and soft roots (different categories) of 'RG' and 'M.9' root systems at the end of the first season of growth (June 2008) after grafting. 97

Table 3.1. Mean internode length, cell length and the total number of cells along the length of the internode in pith tissues sampled along the longitudinal section of the internodes of scions on 'M.9' and 'RG' rootstocks without (-GA) or with (+GA) treatment. 'M9RG' and 'RGRG' represent 'M.9' and 'RG' rootstock with 'RG' scion, respectively, without GA and, M9RG +GA' and 'RGRG +GA' represent GA treatment applied from November (Nov) continued until the end of the growing season The percentage increase in internode length, cell number and cell length were calculated in comparison with control 'RGRG' without GA..... 144

Table 3.2. Over-all rootstock, gibberellin effect and their interactions on internode length, cell length and number along the length of the internode The increase due to interaction and their significant difference (P-values) were given 150

Table 4.1. Treatment effect on the mean length, node number, internode length of the primary shoot for kiwifruit vines *A.chinensis* cultivar 'Hort16A' at

the end of the growing season March, 2008. The values are mean ± standard error..... 181

Table 4.2. Treatment effect on length, node number, internode length of primary shoot; number and length of sylleptic (secondary and tertiary shoots) and total shoot length at the end of the growing season (April, 2010) of kiwifruit vines 'Hort16A' for untreated control vines, auxin inhibitor naphthylphthalamic acid (NPA), indole-3-acetic acid (IAA), indole-3-acetic acid applied below NPA (NPA+IAA) on the stem. 189

Table 4.3 Treatment effect in comparison with experiment one on number of SAS formed for untreated control, auxin inhibitor naphthylphthalamic acid (NPA) and indole-3-acetic acid (IAA) applied below the first node of the primary shoot, indole-3-acetic acid applied below NPA (NPA+IAA) on the stem. Number of SAS for the entire growing season 2007-2008 for experiment one and 2009-2010 for experiment two was recorded. For experiment two bending was done before 2nd week of January, 2010..... 190

Table 5.1. Effect of exogenous auxin (NAA), benzylaminopurine (BAP) gibberellins (GA_3+GA_{4+7}) and untreated control for 'Hort16A' and 'Hayward' on the mean primary shoot length, node number and internode length (cm) at the end of the first growing season, April, 2009 (n=2 for gibberellin treatment (i.e., broken shoots removed) and n=4 for all other treatments for each cultivar). For cultivars, means are averaged over hormone treatment and for hormone treatment means are averaged over cultivars. There were no significant interactions. Data for SAS number and length were log transformed to meet the assumptions of ANOVA. 209

Table 5.2. Effect of foliar sprays of auxin (NAA), benzylaminopurine (BAP) gibberellins (GA_3+GA_{4+7}), auxin transport inhibitor (NPA) and an anti-gibberellin (PBZ) on the mean primary shoot length, node number and length; mean sylleptic axillary shoot number, length and mean total shoot length [primary shoot plus sylleptic axillary shoot in (cm)] for 'Hayward' at the end of their first growing season (April,

2009). Data for SAS number and length were log transformed to meet the assumptions of ANOVA. 217

Table 5.3. Effect of auxin (NAA), benzylaminopurine (BAP) and gibberellins (GA_3+GA_{4+7}) applied to 'Hort16A' and 'Hayward' in summer (Jan-April, 2009) on mean percentage bud break and mean number of flowers during spring of the next growing season (October, 2009). There were no significant interactions in data. Main effects of cultivar and hormone treatment are averaged over hormone treatment and cultivar, respectively. Percentage values were square root transformed before analysis. Data for mean flower number were log transformed to meet the assumptions of ANOVA..... 221

Table 5.4. Effect of auxin (NAA), benzylaminopurine (BAP) gibberellins (GA_3+GA_{4+7}), auxin transport inhibitor (NPA) and gibberellin biosynthesis inhibitor (PBZ) applied to 'Hayward' in summer (Jan-April, 2009) on mean percentage bud break and mean number of flowers during spring of the next growing season (October, 2009). Percentage values were square root transformed before analysis. Data for mean flower number were log transformed to meet the assumptions of ANOVA. 222

Table 6.1. Effect of gibberellin treatment (GA_3 + GA_{4+7}) at 0, 500, 1000, 2000, 4000 mg L^{-1} and anti-gibberellins (PBZ and Ca-Pro) at 0, 400, 600 and 0, 250, 350 mg L^{-1} respectively, on the percentage of shoots of Actinidia deliciosa 'Hayward' that terminated from December, 2009 to February, 2010. 249

Table 6.2. Effect of gibberellin treatment (GA_3 + GA_{4+7}) at 0, 500, 1000, 2000, 4000 mg L^{-1} and anti-gibberellins (PBZ and Ca-Pro) 0, 400, 600 and 0, 250, 350 mg L^{-1} on the length, node number and internode length of shoots of *Actinidia deliciosa* 'Hayward' from 13th Oct, 2009 to 8th Feb, 2010). 254

List of abbreviations

ABA	Abscisic acid
AD	Apical dominance
ANOVA	Analysis of variance
ARD	Apple Replant disease
ATA	Auxin transport auto inhibition
ATIs	Auxin transport inhibitors
BA	Benzyl adenine
BAP	Benzylaminopurine
[^{14}C]-IAA	Carboxyl-labelled indole-3-acetic acid
CLSM	Confocal laser scanning system
CLV	*CLAVATA*
CU	Chilling unit
CZ	Central zone
2-D	Diode-pumped solid state
FAA	Formalin: acetic acid: alcohol
G	Girdling
G.16	Geneva 16
GA (Feb)	Gibberellins applied from February
GA (Nov)	Gibberellins applied from November
GA_{20} *oxi*	GA_{20} Oxidase enzyme
GA_n	Gibberellin $_n$ denotes the number
GAs	Gibberellins
GDC	Geneva double curtain
GLM	General linear model
^{3}H-IAA	Tritiated Indole-3-acetic acid
HRI	Horticutural Research Institute
2ip	Isopentenyladenine

IBA	Indole butyric acid
IPTs	Adenosine phosphate-isopentenyl transferases
IAA	Indole-3-acetic acid
JM	Japanese apple rootstock
KNOX	*KNOTTED*1-like homeobox
LAI	Leaf area index
LSD	Least square means
lsmeans	Least square means
M.793	Merton 793
M.9	Malling 9
MeOX	3-methyleneoxindole
MJ m^{-2}	milliJoule per square metre
MM.106	Malling Merton 106
MPa	Mega Pascal(s) (1 MPa = 10 bars)
NAA	1-naphthalene acetic acid
NPA	1-N-naphthylphthalamic acid
PAT	Polar auxin transport
PGR	Plant growth regulators
PBZ	Paclobutrazol
PIN1	*Pin-protein*
PSA	*Pseudomonas syringae pv*. actinidae
PVC	Poly vinyl chloride
PZ	Peripheral zone
RB	Royal Beauty
RCBD	Randomised complete block design
RDI	Regulated deficit irrigation
RG	Royal Gala
RMS_n	Ramosus $_n$ denotes the number
RR	Root restriction

SADH	Succinic acid dimethyl hydrazide
SAM	Shoot apical meristem
SARD	Specific Apple Replant Disorder
SAS	Sylleptic axillary shoot OR System for statistical analysis
SCA	Shoot cross-sectional area
SL	Strigolactones
SS	Sylleptic shoots
STK	Starkrimson
STM	Shootmeristemless
TG	Trunk girdling
TIBA	2,3,5-Triiodobenzoic acid
USDA	United State Department of Agriculture
WAA	Woolly aphid disease
WUS	*WUSCHEL*
Z	Zeatin
ZR	Zeatin riboside

Chapter 1
General introduction

1.1 Apple and kiwifruit as horticultural crops

Domesticated apples (*Malus domestica* Borkh.) have been grown in New Zealand from the beginning of European settlement. The first apple tree was introduced by Rev. Samuel Marsden in 1819. The first export apples were sent from Christchurch to Chile in 1888 and to the United Kingdom in 1890. New Zealand has produced many new cultivars such as 'Royal Gala' and 'Braeburn' which are still important to New Zealand growers as well as to growers overseas. Recent new introductions of apples such as 'Jazz™' and 'Envy™' were developed by Plant and Food Research, and marketed and sold globally by ENZA, the New Zealand apple and pears marketing board.

Kiwifruit are one of the most recently domesticated fruit crops (Ferguson and Huang, 2007). Kiwifruit of international commerce are selections of two related species *Actinidia chinensis* Planch. and *Actinidia deliciosa* (A.chev.) C.F.Liang et A.R.Ferguson (Ferguson and Seal, 2008). The kiwifruit 'Hayward' and 'Hort16A' were selected from *A. deliciosa* and *A. chinensis*, respectively. Today, the kiwifruit exports of New Zealand have increased from less than half a billion ($488m) in 1999 to more than a billion (1.501bn) (Zespri kiwifruit annual report 2009/10; Bano and Scrimgeour, 2012). Kiwifruit is currently New Zealand's largest horticultural export crop with fruit being exported to over 60 countries.

1.2 Fruit trees – rootstocks

Many fruit crops such as apples, pears, peaches, nectarines, apricots, cherries and plums utilise rootstocks (Webster, 1997). The propagation of fruit tree and vine scions on rootstocks by grafting has been a horticultural practice for over 20 centuries. Grafting a genetically different desirable fruiting scion onto a rootstock produces a composite tree

that consists of two parts: the rootstock and the scion (Fujii and Nito, 1972; Cummins and Aldwinckle, 1983). The rootstock constitutes the root system and a small portion of the lower trunk (shank) of the rootstock and scion the aerial fruiting part of the composite tree. Historically, seedlings were used as rootstocks and grafting was exploited as an asexual propagation method to multiply fruiting cultivars. This is because scion cultivars are not true to type if raised from seeds with significant genetic variation resulting in lack of uniformity (Webster, 2002), and do not propagate easily by layering or cutting (Rosati and Gaggioli, 1989). During the 1600s, using rootstocks in order to dwarf apple and pear scions became a common practice in Europe. In the mid eighteenth century, following the industrial revolution, specialization in fruit growing and orchard plantings increased interest in size control and stock-scion relations (Tukey, 1964).

With significant improvements in propagation techniques, attempts were made to replace rootstocks for propagation of fruit trees with micro-propagated and self-rooted scions. However, self-rooted apple trees have proved slower to establish and precocity was delayed by at least one year (Webster et al., 1986), fruit size was reduced in pears (Carrera and Gómez-Aparisi, 1997), the micro-propagated, self-rooted plums were more vigorous and had reduced fruit size in trials at HRI-East Malling (Webster and Wertheim, 1993). Tissue cultured apple trees were much more vigorous than trees grafted on Malling-Merton series MM.106 and MM.111 (Rosati and Gaggioli, 1989). Another very significant disadvantage of using self-rooted fruit trees compared with trees on selected rootstocks is their lack of tolerance or resistance to unfavourable soil conditions. In the future, the production of genetically modified scions may replace the use of rootstocks, provided they confer all the benefits currently achievable using rootstocks, and achieve consumer acceptability. It is anticipated that due to the above mentioned problems, the breeding of new scion cultivars propagated on their own roots, that confer all the benefits of a rootstock, will not be achieved in the immediate future. Hence, the use of rootstocks will continue due to the additional benefits they confer to the scion (Webster, 2000). Despite the potential of dwarfing rootstocks to increase production efficiency, there still exists a need to breed new improved rootstocks to improve resistance to pests and diseases and tolerance to severe winter cold and

drought, as unfavourable climatic and edaphic (soil) conditions can become a constraint on the production of fruit trees.

1.3 Rootstocks - advantages

The major horticultural benefit a rootstock confers to the scion is reducing tree size by vigour control and providing an easy and inexpensive method for propagation of most temperate fruit trees. Other physiological consequences are precocity of cropping, hardiness and yield efficiency, fruit size and quality, which are an advantage in modern high-density production schemes. According to modern pomological criteria, an ideal rootstock provides options for solving problems related to productivity such as compatibility with scions, tolerance or resistance to unfavourable environmental conditions, such as soil pest, diseases, winter cold injury, drought and stress factors, whilst improving fruit yield and quality (Rom and Carlson, 1987; Webster, 2000).

Grafting a scion onto a dwarfing apple rootstock decreased the final size of the tree at maturity (Tubbs, 1967; Tustin et al., 2001). Decreased final tree size enabled higher planting densities per unit land area (Tustin and Palmer, 2008) that increased light interception in the early life of the orchard (Wagenmakers and Wertheim, 1991; Robinson, 1992) combined with increased floral precocity of the scion on a dwarfing apple rootstock (Tustin and Palmer, 2008), and yield accumulation is greatly increased (Sansavini et al., 1986b). Dwarfing rootstocks can also increase fruit size and colour (Tubbs, 1967; Atkinson et al., 2000; Atkinson and Else, 2001) hence increasing marketable yield. Early bearing (precocity) and high yields per hectare has the most effect on the profitability of orchards (Chalmers, 1986). In New Zealand the intensive planting system increased precocity and fruit quality with tree densities up to 4000 per hectare (Palmer, 1999). However, depending on site and vigour, 2000 to 3000 trees per hectare may be the optimum range of tree densities for ‘M.9’ intensive systems for NZ (Tustin and Palmer, 2008).

In development of an apple orchard, the selection of a suitable size-controlling rootstock is highly important for orchard productivity. Trees on dwarfing rootstocks are normally

planted at high densities in order to quickly fill the allotted space, and flower precociously then allowing an early monetary return on the initial capital investment.

1.3.1 Apple rootstocks

A very large range of rootstocks, either seedling or clonal, is available conferring particular advantages. Clonal rootstocks have the advantage over seedling rootstocks because of their uniformity in controlling tree size. The common attributes of all good clonal rootstocks are long-tem graft compatibility with the scion, pest and disease resistance, ability to control scion vigour to the required level to promote precocity and abundant cropping. The most widely used source of apple rootstocks throughout the world is the Malling and Malling Merton series. The rootstocks in the Malling series were named and given numbers from 'M.1' to 'M.16' (Ferree and Carlson, 1987b; Jackson, 2003). Later a crossing programme between these Malling series and the Woolly aphid disease resistant variety 'Northern Spy' gave rise first to the Merton series (M.778-793), from which only 'M.793' has survived in common use and later to the Malling-Merton series (MM.101-115). Crossing at East Malling between 'M.9' and other Malling series rootstocks produced 'M.26' and 'M.27'. The Merton and Malling-Merton series, though resistant to Woolly aphid, do not reduce the tree size as significantly as the Malling series. All these Malling and MM series are susceptible to winter injury. Therefore, much effort has gone into producing other rootstocks with good vigour control and resistance to winter cold damage and fire blight. From Malling and Malling-Merton (breeding programmes) came the rootstocks Geneva and Michigan series from the USA; the Ottowa clones from Canada; the P-series from Poland and the Budogovsky series from the Soviet Union (Ferree and Carlson, 1987).

Though the dwarfing rootstock 'M.9' is most widely used in many apple producing countries of the world, it is not entirely suited to all environmental conditions. The rootstock 'M.9' is sensitive to winter cold injury. Both 'M.9' and 'M.27' are sensitive to woolly apple aphid (*Eriosoma lanigerum*) and to fireblight (*Erwinia amylovora*) (Webster, 2000). In addition, 'M.9' has poor propagation from stool beds, bad anchorage in soil and brittle roots. This challenged breeders to find clones with better

quality traits than the standard 'M.9', such as NAKB 337-340, Fleuron 56 (Netherlands), Nicolai 29 (Belgium , Burgmer , Germany), Pajam 1 and 2, (France) (Webster, 2000; Kosina, 2010). In Japan a controlled cross of *Malus prunifolia* 'Seishi' x 'M.9' produced 'JM 1', JM 7' and 'JM 8' and were released in 1999 after eleven years of screening and selection'. These are resistant to crown rot and WAA (Soejima et al., 1998; Soejima et al., 2010) and are super dwarfing.

The Cornell-Geneva® apple rootstock breeding programme was initiated in 1968. Later, in 1998, the Cornell University rootstock breeding programme was converted to a joint breeding programme with the United State Department of Agricultural (USDA) and produced the Cornell Geneva rootstock selections, which are now licensed for propagation in several nurseries around the world, but presently only nurseries in the USA and New Zealand have commercial production. These rootstocks have tolerance to pathogens associated with Apple Replant Disease (ARD). The commercialised Geneva rootstocks are: 'G.16', 'G.202', 'G.41', and 'G.935' (Marini et al., 2000 ; Robinson et al., 2004; Fazio et al., 2005; Robinson et al., 2006). Recently a new fire blight resistant, semi-dwarfing apple rootstock 'G.935' has been developed (Fazio et al., 2005).

1.3.2 Apple rootstocks and their utilization in New Zealand

Many rootstocks are now available globally as well as locally in New Zealand. The rootstocks 'MM.106' and 'M.793' are widely used in New Zealand (NZ) because of their resistance to woolly apple aphid (WAA), high productivity and precocity (Palmer and Adams, 1997). In New Zealand, a new apple rootstock 'M.116' was released that exhibits strong resistance to crown rot and has a very similar performance to 'MM.106' (Webster, 2002). A virus free heat-treated clone of 'M.9' was designated as 'NZ9' to distinguish it from the European clones (White and Tustin, 2002). There are two semi dwarfing rootstocks, 'Geneva 202' and 'Geneva 210', both resistant to WAA commercialised in NZ that produce a tree size in the 'M.26' range (Tustin and Palmer, 2008). Also, the dwarfing rootstock 'JM7' from Japan, resistant to crown rot and WAA

diseases and pests, has been commercialised in New Zealand (Tustin and Palmer, 2008) and provides a composite tree between the size of 'M.9' and 'M.26'.

1.3.3 Focus of the present study

Although there are many promising rootstocks that reduce tree size available for apples suitable for the HDP system, there exists a need to breed new rootstocks. Orchards with HDP system are being replanted into land that had been used for pip fruits previously. As a result the soil needs fumigation with chloropicrin for newly planted plants, in order to prevent Specific Apple Replant Disorder (SARD). Therefore, there is a strong need to breed new dwarfing rootstocks with resistance especially to SARD (Tustin and Palmer, 2008) as the use of chloropicrin may not be acceptable by international market in the future. Moreover, the dwarfing rootstocks presently used for increase in production efficiency, for example 'M.9' and 'M.26' are not resistant to WAA and fireblight (*Erwinia amylovora*) and semi-vigorous MM.106 is also susceptible to *Phytophthora* (Ferree and Carlson, 1987b). However, the recently commercialised G.935 (Fazio et al., 2005) is resistant to SARD and confers the tree size of 'M.26'.

In contrast to apples, for kiwifruit, presently there are no suitable vigour controlling rootstocks that confer beneficial characteristics including high marketable yield and resistance to pest and disease, although kiwifruit contribute largely to the New Zealand (NZ) economy. Promising new approaches involve the genetic modification of scions and/or rootstocks by using molecular biology techniques (Webster, 2002). In order to identify dwarfing genes, an understanding of the physiological cause and effect of dwarfing mechanism by rootstocks need to be understood. Breeding programmes using molecular biology techniques would be assisted if the physiology of the dwarfing mechanisms were better understood. In the case of apple trees on dwarfing rootstocks, it was understood that shoot-root-shoot hormonal signalling through endogenous hormones was responsible for reduction in the final size of the tree (van Hooijdonk et al., 2010). In other words, the reduced auxin signal from shoot to root system reduced the root signal to shoot by producing reduced levels of cytokinins and gibberellins. In apples, recently a dwarfing locus (*Dw1)* responsible for dwarfing has been identified

(Rushlome-Pilcher et al., 2008). The transfer of these dwarfing genes is likely to have minimal commercial impact to apples where adequate system of dwarfing already exists (Webster, 2002). However, if these genes could be transferred into rootstocks where dwarfing rootstocks do not exist such as kiwifruit, it would have significant commercial impact. Therefore, the focus of the present study is to increase our current understanding of the physiology of rootstock-induced dwarfing mechanisms in apple, and to elucidate whether the similar mechanisms exist in kiwifruit to help in rootstock breeding for kiwifruit.

1.3.4 Rootstock breeding – constraints and further developments

The major problems in breeding new dwarfing rootstocks is the length of time it takes (between 25 to 30 years), since extensive screening tests are needed for disease resistance and also agronomic traits such as precocity, yield efficiency, fruit size and quality (Webster, 2002). When there is extensive phenotypic variation within a genus it can be easily exploited through conventional breeding to develop new cultivars, but since it takes a long period, gene technology tools such as molecular markers were developed to make the selection cycle shorter and more efficient.

A major locus (*DWI*) involved in the dwarfing trait of the 'M.9' rootstock (Rusholme Pilcher et al., 2008), and genetic marker for WAA resistance in the 'Robusta 5' rootstock (Bus et al., 2008) were identified. The construction of a genetic map of apple progeny from a cross between 'M.9' and 'Robusta 5' has potential application to further elucidate the genetic control of rootstock-induced dwarfing of the scion by the 'M.9' rootstock (Celton et al., 2009). In the case of kiwifruit rootstock breeding, the main problems are long generation time and vigorous vegetative growth (Ferguson and Seal, 2008). In addition, male plant selection and ploidy differences, makes the crossing options difficult (Seal, 2003). Hence, molecular techniques could be very useful. However, in order to identify the genes responsible for desired characters such as controlling vigour and increasing yield efficiency, understanding the biological processes and physiological mechanisms that particular genes control is essential. Therefore, it is important to understand the first architectural changes imposed by

dwarfing rootstocks on scions and clearly identify the physiological causes and subsequent developmental effects (van Hooijdonk et al., 2010).

1.3.5 Apple dwarfing rootstock – effect on scion architecture

The study of tree architecture is considered to be a key factor for understanding growth strategies and provides powerful tools for analysis of plant structure (Hallé et al., 1978; Barthélémy and Caraglio, 2007). Though the use of dwarfing rootstocks is wide spread in fruit industry, their impact on the tree architecture is poorly understood (Atkinson and Else, 2001). This has become the main focus of several research studies (Seleznyova et al., 2003; Costes and García-Villanueva, 2007; Seleznyova et al., 2007; Seleznyova et al., 2008; van Hooijdonk et al., 2010). This section will discuss the architectural changes, imposed by a dwarfing rootstock on a scion and the timing of the occurrence of those changes.

Dwarfing rootstocks are thought to reduce tree size by restricting tree volume and promoting flowering (Lockard and Schneider, 1981; Fallahi et al., 2002). Costes, (2001) observed the growth and branching patterns of annual shoots of two cultivars ‘Royal Beauty’ (RB) and ‘Starkrimson’ (STK) grafted on semi-dwarfing (‘M.7’) and dwarfing (‘M.9’) rootstocks for about six successive years. It was also mentioned that the rootstock effect was cumulative and the dwarfing rootstock (‘M.9’) reduced the proportion of long laterals over the first two years of growth while the number of buds bursts in spring remained the same, and the same effect was shown on the second order branching thereby reducing the length of the growing period. It was also observed that the number of long laterals was reduced in the first two years of growth and this reduction was compensated by an increase in both medium and short (spurs) laterals.

When examined, the structure of 3-year-old branches on five year old ‘Royal Gala’ trees grafted on dwarfing and non dwarfing rootstocks, it was found that the dwarfing rootstock ‘M.9’ decreased vegetative vigour of the scion by reducing the number of neoformed nodes (Seleznyova et al., 2003) indicating that ‘M.9’ does not affect the relationship between node numbers and shoot length i.e., dwarfing rootstock does not

decrease internode length when similar classes of shoots are compared. Selezynova et al., (2008) reported that by the end of the first year growing season, the mean length, node number and internode length of primary shoot were not different for scions on 'M.9' and 'MM.106' rootstocks. It was also reported that heavy flowering in the following spring i.e., year two for the scion on 'M.9' reduced its vegetative growth. However, work at Massey University, New Zealand showed that 'M.9' imposed significant changes in scion architecture during the first year from grafting (Figure 1.1) of 'RG' scion on different size controlling rootstocks (van Hooijdonk et al., 2010). They also stated that uniformity of the material is very important because the subtle changes that appear on the primary shoot on different size-controlling rootstocks may not be detected if large initial variation exists. The increased flowering of the scion in the second year after grafting may be the result of physiological changes that occurred during the previous growing season (van Hooijdonk et al., 2010), as the evocation of floral bud in apple occurs in the previous year (Forshey and Elfving, 1989). Therefore, the first year appeared to be a critical year of development when the first physiological changes due to rootstock occur in the scion (van Hooijdonk et al., 2010). It was also mentioned that the initial occurrence of scion dwarfing following grafting of the composite tree on a dwarfing apple rootstock could occur in either year one or two depending on the growing environment and the method of propagation used to grow tree material (van Hooijdonk et al., 2010). Dwarfing rootstock 'M.9' had a slower rate of growth during the first growing season, followed by early shoot apical meristem termination leading to short primary shoots with fewer neoformed nodes. The 'M.9' rootstock also reduced the number of secondary shoots formed on the primary shoot and caused a greater proportion of secondary shoots to terminate early (van Hooijdonk et al., 2010). Therefore, the earlier growth termination of shoot may in part account for the increased number and quality of floral buds formed on trees on dwarfing rootstocks (Swarbrick, 1929; Webster, 2002). These architectural responses are typical of modified hormone transport effects (van Hooijdonk et al., 2010) and will be discussed further in the following section (section 1.3).

Figure 1.1 Effect of 'M.9' (dwarfing), 'MM.106' (semi-vigorous), 'M.793' (vigorous) and 'Royal Gala' (very vigorous control) rootstocks (from left to right, respectively) on the architecture of 'Royal Gala' apple scions by the end of the first growing season from grafting. Yellow rule is 1 m. (source: van Hooijdonk, 2009). The final length of the primary shoot and number and length of sylleptic axillary shoots were reduced for scion on 'M.9' rootstock by promoting early termination.

1.4 Suggested mechanism of vigour control by dwarfing apple rootstocks

The interdependence of shoot and root is apparent since roots absorb water and minerals and shoots perform photosynthesis to provide metabolites required for root growth. A dynamic balance is maintained between these two organs. The shoot-root ratio of a given cultivar is maintained irrespective of the vigour of the rootstock (Lockard and Schneider, 1981). In apple, root growth was concurrent with shoot growth (Cripps, 1970). The following sections introduce the physiological mechanisms underlying shoot-root interactions involving dwarfing rootstocks as proposed in the literature.

1.4.1 Nutrients

Rootstocks, apart from providing anchorage, supply nutrients to scions necessary for growth**.** Anatomically the root system of a dwarfing rootstock has a low xylem to

phloem ratio (Beakbane and Thompson, 1947) and a higher proportion of living parenchymatous xylem and phloem. These characters are genetically fixed and have been associated with dwarfing (Elfving et al., 1993). These parenchymatous cells may have storage function and might be expected to have a high rate of respiration, thus utilising the nutrients which could be linked with the potential of the root system to dwarf the shoot perhaps, by utilising a greater portion of the total assimilates (Beakbane and Thompson, 1947; Miller et al., 1961). This assumption that the dwarfing effect of rootstock was due to reduced supply of nutrients was not consistent with the observation that scion leaf nutrients differed very little among different rootstocks (Awad and Kenworthy, 1963; Chaplin and Westwood, 1980). Moreover, analytical studies revealed that dwarfing trees contain higher concentrations of minerals and organic nutrients than vigorous ones (Colby, 1935; Rao and Berry, 1941). Therefore, it can be assumed that the reduction in growth of the scion on a dwarfing rootstock is not due to lack of nutrients.

1.4.2 Photosynthesis

There is much inconsistency in the findings of rootstock effect on the rate of photosynthesis: Titova and Shishkanu (1977) studied the photosynthesis of several rootstocks for three years and reported higher rates of photosynthesis in the leaves of dwarfing than vigorous rootstocks, Avery (1977) reported that there were no differences in the rates of photosynthesis of leaves of pot-grown dwarfing and vigorous rootstock plants, Barden and Ferree (1979), reported similar results in young 'Starking Delicious' scions on rootstocks with a range of dwarfing nature, and Schechter et al., (1991) reported higher rates in vigorous than dwarfing rootstocks. Therefore, it can be assumed that although, photosynthetic potentials are genetically limited, they are generally determined by leaf exposure during its development (Lakso, 1980). Due to this inconsistency, it would be difficult to draw definite conclusions on how the rate of photosynthesis affects the dwarfing effect.

Generally in apples the rate of photosynthesis per unit leaf area is found to be ≈15-22μmol.m^{-2}.s^{-1} (Flore and Lakso, 1989), which is an extremely high photosynthetic rate per unit leaf area. The photosynthetic rate of apple leaves is maximal shortly after full expansion and declines slowly provided the leaf remains healthy and fully exposed to light (Fujii and Kennedy, 1985). Therefore, the significance of slow photosynthetic ageing may be that the apple tree canopy can remain productive without continuously producing young leaves over the entire season. This lowers the respiratory costs because respiratory maintenance of mature leaves is lower (Lakso et al., 1999) than that of newly formed leaves. Monteith (1977) elucidated that dry matter productivity of apples is essentially a linear function of total radiant energy interception over the season. High yield of apple orchards are correlated with high light interception but excessive shade within the canopy lowers marketable fruit yield (Jackson, 1988; Palmer, 1999; Lakso, 1999). Open tree canopies with the fruit bearing spurs well exposed throughout the growing season enhance fruit number, size, colour and quality (Wünsche and Lakso, 2000). Fujii, (1985) reported that fruiting apple trees (*Malus domestica* Borkh.) show higher photosynthetic efficiency as they exhibit reduced leaf weight (Proctor et al., 1976) and leaf areas (Avery, 1969) but more dry matter than non-fruiting trees (Hansen, 1971). The increased fruiting of apple trees on dwarfing rootstocks may be due to its higher photosynthetic efficiency, which again may be due to low vegetative vigour with reduced leaf area. Therefore, dwarfing is not a result of reduced photosynthesis.

1.4.3 Hydraulic capacity - graft union

One of the hypotheses concerning the mechanisms of dwarfing is that restricted supply of water from roots to the scion reduced shoot growth (Beakbane, 1956; Tubbs, 1973; Olien and Lakso, 1986). It was assumed that flow of water and minerals was restricted due to partial blockage of graft union (Simons, 1987), or due to small vessels characteristic of dwarfing rootstock (Beakbane, 1956). Atkinson and Else (2003) reported that in apples the hydraulic conductivity through the rootstock stem and graft union to the scion was related to the rootstock vigour and increased as the vigour of the rootstock increased. For example, the stem tissue of ‘M.9’ had a lower hydraulic

conductivity than ‘MM.106’. Though recent data suggested that dwarfing root systems have lower hydraulic conductivity than vigorous ones, Atkinson et al., (2003) reported such differences were lost when root mass was accounted for. Other possible reasons that contribute to the dwarfing effect of a dwarfing rootstock are the morphological abnormalities affecting the vascular system of dwarfing rootstock with excessive non-conductive phloem (Simons, 1987).

The anatomical changes in the graft union may be due to limitation of polar auxin transport across the graft and its accumulation at the graft union (Simons, 1986; Soumelidou et al., 1994a). IAA is key leaf derived regulator of xylem cell differentiation and division within cambial zone to initiate vascular redifferentiation across graft union (Parkinson and Yeoman, 1982). Therefore the reduced flow of auxin could provide an explanation of the observed changes in the xylem to phloem ratio in apple dwarfing rootstocks. In addition hydraulic limitations imposed by the graft tissue on the scion grafted on a dwarfing rootstock may be overcome by an increase in the diameter of the graft union due to auxin accumulation (Atkinson et al., 2003).

With-holding water to reduce shoot growth is well proven and became the basis of regulated deficit irrigation (RDI) techniques. For deficit irrigated apple trees, a midday water potential greater than -1.5 MPa was required to significantly reduce vegetative growth of the scion, compared with fully irrigated controls (Irving and Drost, 1987). The reduction in water potential imposed by dwarfing rootstock ‘M.9’ in the study of Olien and Lakso (1986) was relatively small and insufficient to decrease shoot growth, however, if these imposed water deficits are maintained throughout the summer season, fruit size is invariably reduced (Webster, 2002). Such reduction in fruit size is not associated with the use of dwarfing rootstocks. In fact, the size of the fruit can sometimes be increased with the use of dwarfing rootstocks (Webster, 2002).

Some authors suggested that the graft union influences vegetative shoot growth by restricting plant growth regulators (cytokinins) from the transpiration stream (Knight, 1925; Jones, 1974a; Jones, 1986) assuming that it filters the contents of the xylem sap from root to the shoot. However Kamboj et al., (1999a) showed that the concentration of zeatin and zeatin riboside in the sap from above and below the graft union collected

from composite trees of 'Fiesta' grafted onto different size controlling rootstocks were similar. Therefore these observations do not support the theory of Jones, that dwarfing may result from depletion of xylem sap solutes by the graft union between a scion and a dwarfing rootstock.

In summary, dwarfing rootstocks do not reduce vegetative growth by reducing hydraulic capacity or by depleting the flow of minerals and/or plant growth regulators through the graft union but it may communicate to the scion, probably through the endogenous plant hormones that control root and shoot growth.

1.4.4 Hormonal regulation of scion vigour

Plant hormones may control root and shoot growth and the functional relationship between the sizes of these plant parts (Vaadia and Itai, 1968). The mechanism by which the rootstock affects the growth of the composite tree as a whole may be due to a growth substance inter relationship between root and shoot that coordinates activities in the plant (Wareing, 1977), or due to a coordinated role of hormonal system (Jones, 1986; Steffens and Hedden, 1992; Soumelidou et al., 1994a; Sorce et al., 2002).

Reduction in scion vigour by dwarfing rootstock may result from modified transport of growth hormones (Rogers and Beakbane, 1957; Lockard and Schneider, 1981; Webster, 1995; Singh Kamboj and Quinlan, 1997; van Hooijdonk et al., 2010). Lockard and Schneider, (1981) hypothesised that decreased IAA basipetal transport through the cambial and phloem cells of the rootstock stem, to the root system may reduce the amounts of shoot-produced IAA reaching the root. Soumelidou et al., (1994a) reported reduced basipetal transport of radio-labelled IAA through the stem of dwarfing rootstocks, Kamboj, (1997) observed reduced transport of ^{3}H to the root system of 'M.9' dwarfing rootstock when ^{3}H-IAA was applied to a mature basal leaf of 'Fiesta' scion grafted on to 'M.9' rootstock and Michalczuk, (2002) observed levels of endogenous IAA in cambial sap from dwarfing rootstock was significantly lower compared to the levels in vigorous rootstocks. Recent studies proved that decreased levels of IAA to the root system after NPA application to the rootstock stem reduced

root formation and affected the production of root produced hormone such as cytokinins and gibberellins (van Hooijdonk et al., 2010).

The concept of physiological activity under hormonal control is closely associated with changes in the concentration at the site of action, changes in the tissue's sensitivity, and interaction with other phytohormones (Gaspar et al., 2003). Therefore, in order to understand the hormonal control in maintaining the shoot-root ratio and its influence in shoot-root-shoot signalling (van Hooijdonk et al., 2010), the site of synthesis, mode of action and effects of plant hormones need to be understood.

In the following section, how shoot-root-shoot signalling of endogenous hormones may play a role in the dwarfing of the scion on a dwarfing rootstock will be discussed in detail.

1.4.4.1 *Auxin transport and shoot-root-shoot signalling*

The plant hormone auxin is critical for plant growth and coordinates many developmental processes between shoot and root (Wareing, 1977; Woodward and Bartel, 2005). Indole-3-acetic acid (IAA) is recognised as the key auxin in most plants. Nearly six decades after the structural elucidation of IAA, a few fundamental questions and many small details remain unresolved, although many aspects of its metabolism transport and signalling are well established (Woodward and Bartel, 2005). Auxin interaction with other hormones adds to the present challenge of understanding auxin response.

IAA is synthesised in the shoot apex from the amino acid tryptophan (Moore, 1979) which is present in abundance, three degrees of magnitude higher than endogenous auxin in plant tissue (Reinecke and Bandurski, 1987), therefore tryptophan is not a limiting factor in auxin synthesis. Indole-3-acetic acid (IAA) synthesised in shoot apex translocates basipetally through a polar auxin transport (PAT) mechanism and influences the initiation and growth of lateral roots. Conjugation between endogenous IAA and amino acids leads to the synthesis of the specific protein necessary for formation of root initials (Ryugo and Breen, 1974). The decapitation (removal of auxin

supply) (Fu and Harberd, 2003) and NPA application to the root-shoot junction of *Arabidopsis* seedling (Reed et al., 1998) reduced the number and density of lateral roots and supply of auxin (IAA) to the decapitated stem restored the growth of lateral roots. As the roots of decapitated *gal-3 Arabidopsis* plants, GA biosynthetic mutants, were much less responsive to GA than the intact *gal-3* plants, and recovered their response after IAA application to the decapitated stem, it was assumed that auxin controls the growth of roots by modulating cellular responses to the phytohormones gibberellins (Fu and Harberd, 2003). Thus, the shoot apex-derived auxin promoted root growth by facilitating the response of root cells to gibberellins (Fu and Harberd, 2003). Therefore, it can be assumed that auxin supply from the shoot influences root growth. To fully understand auxin regulation, action and interaction, many aspects of plant growth and development need to be understood.

To explain the effect of dwarfing rootstock on scion growth, main concept focuses on the flow of auxin, from shoot to root and its influence on root system. It was reported that the velocity of basipetal auxin transport was lower in the stem tissue of the 'M.9' dwarfing rootstock compared to the more vigorous 'MM.111' rootstock (Soumelidou et al., 1994a). Therefore, low levels of IAA transported to the root system may decrease root growth (Davies, 1995; Sachs, 2005) and may show influence on the production of root derived phytohormones such as cytokinins (Kamboj et al., 1999a). Kamboj et al., (1999a), reported that high levels of cytokinins in the xylem sap of vigorous rootstocks compared to less vigorous rootstocks. These high levels of cytokinins may explain the increased rate of scion growth on vigorous rootstocks.

When polar auxin transport was inhibited by applying an auxin inhibitor naphthylphthalamic acid (NPA), on the stem region of rootstocks 'M.9', 'MM 106', 'M. 793' and 'Royal Gala' (control) each grafted to 'Royal Gala' scion separately, node production of the primary shoot was reduced (Figure 1.2) and the growth appeared to be similar irrespective of the vigour of the rootstocks (van Hooijdonk et al., 2010). Therefore, it was understood that auxin transport inhibition restricted the growth of the scion grafted onto the rootstock. Thus, shoot-root-shoot hormonal signalling may play a role in the rootstock dwarfing mechanism. Therefore, it can be assumed that the focus for rootstock dwarfing lies in the auxin supply from shoot to the root system. But the mechanism(s) behind the auxin effect needs elucidating.

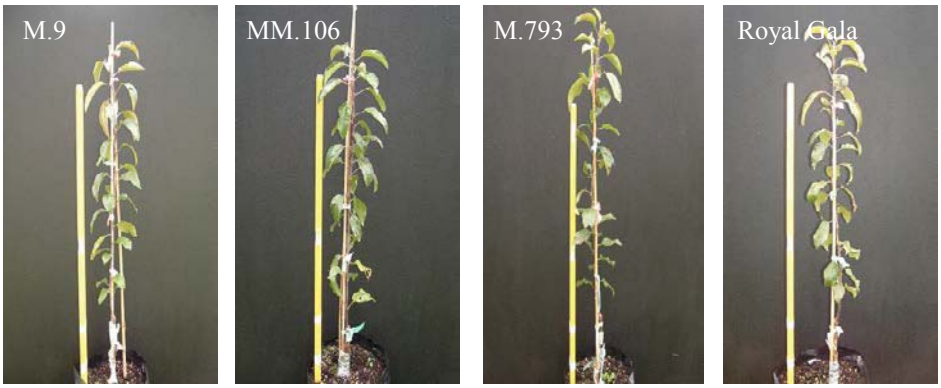

Figure 1.2 Effect of the auxin transport inhibitor 1-N-naphthylphthalamic acid (NPA) applied to the stem of 'M.9', 'MM.106', 'M.793', and 'Royal Gala' from left to right, respectively) on scion architecture of 'Royal Gala' apple trees by the end of their first growing season from grafting (source: van Hooijdonk et al., 2009). Yellow rule is 1 m. Observe reduced secondary shoot formation and early termination similar to the effects of dwarfing rootstock, thereby indicating the basipetal transport of auxin is an important physiological signal regulating rootstock-induced scion dwarfing.

The actual mechanism of how the rootstock stem reduces the basipetal transport of IAA is not known. The growth inhibiting property of a dwarfing rootstock may be associated with phenols present in the bark. Transfer of a piece of bark from dwarfing rootstock 'M.26' to a fast growing tree 'Gravenstein'/'MM111' resulted in dwarfing similar to dwarfing interstock (Lockard et al., 1982). When a stem piece of the dwarfing 'M.9' rootstock (interstock) was inserted between a vigorous rootstock and a vigorous scion, it reduced vegetative growth of the composite tree and stimulated flowering and fruiting compared to the tree without the interstock (Roberts and Blaney 1967). Basler and McBride, (1977) found a direct relationship between vigour and total phenol content in dwarfing cherry varieties. It was proposed by Leopold and Kriedemann, (1975) that some of the auxin was degraded in the bark, and the concentration decreased as it proceeds down through the bark to reach the root system of a dwarfing rootstock. The amount of degradation depended on the amount of IAA oxidases, peroxidises and phenols present in the bark of apple dwarfing rootstocks. The inactivation of IAA was achieved by conjugation and direct oxidation (Woodward and Bartel, 2005). The other reason may be PAT interaction with inhibitors such as abscisic acid (ABA) (Pilet and Galston, 1955).

The 'M.9' rootstock had higher rates of IAA degradation (Alvarez et al., 1989) as it required 10 times more IAA to produce the same number of roots as 'M.26' (James and Thurbon, 1981) and 'M.9' produced one-third of the roots after absorbing twice as much [I-^{14}C]IAA as 'M.26' in '*in vitro*' rooting. It was also mentioned that the difference in rooting capacity between 'M.9' and 'M.26' resulted from difference in endogenous auxin levels and auxin metabolism (James, 1983). Therefore, the nature of 'M.9' rootstock may be such that it requires higher levels of auxin than other rootstocks to produce roots. It was also reported by Alvarez et al., (1989) that conjugated IAA levels in 'M.9' were significantly higher than those in 'M.26', which could be attributed to the reason for the difference in rooting between 'M.9' and 'M.26'. So, by its nature 'M.9' rootstock requires higher levels of auxin for initiation of roots.

Therefore, the polar auxin transport was inhibited in the dwarfing rootstock and IAA levels were decreased as they were destroyed or metabolised while transporting through the stem portion of the dwarfing rootstock. This decreased basipetal auxin transport influences root metabolism and reduces the production of root derived hormone cytokinins and gibberellins transported to the scion through xylem vasculature.

1.4.4.2 *Shoot-root-shoot signalling via cytokinins and gibberellins*

As previously discussed in section 1.4.4.1, the reduction in the supply of auxins from scion to root system of a dwarfing rootstock may reduce the synthesis of root-produced gibberellins and cytokinins. There is evidence to confirm that root synthesised cytokinins translocated through the xylem (Jones, 1974b; Cutting et al., 1991; Kamboj et al., 1997; Kamboj et al., 1999a) to shoot where they influence shoot growth by activating the axillary buds (Cook et al., 2001). Kamboj, (1999a) mentioned the scion 'Fiesta' grafted on 'M.9' rootstock had lower concentration of cytokinins (zeatin and zeatin riboside) in the xylem sap compared with that of 'MM 106' a semi-vigorous rootstock. The concentration of cytokinins was more in the scion on more vigorous rootstock. Thus it can be hypothesised that increase in IAA levels from shoot to root in vigorous rootstocks may have resulted in an increase in the levels of cytokinins.

To understand the nature of hormones, their sources, sites and uses must be considered in the context of the whole organism, rather than isolated parts (Crozier and Reid, 1971). Crozier and Reid extracted gibberellins from all parts of the plant and characterised different gibberellins and their changing distribution within the plant. Their results showed that gibberellins (GAs) from roots have an effect on stem elongation. They suggested an involvement of shoot-root-shoot recycling system for gibberellin synthesis. According to this hypothesis, GA_{19} may be synthesised in the shoot, primarily in the primary leaves, move to the root tip where it is converted to a different gibberellin and then re-circulated to the shoot. Therefore, it can be assumed that there is shoot-root-shoot relationship in the mechanism of dwarfing as the removal of root apices resulted in the complete disappearance of GA_1 in leaves and apical buds, which prevented stem elongation. It was reported, the young and rapidly growing shoot tissues contain higher levels of bioactive gibberellins than mature tissues (Smith et al., 1992b; Ross et al., 2002). From grafting studies between gibberellins mutant and wild type pea plants it was understood that GA_1 is not a mobile gibberellin, only precursors of the bio-active gibberellins are transported (Reid et al., 1983; Hedden and Stephen, 2006) from mature tissues to young leaves, although the contribution from roots for gibberellin precursors cannot be ruled out. Moreover, gibberellin-like substances have been detected in the xylem sap from stems of apple and the quantities were found to be sufficient to produce an important effect on shoot development (Jones and Lacey, 1968).

Dwarfing rootstock ‘M.9’ reduced node production, promoted early termination and reduced axillary bud activation. Similarly, NPA application to the rootstock stem reduced node production and axillary bud activation irrespective of the vigour of the rootstock (Figure 1.2). These effects of restricting IAA transport (Figure 1.2) could be reversed with shoot applied gibberellins (Figure 1.3) and the shoot apical meristem could be re-stimulated to grow, which suggested the involvement of the shoot-root-shoot signalling mechanism. The application of gibberellins delayed termination of growth and increased node production and showed that scions on dwarfing rootstocks were deficient in gibberellins.

Figure 1.3. Interaction of NPA and GA_{4+7} on the growth of 'Royal Gala' scion grafted on 'M.9', 'MM.106', 'M.793' and 'Royal Gala' rootstocks by the end of their first growing season from grafting. The early termination imposed by NPA was prevented by GA_{4+7} thus alleviating the effects of probable impaired auxin transport. Yellow rule is 1 m. (van Hooijdonk et al., 2010).

The auxin transport inhibitor (NPA) decreased branching which was reinstated by applying cytokinin to the scion (Figure 1.4). This suggests that the scions on NPA treated rootstocks decreased basipetal auxin transport from shoot to root which in turn reduced cytokinin, which reduced the sylleptic axillary shoot formation (branching). Therefore, the NPA effect was similar to 'M.9' dwarfing rootstock effect and NPA converted 'RG' plant (vigorous) to a dwarf form, while that vigorous form could be re-instated with cytokinins and gibberellins.

Figure 1.4. Interaction of NPA and BAP on the growth of 'Royal Gala' scion grafted on 'M.9', 'MM.106', 'M.793' and 'Royal Gala' rootstocks by the end of their first growing season from grafting. The decreased secondary axes formation with NPA could be reinstated by applying cytokinin. Yellow rule is 1 m (van Hooijdonk et al., 2010).

1.5 Apple tree shoot apical meristem (SAM)

The architecture of a tree results from the activity of meristems. All organs in a plant are made of cells and tissues (groups of cells) that originate initially from meristems. Therefore the final architecture of the tree depends on the activity of meristems. Architectural diversity resulted from difference in the growth patterns of apical and axillary meristems are regulated genetically and hormonally (Sussex and Kerk, 2001).

The three main aspects of vegetative growth are: the quantity of primary growth, number and nature of developing axillary shoots and meristem death or termination. In the process of growth addition of basic elementary building blocks, the metamers form a complete leafy shoot of repeating units. Each metamer comprises a node, a leaf, axillary bud and subtending internode (White, 1979). The leafy axis extension can be continuous or rhythmic. Rhythmic growth occurs in successive growth flushes interrupted by a period of rest. These morphologically distinct growth increments are referred to as growth units (Seleznyova et al., 2008), which consists of a ring of bud scales, bud scale scars and/or zone of short internodes at its base that morphologically marks a period of rest (Barthélémy and Caraglio, 2007).

The growth of an apple tree results from monopodial growth where all the metamers are produced by the same meristem and from sympodial growth when the terminal bud differentiates into an inflorescence (Crabbé, 1983; Costes et al., 2003). Floral differentiation occurs in 1-year-old wood in the resting buds in either terminal or axillary positions. Thus, the floral growth unit that develops from the resting bud is a mixed unit composed of vegetative organ at its proximal part and floral organ at its distal part. This floral unit is called ‘Bourse’ which usually bears a short axillary shoot, which starts developing without any resting period, the so-called ‘bourse shoot’. The growth of shoot apical meristem and the growth of young fruits in a cluster of fruits are influenced by the action and interaction of important phytohormones, auxin and cytokinins. The following section will discuss the role of auxin and cytokinin and their interaction in apical dominance or correlative inhibition.

1.5.1 Role of auxin and cytokinin in apical dominance / correlative inhibition - apple tree

1.5.1.1 *Apical dominance / correlative inhibition*

The correlative events, the vegetative apical dominance (AD) and correlative inhibition of young fruits are regulated in a complex way. The main shoot in an intact plant grows predominantly while suppressing the outgrowth of axillary buds, which is called as apical dominance. Though the external conditions are favourable, the axillary bud is said to be in a state of paradormancy. The removal of shoot apex releases the axillary buds from apical dominance. The apical dominance is a correlative inhibition of shoot tip (apical bud) on the outgrowth of axillary bud (Cline, 1997; Cline, 2000). The correlative inhibition of young fruits is the suppression of growth of lateral fruits by the king fruit and/or by bourse shoots (Bangerth et al., 2000). The removal of the dominant fruit (king fruit) or bourse shoot at an early stage allows lateral fruit to increase their IAA export.

Plant shape and form are determined by differential elongation of buds and branches. The mechanism and role of auxin in this correlative inhibition is still under considerable scrutiny. Ample evidence exists in literature to say apical dominance is a correlative mechanism mediated by auxin and cytokinins (Kozlowski, 1964; Cline, 2000). In an intact plant, auxin from the shoot apex suppresses axillary bud outgrowth and auxin and cytokinins interact with each other to control shoot branching (Shimizu-Sato et al., 2009). In the branching process, new internodes produced by the terminal bud possess nodes with axillary buds in their axils. The branching can be sylleptic or proleptic. Sylleptic branches are those that develop within the current season, without any period of dormancy and proleptic are the delayed branches which develop after a period of bud dormancy. Sylleptic branching often occurs in the middle portion of shoots (mesotony) of the current season growth (Crabbé, 1983), when the growth rate is fast (Champagnat, 1954). Thus, sylleptic branches are those that develop from the buds that develop in the same growing year from the current year's shoot, without undergoing a period of rest (Tworkoski and Miller, 2007). Proleptic and sylleptic branch developments are

regulated by auxin/cytokinin concentration (Bangerth et al., 2000; Cline and Dong, 2002).

1.5.1.2 *Role of auxin in apical dominance*

It has been known for a long time that auxin is a major signal for apical dominance. Almost 70 years ago Thimann et al (1937) reported decapitation in *Vicia* species induced axillary bud outgrowth and application of indole-3-acetic acid (IAA) to the stump prevented it. Axillary bud growth cannot be prevented by the direct application of auxin to the axillary buds after decapitation (Shimizu-Sato et al., 2009). There is evidence to say that auxin does not directly prevent axillary bud out-growth as radioactive labeled auxin applied to the stump was not transported into the axillary bud (Hall and Hillman 1975). However, the auxin transport inhibitor 2, 3, 5-triiodobenzoic acid (TIBA) in lanolin paste applied to the stem of an intact plant, reduced or abolished apical dominance (Snyder, 1949). These observations indicate that basipetal auxin flow from a shoot apex promotes apical dominance. Auxin, produced mainly in young leaves and in the shoot apex (Ljung et al., 2001) is transported basipetally down in the stem in a polar manner by active transport in the vascular parenchyma (Lomax et al., 1995). Using vegetative pea shoot and apple fruit system, Bangerth (2000) demonstrated that auxin transport auto-inhibition (ATA) is produced whenever two streams of auxin; one from apical bud and another from lateral bud are converged. This may be the reason for the inhibition of axillary bud growth (Figure 1.5A). To inhibit lateral bud inhibition ATA alone was sufficient, but to release the lateral bud from inhibition only removal of ATA was not sufficient, but cytokinins were needed (Bangerth et al., 2000). In fact, Morris et al., (2005) reported that IAA depletion was not the first trigger for bud outgrowth, but the presence of cytokinins was needed for bud outgrowth (Figure 1.5B) (Foo et al., 2007; Dun et al., 2009; Ferguson and Beveridge, 2009; Shimizu-Sato et al., 2009). In addition, the trigger for branching after decapitation must be rapid (Morris et al., 2005) and this may be a physical response to change in the turgor pressure (Reiser et al., 2003).

1.5.1.3 *Role of cytokinin in promoting axillary bud outgrowth*

The effects of cytokinin in apical dominance are antagonistic to those of auxins as direct application of cytokinin to axillary buds promotes axillary bud outgrowth even in intact plants (Wickson and Thimann, 1958). To date cytokinin is the only chemical known to release axillary buds from para-dormancy (Shimizu-Sato et al., 2009). After decapitation in chickpea, the levels of cytokinin in the axillary buds increased 7-fold and the axillary bud outgrowth correlated with cytokinin levels in the axillary buds (Turnbull, 1997). Root apex appears to be the major site of cytokinin, gibberellin and abscisic acid synthesis (Little and Savidge, 1987) and there is evidence that cytokinins exist in roots xylem exudates (Kende and Sitton, 1967; Skene, 1972). Application of 1-naphthaleneacetic acid (NAA) to the stump prevented the increase in cytokinin in the bean xylem exudates (Li et al., 1995). These observations led to the suggestion that CK derived from roots promotes axillary bud outgrowth after decapitation, and auxin derived from shoot apex regulates CK transport in plants (Woolley and Warens, 1972; Letham, 1994). However, there are a few studies that support the view that CK are synthesised in the nodal stem. The axillary bud outgrowth in the excise stem segment of *Phaseolus vulgaris* (Tamas et al., 1989) suggested that CK are synthesised in the nodal stem. Recent studies reported that CK are synthesised throughout the plant as adenosine phosphate–isopentenyltransferases (IPTs) that catalyses the first step of CK biosynthesis, which are expressed throughout the plant (Nordstrom et al., 2004). There is also evidence for cytokinins to be present in cambium and all actively dividing tissues (Chen et al., 1985), as cultured rootless tobacco plants supplied with radioactive adenine (8-^{14}C), were capable of synthesising radioactive cytokinins. Moreover, plant tissues contain enzymes to interconvert cytokinin bases, nucleotides and nucleosides (Chen, 1981).

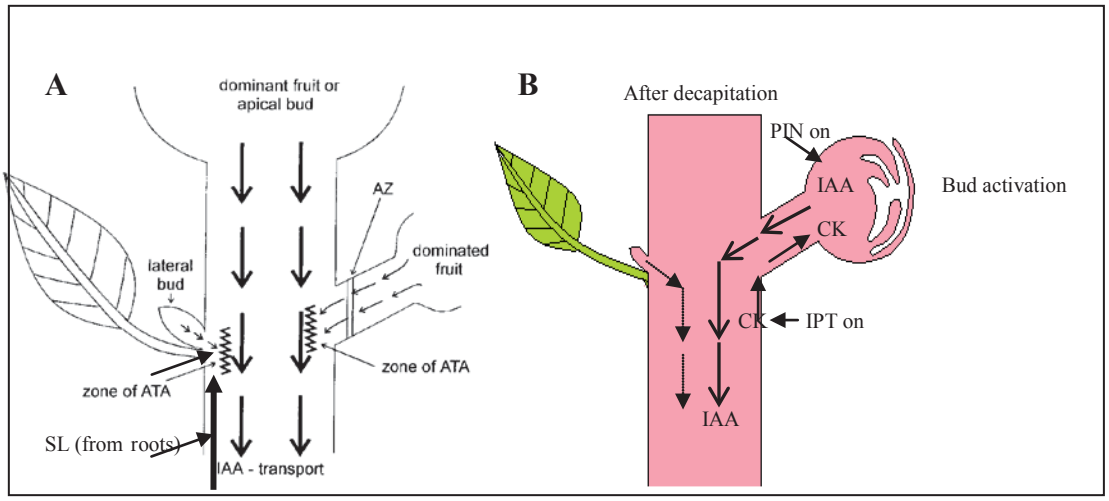

Figure 1.5. A. Schematic diagram representing a junction of basipolar IAA from apical bud and axillary bud. The wavy lines mark the area of auto-transport inhibition (ATA) from where strong inhibition signal extents acropetally into axillary bud [source: Bangerth et al., (2000)] and strigolactone (SL) signal from roots to inhibit branching (Shimizu-Sato et al., 2009) B. Model showing the interaction between auxin and cytokinin in shoot branching through *PIN* and *IPT* [source: Shimizu-Sato et al., (2009); Müller and Leyser, (2011)] after decapitation. PIN: IAA efflux proteins and IPT: Isopentenyl transferase.

1.5.1.4 *Role of auxin - cytokinin interaction in promoting axillary bud outgrowth*

There is evidence that auxin/cytokinin ratio may control lateral bud outgrowth, where auxins from apical bud plays a repressive role and cytokinins from the root plays a promotive role in herbaceous plants (Cline, 1994; Cline, 1997). There is evidence of a repressive effect of auxin (Cline, 2000) and promotive effect of cytokinins (Williams and Stahly, 1968) on proleptic branching. Axillary meristems escape from apical dominance when root factors overcome inhibition from distal auxin export (Tromp, 1996). This suggested that axillary buds were released from apical dominance directly because of increased root cytokinin production and only indirectly due to reduced auxin export by the shoot apex. Moreover, in apple, shoot-derived cytokinin may be directly involved with spring burst (Tromp and Ovaa, 1990; Cutting et al., 1991; Faust et al., 1997; Cook et al., 2001). Therefore, in the case of apple trees, cytokinins may play an important role in stimulating axillary buds to grow out (Jones, 1973; Young, 1989; Tromp and Ovaa, 1990; Kamboj et al., 1997; Kamboj et al., 1999a; Cook et al., 2001).

Moreover, how these hormones act in the sylleptic axillary shoot formation in woody species is not yet fully understood.

1.5.1.5 *A third plant hormone – shoot branching*

Recently a novel hormone, which is a group of carotenoid derived signalling molecules that controls shoot branching, called strigolactones (SL), was discovered. The levels of SL are significantly reduced in the roots of enhanced shoot branching mutants of pea (*max/rms/dwarf*) and that application of a synthetic strigolactone restored towards wild type (Gomez-Roldan et al., 2008; Umehara et al., 2008). Strigolactones are identified in connection with induction of germination of parasitic plants, released from the roots of the host plant (Akiyama and Hayashi, 2006). Strigolactones may be a part of the mechanism that contributes to apical dominance. In pea, the branching genes, RAMOSUS: RMS1 and RMS5 were regulated by IAA for the synthesis and perception of long-distance inhibitory branching signals, the strigolactones produced in stem and roots (Bainbridge et al., 2005; Foo et al., 2005; Johnson et al., 2006). The expression of these branching genes RMS1 and RMS5 increased when IAA applied was reduced either by decapitation or by applying auxin inhibitors (Foo et al., 2005; Johnson et al., 2006). Though decapitation removes apical dominance and releases the suppression of axillary buds (Van Overbeek, 1935; White et al., 1975; Morris et al., 2005), the auxin levels transport and flow or the reduction in the expression of RMS genes were not always sufficient to promote bud outgrowth (Beveridge, 2006). It was also reported that this signal also does not require living tissue for transport as the decapitation-induced signal is perceived by buds located below a girdled internode.

Therefore, in summary apical dominance plays a major role in the formation of sylleptic axillary shoot formation (branching). In this apical dominance the interaction between endogenous hormones auxin and cytokinins plays a very important role. In addition, in apple trees the branching and its angle with primary shoot (crotch angle) is another important horticultural character (see below).

1.5.1.6 *Branching in apple tree and crotch angle*

The branching process in apple trees have been studied by counting number of spurs (branches <2.5cm) per meter (Ketchie, 1984; Warrington et al., 1990). Spur density (20-21spurs/m) was found to be significantly positively correlated with yield efficiency, when 1-year-old vegetative spurs growing on 2-year-old branches were measured on 'delicious' apple strain on 'M.7'rootstock (Warrington et al., 1990). It was also found that the spur leaf number and area were high with vigorous rootstocks, but the spur density was low. Rootstock genetics have a significant effect on the number of branches (feathers) and angle of branches. The important criteria in evaluating tree quality are the number of branches and the angle of the branches (crotch angle). Wide crotch angle and flat branches have been associated with higher productivity (Fazio and Robinson, 2008). Rootstocks are found to show stability in the number of branches and in the branch angle in different environments. Crotch angles are found to be much wider in Geneva rootstocks than in Malling rootstocks. For high density orchard systems, the rootstock producing flatter branch angles would be a significant advantage (branch angle 0 = flat; 90 = upright). Generally, dwarfing ability of the rootstock is inversely correlated to the vegetative vigour. The apple trees on 'G.16' rootstocks are much larger with more feathers than those on 'M.9', 'B.9' and 'G.41', but 'G.16' is well known to become productive and switch resources to fruit growth and maintain dwarfing. The ability to fill its space quickly and then divert the resources towards yield by reducing the rate of vegetative growth is another characteristic of a good rootstock. High productivity of Geneva rootstock was associated with high number of feathers. This character is highly valued in high density orchard production systems where the goal is to maintain one major trunk with many emanating productive branches (Fazio and Robinson, 2008).

While the interaction between auxin and cytokinins promote or inhibit shoot branching i.e., sylleptic axillary shoot formation, another important hormone that allows these shoots to promote extension growth to produce long sylleptic shoots that increases vegetative growth are gibberellins. Although the role of gibberellins in the dwarfing mechanism was questioned by the scientists at East Malling (Webster, 2004; East Malling, 2005), in this study attempts were made to support previous studies that

reported the role of gibberellins in dwarfing mechanism. Therefore, the following section explains their role in shoot extension and termination.

1.6 Apple tree shoot extension and termination – involvement of gibberellins

Hormonal shoot-root-shoot signalling in a dwarfing rootstock appears to reduce the shoot length of the scion grafted onto it by decreasing the number of neo-formed nodes and by reducing the duration of shoot extension growth. Exogenous gibberellins increased the proportion of shoots growing on 'M.9' late in the season and therefore increased neo-formed nodes and shoot length, whereas exogenous cytokinins increased sylleptic axillary shoot formation (van Hooijdonk et al., 2010). Moreover, exogenous cytokinins combined with GA_{4+7} increased total shoot growth but in the absence of GA_{4+7}, buds formed only short spurs (Miller and Eldridge, 1986). Thus it may be suggested that since exogenous cytokinins and gibberellins changed the architecture of the scion by increasing shoot length and neo-formed nodes, dwarfing rootstocks produced less endogenous gibberellins and cytokinins.

In regulating plant growth, auxin and gibberellins play a very important role. Decapitation (Sheriff et al., 1994) and auxin transport inhibitors applied to elongating internodes (Ross, 1998) dramatically reduced the concentration of GA_1 in internode tissue below the excision site (Wolbang and Ross, 2001) i.e., in the stem. In a decapitated stem, application of IAA to the stump substituted for the apical bud in maintaining stem GA_1 content. This clearly supported the hypothesis that GA_1 biosynthesis occurs elsewhere other than the apical bud i.e., young leaves but only when IAA levels are adequate (Ross et al., 2001). Therefore, it is possible that part of the growth response due to auxin is mediated by GA_1. The growth of the primary and secondary shoots on 'M.9' tended to be slow and terminate earlier in the growing season (van Hooijdonk et al., 2010), as the growth of the apple meristem may be somewhat limited by endogenous gibberellins because gibberellins applied to apple shoots increased shoot growth (Steffens et al., 1985; van Hooijdonk et al., 2010). It was

also observed that growth of apple shoots rapidly ceased when IAA transport was inhibited by TIBA an auxin inhibitor that was applied to the root collar of apple seedlings (Grochowska et al., 1984) and NPA to the stem of vigorous rootstocks (van Hooijdonk et al., 2010). Application of NPA to the rootstock stem region of vigorous rootstocks significantly reduced node production by primary shoot because the shoot apical meristem on the primary shoot ceased to grow within 14-21 days after NPA treatment (Figure:1.2). From the above observations, it can be assumed that IAA transport to the roots, and/or, gibberellin transport to the shoot may be important signals that regulate meristematic activity of the scion on a dwarfing apple rootstock. While root derived cytokinins appear to play an important role in releasing apical dominance, root derived gibberellins and other substances of regulatory and nutritive nature may play a role in enhancing subsequent elongation (Prochazka and Jacobs, 1984; van Hooijdonk et al., 2010 and 2011).

Among many gibberellins so far identified from plants, only a few of them such as GA_1, GA_4 and GA_7 are thought to function as bioactive hormones, whereas many other non-bioactive GAs exist as precursors for the bioactive forms or exist as deactivated metabolites (Yamaguchi, 2008). The bioactive GAs are synthesised at the site of their action. In the biosynthesis of gibberellins, for the activation of GAs the enzymes *GA_{20} oxidase* and *GA_3 oxidase* and for deactivation, the enzyme *GA_2 oxidase* are essential (Figure 1.6). *GA_{20} oxidase* activity is the major determinant for the production of active GAs in plants (Xu et al., 1995; Fagoaga et al., 2007). An apple scion variety in which the gene encoding for *GA_{20} oxidase* was suppressed resulted in significant reductions in height of the primary shoot (Bulley et al., 2005). Therefore, the penultimate step in the formation of bioactive GAs requires the presence of the enzyme *GA_{20} oxidase*.

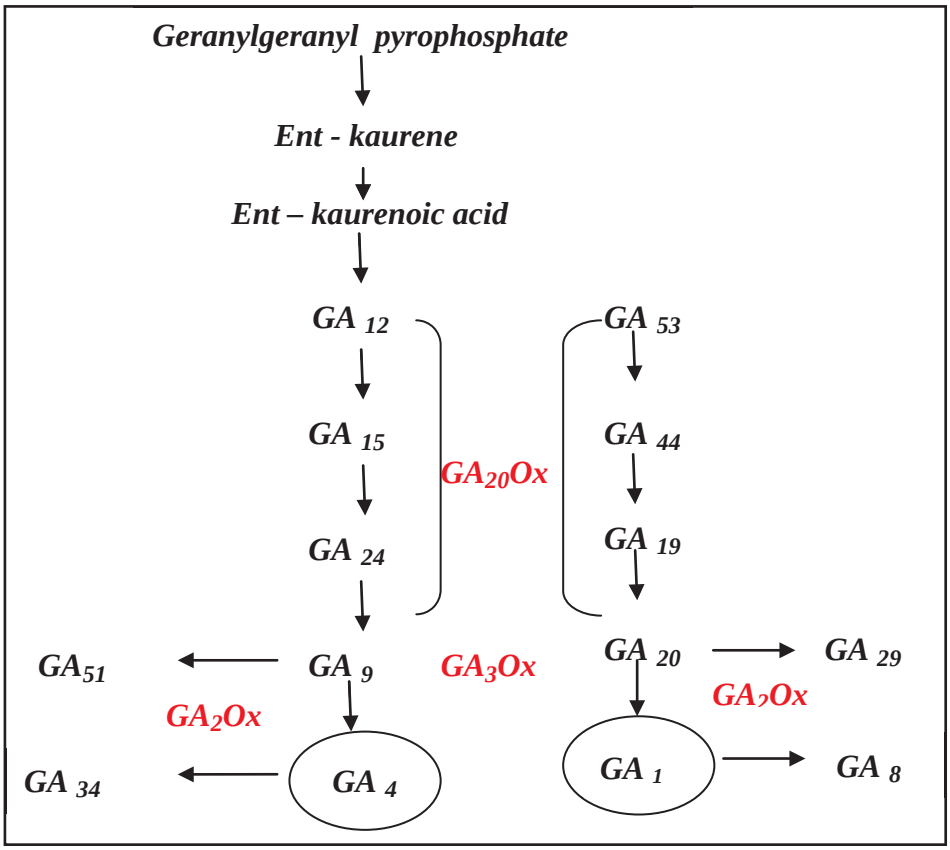

Figure 1.6. Scheme of gibberellin biosynthetic pathway showing the last reactions catalysed by GA deoxygenases source:(Fagoaga et al., 2007). *GA20ox* and *GA3ox* are the activating enzymes and *GA2ox* is the deactivating enzyme. GA_1 and GA_4 are bioactive enzymes.

In apple stem apices the presence of many gibberellins such as GA_1, GA_3, GA_4, GA_7, GA_8, GA_9, GA_{19}, GA_{20}, and GA_{29} has been demonstrated (Steffens and Hedden, 1992). As Lockhard and Schneider, (1981) mentioned, shoot synthesised GAs might be translocated to roots, where they are converted to other gibberellins and then recirculated through xylem sap to the stem tip. Since cultivated apples are generally grafted onto rootstocks, the metabolism of gibberellins may be different depending upon the size controlling rootstocks and the rootstocks may consequently change the effect of the gibberellins which could be activated by shoot metabolism (Motosugi et al., 1996).

In summary, it was understood from the literature that the architectural changes a dwarfing rootstock imposed on the scion grafted onto it, appeared to be more involved

with endogenous hormones rather than other causes such as graft union, scion photosynthetic rate and root hydraulic potential. Previous studies on rootstock dwarfing mechanism on composite apple trees revealed that the polar auxin transport signal to root is essential in maintaining the root to shoot hormonal signalling. The decreased IAA supply to roots from a scion on a dwarfing rootstock decreased root-produced hormones the GAs and cytokinins, thereby, reducing the final tree size of the scion on a dwarfing rootstock. In comparison with vigorous rootstocks, the dwarfing rootstock reduces the speed of extension growth throughout the season and generally promotes early termination of shoot extension early in summer, which finally reduces the tree size. Arguably, this slow rate of shoot extension and early shoot termination could be related to decreased gibberellins from root as auxin from shoot decreased, which intern establishes the shoot-root-shoot hormonal signalling the causative factor for dwarfing of a scion on the dwarfing rootstock.

As the dwarfing mechanism of composite apple tree has been unveiled in terms of shoot-root-shoot hormonal signalling recently was convincing, now it is time to understand the physiology behind vigorous growth of certain fruit crops, such as kiwifruit vine, and also to elucidate whether the similar hormonal mechanism does exist, in order to breed rootstock that reduce vigour and increase harvest index. As there are many advantages a dwarfing rootstock imposes on to the scion towards many desired agronomic traits, it would be good to pay attention to fruit crops where there are no promising size-controlling rootstocks such as kiwifruit. New Zealand is currently the third largest global producer of kiwifruit and it is the most important fruit crop to the New Zealand economy. As one of the major objectives in this study was to implement the knowledge so far obtained for composite apple tree dwarfing mechanism onto kiwifruit vine, it would be proper to introduce this fruit crop in detail at this stage.

1.7 Kiwifruit vines

1.7.1 Introduction

Kiwifruit [*Actinidia deliciosa* (A. Chev.) C.F. Liang et A. R. Ferguson var. *deliciosa*] a vigorous, climbing, deciduous, dioecious, perennial vine, belongs to class: *Magnoliopsida*, order: *Ericales* (Cronquist) or Theale (Thakhtajan) and family: *Actinidiaceae* (Ferguson, 1984). Kiwifruit is a native of China, and the genus *Actinidia* originated from the mountain ranges of south western China (Ferguson, 1984). The genus *Actinidia* has 66 species and 118 taxa. Kiwifruit as a horticultural crop has been subjected to very little selection pressure, and are still very similar to plants in the wild (Huang and Ferguson, 2006). Germplasm collection provides raw material for plant breeding and crop improvement. Kiwifruit germplasm collection by the New Zealand Institute for Plant and Food Research Ltd. holds about 3500 distinct genotypes of which 1000 have been selected for long term retention (Ferguson, 2007). There are about 80 cultivars and named selections which can be of great potential for breeding programmes, though they are of little value in their own right. The ultimate use of kiwifruit germplasm is for the production of new cultivars that bring the New Zealand industry a competitive advantage.

The two main economically important species of *Actinidia* are *A. deliciosa* and *A. chinensis*. In 1904, seeds of *Actinidia deliciosa* were collected from China and 'Hayward' was produced by New Zealand horticulturist Hayward Wright in 1925, and is now known as green kiwifruit. In the 1940s, the first commercial orchard produced fruit for the domestic market. The 'Hayward' has become world famous and dominated the international kiwifruit market for a long period since it was first selected and commercialised in New Zealand. Later in 1987, seeds of *A. chinensis* were collected from China and an 11 year process of cross pollination and grafting produced the 'Hort16A', or gold kiwifruit, which is marketed worldwide under the brand name 'ZESPRI^{TM} Gold' kiwifruit. The 'Tomua' of *A. deliciosa* was produced in 1983 by Plant and Food Research in Te Puke, New Zealand and was established in 1989. This cultivar flowers two weeks earlier than 'Hayward' and fruits mature four weeks ahead (Lowe and Marsh, 1999). These two cultivars entered the kiwifruit market; the

production of kiwifruit 'Tomua' was at low ebb and ceased and 'Hort16A' became strong and has quickly become established. Therefore 'Tomua' is no longer in production. At the Guangxi Institute of Botany China, new cultivars such as 'Guihaia 4' and 'Shimei' were produced from *A.chinensis*. The kiwifruit 'Shimei' has become a competitive cultivar in the market because of its large fruit, fine shape, early fruiting, high quality and adaptability (Li et al., 2003). This cultivar grows vigorously with strong hard branches and 88% of these were fruiting laterals. Other cultivars such as '51-1785' (Lowe, 2005) and 'Hort22D' (Lowe, 2008) were also produced. Zespri's kiwifruit programme with Plant and Food Research is the world's largest and most advanced and promised to deliver three substantial new varieties. They are 'Gold3' 'Gold9' and 'Green14'.Though promising high yielding kiwifruit cultivars are produced, still growers are facing a challenging problem of controlling vegetative vigour. Another new and distinct cultivar produced from controlled pollination between 'Hort16A' and an unreleased selection CK39_16 is a male plant that has been (Lowe and Hofstee, 2011) named 'Bruce' which appears suitable for use as a pollinizer in commercial kiwifruit production. So far kiwifruit vigour controlling rootstock has not yet been bred. As kiwifruit vines grow vigorously, growers are finding it difficult to control as the cultural practices were found to be expensive. Thorough knowledge of its biology and hormonal physiology behind its vigorous growth is required in assisting breeding programmes to breed vigour controlling rootstocks. The following section will explain vegetative growth of kiwifruit vines and the cultural practices growers employ to maintain the orchard.

1.7.2 Kiwifruit Rootstocks - vegetative growth

All *Actinidia* species are perennial climbers, having long vigorous shoots twisting tightly, usually upwards in a clockwise direction, around supports. Shoots of current season (annual shoots) come from axillary buds of the previous season's growth. A mature vine in a commercial orchard has a main trunk and a permanent leader or 'cordon' growing in the opposite direction (Figure 1.7). The fruiting 'arms' or canes are one year old canes, developed from leaders are tied down on to the supporting wires. In

spring, bud break occurs along these canes. Kiwifruit vines exhibit strongly vigorous vegetative growth and they need heavy pruning strategies to control and maintain them in a manageable state. Clearwater, (2004) reported that there are no size-controlling rootstocks and there is no information on the physiological mechanism of vigorous growth for kiwifruit. There are however a few rootstocks that are known to enhance precocity and productivity.

The rootstock, 'Kaimai' has been reported to confer enhanced precocity in the scion variety especially 'Hayward' young vines (Warrington, 2000). For many years, 'Bruno' seedlings were used as rootstocks for kiwifruit in New Zealand and growers believed that vines on seedling rootstocks grew rapidly and were more productive (Lawes, 1990). Though, Lawes, (1990) reported that the vines propagated by rooted stem cuttings were not inferior to grafted kiwifruit vines, growers were reluctant to use them. The 'Hayward' scion grafted to a Te Puke (New Zealand) selection of A. hemsleyana (TR2, Kaimai) produced twice as many flowers as seedling rootstock and maintained a similar average fruit size despite carrying a 20% higher crop load (Lowe et al., 1992). Similar increase in flower number was observed on the rootstock selections of A. eriantha and A. rufa (Wang et al., 1994). Thus, the vine performance was found to be dependent on the selection of rootstock (Cruz-Castillo et al., 1997). Nevertheless, until now vigour controlling rootstocks for kiwifruit to reduce vigour and to promote floral precocity were not available. However, the trials were conducted at Plant and Food Research, Te Puke, New Zealand on the fruiting and vegetative behaviour of 'Hort16A' scion on the rootstock 'Bounty 71'. The rootstock 'Bounty 71' was found to have benefits relative to two known commercial rootstocks, 'Hayward' and 'Kaimai' in reducing the vegetative vigour slightly, producing a more open canopy and promoting flower number and fruit size, thus resulting in higher yields of export quality fruit. The 'Bounty 71' rootstock caused 'Hort16A' to produce slightly less vigorous and more open canopy (Thorp et al., 2012).

Various *Actinidia* species have been investigated for vigour. Clearwater et al., (2006) suggested that symptoms of vigour due to rootstock in kiwifruit vines are visible in the earliest periods of shoot development in spring, immediately after bud break. Three types of shoots were identified based on the early growth patterns. They are short,

medium and long shoots. The short and medium shoots: self terminating, produce nine or more than nine nodes and the long shoots: non-terminating and produce many neo-formed nodes. The vigour of rootstocks was measured according to the proportion of short self terminating shoots to long non-terminating shoots.

Figure 1.7 Excessive vegetative growth that kiwifruit 'Hayward' normally exhibits in an orchard system.

The self-terminating shoots stop shoot extension growth and produce only the pre-formed nodes in parent axillary bud (Seleznyova et al., 2002). The scions that produced 50% less leaf area with higher proportions of short and medium shoots on rootstocks were considered to be low vigour. When these short shoots were retained during pruning they produced a higher proportion of short shoots in the following year, thus reinforcing the effect of low vigour rootstocks (Clearwater et al., 2006). Hence, for vines on these low vigour rootstocks, there is a high percentage of short early terminating shoots. It was also mentioned that increased numbers of long non-terminating shoots decreased fruit yield (Chesoniene, 1999).

Clearwater et al., (2007) measured root pressure continuously over spring in eight clonal kiwifruit rootstocks: *A.chrysantha, A. deliciosa, A. eriantha, A. hemsleyana, A. kolomikta, A.macrosperma, A.polygama*. The scion grafted on each of these rootstocks was *Actinidia chinensis* 'Hort16A', a commercial cultivar of yellow fleshed kiwifruit (Ferguson and McNeilage, 1999). *A. hemsleyana* Dunn 'Kaimai" (known as 'TR2') is known to promote flowering and vigour in green kiwifruit (Wang et al., 1994; Warrington, 2000). The rootstocks *A. hemsleyana, A. macrosperma, and A. deliciosa* developed high root pressure before or during bud burst and the scion growth was not water stressed and *A. eriantha* and *A. chrysantha* also developed root pressure and all these rootstocks were classified as high vigour rootstocks (Clearwater et al., 2006). The rootstocks *A. polygama, A .kolomikta* developed root pressure after bud burst and their scions exhibited water stress and were classified as low vigour rootstocks. Root pressure in rootstocks that promoted vigour was higher (0.15 MPa) than that in rootstocks that reduced scion vigour (0.05 MPa). In low vigour rootstocks, the flower number was not reduced (Clearwater, 2004), even though kiwifruit flower development is known to be sensitive to carbohydrate reserve availability (Grant and Ryugo, 1982; Snelgar et al., 1992). Therefore, the effect of rootstock on scion vigour may be independent of flowering effect. It was also suggested that the availability of carbohydrates and minerals from the roots are not the cause for the early differences in the scion shoot growth between rootstocks (Clearwater et al., 2007). The early cessation of growth and shoot tip abortion of short and medium shoots and non-terminating long shoot organogenesis are controlled independently (Foster et al., 2007b). *Actinidia* species differ in the timing of bud burst and also differ in their proportion of short terminating shoots. The underlying physiology and the hormonal impact on these differences in timings of bud break and growth patterns need to be understood. Moreover, as there are no rootstocks to reduce vigour, growers had a great deal of expense for various pruning strategies to produce marketable quality fruit.

1.7.3 Techniques to control vigour

1.7.3.1 *Canopy management- Crop load*

Crop productivity is related to the balance between vegetative and reproductive growth. Growers need to manage the plant so that the current year's dry matter production is distributed strongly into fruits and not unnecessarily into shoots and roots. Apple trees on dwarfing rootstocks are more efficient in diverting the dry matter into fruits rather than into wood, thereby increasing the harvest index (Forshey and Elfving, 1989). Total dry matter production is a function of light interception by the tree and its distribution within the tree (Jackson, 1988), which depends on the leaf area index (LAI). Generally, in order to have an orchard with evenly distributed canopy, dwarf trees are used, which are trained on horizontal or angled trellis systems. If trees are widely spaced, much higher LAI is needed to give the same light interception; then again the tree volume is densely shaded. Dense planting with inadequate control of vegetative growth again causes serious shading problems (Sansavini et al., 1980).

In kiwifruit, the final fruit size, export yield and return bloom are influenced by both crop load and canopy management. Crop loads were adjusted by manipulating winter pruning and flower or fruit-let thinning 5-28 days after full bloom (Cooper and Marshall, 1991). Cooper and Marshall, (1991) mentioned that a crop load of around 40 fruit/m^2 and a 3:1 leaf to fruit ratio on the fruiting cane are suitable for the best fruit size of export yield. Carrying a lighter crop load (i.e., less fruit in relation to leaf area) results in greater average fruit size. The total radiation intercepted by the canopy during the growing season greatly influences the energy balance of kiwifruit vines and yield, growth, quality and storage life of kiwifruit (Grant and Ryugo, 1982; Snelgar and Hopkirk, 1988; Tombesi et al., 1993).

High vigour canopies that are dense and with low light levels are reported to have premature fruit fall, fruit softening at maturity, reduction in dry matter accumulation in fruit during fruit maturation (Snelgar et al., 1998) and high losses of fruits during cool storage. Such losses significantly reduce orchard profitability. In kiwifruit a leaf area index (LAI) around 3.5 is necessary to obtain regular vine growth, optimum yield, fruit size and quality and return bloom for the following season (Tombesi et al., 1993). Fruits

from the shaded part of the canopy were smaller (Tombesi et al., 1993) with lower chlorophyll content at harvest. These fruits were with lower soluble solid concentration and less firm compared with the fruits grown in exposed positions. Therefore, kiwifruit orchards need careful canopy management to ensure good fruit quality. Comparatively low levels of light under kiwifruit vines (0.1%) compared to other crops (>1%) suggested that kiwifruit vine canopies are effective at absorbing light (Snelgar et al., 1998) may be due to very large leaves with average area 130 cm^2, (Snelgar and Hopkirk, 1988). Therefore, the combination of high LAI and low transmission resulted in the fruit on kiwifruit vines being more shaded than the fruit of other horticultural crops such as apples (Snelgar et al., 1998).

1.7.3.2 *Orchard manipulations to reduce vegetative vigour*

The major problem in modern kiwifruit orchard management is maintaining the most cost-effective balance between vegetative and reproductive growth. A number of strategies may be used to control vine vigour. Use of chemical growth regulators never received public acceptance due to side effects and residues (Labonte, 1989; Igbedioh, 1991). In apples alternative techniques to control the vegetative growth are restriction of effective root volume and root pruning (Ferree et al., 1991; Atkinson et al., 1996; Atkinson et al., 2000), manipulation of plant densities (Ernani et al., 2008; Yuri et al., 2010), competition by cover crops, and regulation of water availability (Dry and Loveys, 1998; Palmer, 1999). As partial drying of the root system resulted in a significant reduction in shoot growth in grape vines (Dry and Loveys, 1998) and, as the shoot elongation rate of young grape vine was regulated by controlling the size of the root system by root pruning (Buttrose and Mullins, 1968), signals from root may be important in controlling vegetative growth. Therefore, further in this section various methods to manipulate kiwifruit orchards to reduce vegetative vigour and to increase yield of quality fruit will be discussed.

1.7.3.2.1 High density planting

In many fruit crops, introduction of high density planting (HDP) on dwarfing rootstocks achieved successful economic results. The economic output in terms of yield and quality and the inputs of capital, labour and land can all be expressed in cash terms (Jackson, 1988). Increasing yield but decreasing quality is not acceptable if it cannot repay the grower. A well illuminated canopy maintains proper exposure of fruits to light and hence fruit quality. For example, using dwarfing apple rootstocks in HDP increased cumulative yield, especially in the early life of the orchard (Preston, 1959; Tustin et al., 2001). HDP greatly increased the light interception in the early life of the orchard (Robinson, 1992) and reduced the time taken for the scion canopy to fill the allotted space. Low vigour and the compact nature of scions on dwarfing rootstocks in HDP can decrease the labour cost in fruit picking and pruning (Palmer, 1999; Tustin and Palmer, 2008). The major risk in HDP is the loss of fruit quality due to over shading if growth is not controlled (Lang, 2005).

Fruit crops such as kiwifruit, where there are no dwarfing rootstocks, do not adapt well to high density planting. It seems unlikely that canopy development in kiwifruit can be controlled in the near future (Massai et al., 1997). In kiwifruit it was found that the vines planted at greater spacing had unsatisfactory number of canes and subsequently the yield per hectare was low (Testolin, 1990). High density planting of kiwifruit has been attempted in many places, following specific training techniques. For example, kiwifruit in Italy are generally trained to a T-bar or to a pergola (Costa and Testolin, 1990) with planting density ranging from 500 to 740 vines/ha and 400-625 vines/ha respectively. In order to increase the orchard production and to enhance yield, much higher densities of planting were allowed following other training systems such as Geneva double curtain (GDC), free spindle and “Tatura” (V-trellis). Experimental comparisons for kiwifruit showed that training systems such as T-bar and GDC (740 vines/ha and 987 vines/ha respectively) with canopies trained on a horizontal plane at a height of 1.8 m performed generally better (Costa and Biasi, 1991) than either the free spindle (1333vines/ha) or V-trellis (1782vines/ha) with canopies trained to a vertical plane up to 4.0 m in height. In the latter systems, both fruit quality and yields were negatively affected and the number of renewable shoots for next year’s crop was also

reduced. Systems featuring vertical cane-orientation gave poor results (Sale and Lyford, 1990). The T-bar with downward oriented canopy keeps the canes spaced further apart from one another, increased the light penetration, increased the yield and quality of fruit and increased the number of renewable canes (Snelgar and Thorp, 1988; Costa, 1999).

1.7.3.2.2 Pruning

Pruning is removal of extra vegetative growth to improve light interception and air penetration through the canopy. Each year at the end of autumn, winter pruning, and during the growing season summer pruning are carried out. Summer pruning is done until a few days before harvesting (Salinero et al., 2009). Physiological basis of pruning is: temporary loss of apical dominance, accelerating axillary bud activity; and increase in cytokinins presumably by increased export from roots (Saure, 1987). Pruning removes the sites of auxin production and releases the cytokinins activity. Pruning interferes with endogenous hormones of trees and the effect is subjected to reversion because of the inherent capacity of the tree to restore the previous situation by their endogenous hormone system (Saure, 1992). Therefore, various ways of pruning have been developed to inhibit further active growth.

Vines are pruned in various ways according to local and traditional practices. In winter pruning, cane replacement has been practised to remove spent fruiting wood (Miller et al., 2001) as new fruiting wood is produced on 1-year-old canes developed during the previous growing season. In ‘leader pruning’ all vigorous vegetative shoots close to the central leader were snapped from the parent structure, rather than cut to prevent re-growth from basal buds throughout the growing season at regular intervals (14-21 days). This led to a more open canopy and increased fruit yield and soluble solids concentration in kiwifruit orchards (Miller et al., 2001; Gerasopoulos and Drogoudi, 2005). As fruits in the leader zone were exposed to higher light levels, fruit quality was increased (Miller et al., 2001). In kiwifruit it was noted that both leaves and fruit need exposure to light for high quality fruit (Biasi et al., 1993).

The purpose of pruning is to maximise the dry matter in the fruit. Simply cutting vegetative shoots will produce several new vegetative buds in the adjacent leaf axels, further compounding the problem (Torr, 2009). Consequently, this can be avoided by 'Zero leaf pruning' in which the fruiting shoot is cut back to the leaf next to the last fruit let (Snelgar and Thorp, 1988; Buwalda et al., 1991; Cooper and Marshall, 1991; Smith et al., 1992a; Torr, 2009). This is used extensively by 'Hort16A' growers as an alternative for summer growth control. Gel pruning is when the vigorous shoot is cut back to seven or nine leaves past the last fruit and the cut end is treated with growth inhibiting gel with Naphthalene acetic acid (Ward et al., 1999; Ward et al., 2004). This 'Tipit' gel pruner was developed in the late 1980s and early 1990s by plant and Food Research to control summer growth on kiwifruit vines. The other way of controlling vegetative growth of 'Hort16A' is by 'Crush tipping' which could be as effective as application of NAA pruning gel. In this method squeezing each tip gently is critical so that they do not break or die quickly. The squeezed tip needs to be living, allowing PAT of IAA promoting apical dominance (Torr, 2009). Therefore, in kiwifruit vine management, pruning is one of the most important aspects. It plays a major role in obtaining consistently good yield of fruit each season. Therefore correct and on time winter and summer pruning practices are important (Assar et al., 2009).

Root pruning is another way of reducing shoot growth in fruit trees. Recent work with root pruning in mature 'Hayward' and 'Hort16A' vines demonstrated the potential to adjust the partitioning of carbohydrate resources between roots and fruit and increased fruit size, dry matter content and advanced fruit maturity (Patterson et al., 2009). Shoot growth rate decreased with increasing root pruning on 'Golden Delicious' apple trees grown in large containers (Geisler and Ferree, 1984), though it is a short term response compared with field conditions, single pruning resulted in season-long growth control, which lasted for the entire growing season.

1.7.3.2.3 Girdling

Girdling is a procedure by which a ring of bark is removed from the trunk or branch of a tree. This is basically a disruption in the phloem transport between canopy and roots, in

an attempt to manipulate the distribution of photosynthates, mineral nutrients and plant bio- regulators (Goren et al., 2003).

1.7.3.2.3.1 Girdling in kiwifruit

One of the methods by which a kiwifruit grower can produce high dry matter is girdling, which can be done by trunk girdling (TG) or cane girdling (CG). Trunk girdling has been practised worldwide for centuries to increase fruit size, yield and flavour of grapes, stone fruits, pip fruits and persimmon. Kiwifruit research showed that trunk girdling increased dry matter, fruit size but in the long term resulted in poor vine health and reduced productivity. In trunk girdling, leaf produced photosynthates are trapped in the canopy and supports fruit development and increases dry matter while roots are completely deprived of food material till the girdle is healed. It was found that carbohydrate reserves in fine roots were reduced when trunk girdling was followed for two consecutive seasons. The consequences of low root reserves are reduced shoot growth in spring and decreased canopy photosynthesis. TC can be used to increase dry matter of Green and Gold kiwifruit, if growers are cautious not to do it every season as it can result in root starvation, reduced canopy development in spring and excessive flower loads (Currie. et al., 2005).

Cane girdling has been developed for the last six years to influence the fruit size and dry matter. Fruit size and dry matter content of the fruit were increased by summer cane girdling, but not as much as TG. In CG roots are not fully isolated, and some carbohydrate can travel to roots while girdles are healing. This may be the reason for lower fruit responses to CG compared with TG. The cane girdling may be safe for vines, but is also less effective. For the growers who are looking for a maximum lift in the fruit size and dry matter, TG would probably the best option as this also can increase return bloom (Currie et al., 2005). However, if there are concerns over vine vigour, root health or trunk diseases, CG may be a more suitable option.

Trunk girdling, one centimetre wide, performed four times every seven days in the middle and later half of July and repeated yearly showed a significant increase on the

size of the fruit (Moriguchi, 2002). He also reported that the earlier the girdling treatment, the larger the fruit. The yearly treatment exhibited significant effect on the size of the fruit compared to the single year treatment and also the early elongation of the current shoot in the year after girdling was reduced (Moriguchi et al., 2002).

1.7.3.2.3.2 Physiology of girdling

Girdling induced interruption of phloem transport affects not only assimilates but also plant hormones that play a regulatory role in growth and development. It has been suggested that girdling leads to reduced vegetative growth of the shoot system (Dann et al., 1984; Cutting and Lyne, 1993; Theron and Steyn, 2007) by altering endogenous hormones. Auxins synthesised in young leaves, fruits and shoot tips are transported basipetally through the living tissue associated with phloem (Goldsmith et al., 1974; Morris and Thomas, 1978) to the roots. An increase in the IAA concentration in bark immediately above a girdle and a permanent decrease in IAA concentration below the girdle in peach (Dann et al., 1984) was observed. Dann et al., (1984) proposed that reduced auxin signal to roots leads to a reduction in a coordinating signal from the root system. As girdling reduced the auxin supply to the roots there was coordinating signal from root in reducing levels of cytokinin zeatin-riboside, and gibberellin GA_1 and GA_3 in the xylem sap of peach shoots above the girdle and a corresponding reduction in shoot growth (Cutting and Lyne, 1993). Similar decrease in cytokinin was found in grape shoots (Skene, 1972).

However, xylem sap cytokinin levels were actually enhanced on girdled kiwifruit canes, (Currie, 1997), which suggested that girdling of kiwifruit shoots was unlikely to result in a deficiency of cytokinins. He also reported that the application of a girdle to a kiwifruit cutting showed similar response of decapitation resulting in elevated levels of cytokinin in root xylem exudates. This provided strong evidence that girdling of kiwifruit eliminates the auxin signal from shoots past the position of a girdle.

There are a few ways to manipulate kiwifruit orchard system by various training techniques such as HDP, pruning: zero leaf pruning, gel pruning and crush tipping to

enhance yield of quality fruit, yet there is need for proper vigour controlling rootstocks that not only confer vigour reduction but also confer resistance against several pests and diseases such as the recent PSA (*Pseudomonas syringae Actinidae*) disease which attacked kiwifruit orchards and became a threat to the New Zealand economy. Peach rootstock 'GF677' conferred resistance to *phytophthora syringae* (Thomidis, 2007). It has been reported that the bacterial canker disease of kiwifruit, *Pseudomonas syringae* pv. *Actinidae* was originally transmitted from wild *Actinidia* plants in Japan (Ushiyama et al., 1992), and work has been conducted on rootstocks resistant to bacterial cankers and PSA disease in Korea (Shim and Ha, 1999). Because of relative amenability to *in vitro* multiplication such as protoplast culture, somatic hybridization, rapid progress may be possible in developing resistance or tolerance in kiwifruit. Also transegenic plants of A. deliciosa have been recovered with some foreign genes of interest: rolABC, rolB, whole T-DNA, some of which are under field evaluation (Gutiérrez-Pesce and Rugini, 2009).

In summary it can be seen that, there is a need to understand the hormonal influence on the vigorous vegetative growth of kiwifruit vines and how it may be applied to improved management techniques. Even though this research area is clearly important there has been surprisingly little research done on physiology in relation to vigour in kiwifruit, except for a few *in vitro* studies, which may give an insight into the role of hormones in vine growth.

1.7.4 Role of hormones in kiwifruit vine vigour

1.7.4.1 *In vitro studies an insight into the role of hormones in vine growth*

Addition of benzyl adenine (BA) to the culture medium became essential as explants did not possess roots that are the main sites of cytokinin (Ck) biosynthesis (Arigita et al., 2005). When Akbas et al., (2007) used shoot meristem from young seedlings of matured seeds of kiwifruit as starting material; the results showed that cytokinins (BA) in the medium were found to be essential for shoot proliferation of kiwifruit. Synthetic cytokinins added to the cell suspensions derived from leaf callus of *A. chinensis* induced

adventitious buds (Suezawa et al., 1988). When leaves of axillary bud of a shoot were culture in darkness for 1.5 months on the medium with only zeatin as the sole growth regulator developed embryos. Axillary shoot formation was influenced by the type and concentration of cytokinin used. Furthermore, Argita et al., (2005) found that the continuous presence of cytokinins did not allow further development of axillary buds of kiwifruit explants and elimination of cytokinins from the medium allowed GA_3 present in the culture medium to promote cell division and elongation in the sub apical region of the newly formed shoots (Moncalean et al., 2003). Although the most critical growth hormone to promote axillary shoot proliferation is cytokinins, low amounts of GA_3 may be useful to stimulate shoot extension growth (Krikorian et al., 1995; Moncalean et al., 2003).

Gibberellic acid uptake was studied by Moncalean et al., (2003) using a kiwifruit shoot containing the apical meristem and three axillary buds. It was found that GA_3 absorption by kiwifruit explants occurred gradually throughout the 35-day culture period, with 17% of GA_3 absorption during the first hour and then after the first 8 h of culture. At the end of the culture period, kiwifruit explants consumed 33% of the GA_3 initially contained in the medium. Other studies using the same species and culture medium showed that 20% of the BA initially added to the medium was quickly taken up during the first 30 min of culture, thus indicating that kiwifruit explants take up similar amounts of GA_3 and BA during the initial period of culture (Moncaleán et al., 1999). From this observation, it can be assumed that in kiwifruit vines cytokinins are involved in axillary bud activation and gibberellins are involved for the extension growth of laterals. However, surprisingly no such studies have been carried out on kiwifruit. The *in vitro* studies on the involvement of hormones in the axillary bud activation and elongation can be supported by other study, on other crops. For example, in apples that cytokinin stimulated the outgrowth of rootless apple shoots in vitro (Jones, 1973).

1.7.4.2 *Use of growth retardants to reduce kiwifruit vigour*

Growth retardants that inhibit GA biosynthesis such as paclobutrazol (3000 mg L^{-1}) and succinic acid dimethyl hydrazide (SADH), sprayed on 'Hayward' vines 10 days after

bud burst had little effect on decreasing shoot growth (Biasi et al., 1987). Also, there was no effect when 3000 mg L^{-1} CCC (2-chloroethyl-trimethylammoniumchloride) was sprayed (Biasi et al., 1987). However, these growth retardants decreased flower abscission. Therefore, Biasi et al., (1987) concluded that it is very difficult to control kiwifruit vigour with growth retardants. Small reductions in vegetative growth rate imposed using SADH and paclobutrazol (PBZ) during the first phase of the shoot growth tended to increase vine yield. However, fruit shape was affected, which reduced marketability. On the other hand, the application of PBZ as a soil drench reduced vegetative shoot growth, increased fruit length and decreased the weight of winter pruning.

In an attempt to reduce the cost of maintaining 'Hort16A', the antigibberellin REGALIS® (calcium 3, 5-dioxo-4-propionyl-cyclohexane-carboxylate or prohexadione-Calcium) was applied three times to expanding axillary shoots at 14 day intervals at a concentration of 2.5 g L^{-1} of water with the addition of 2 ml of REGULAID® sticker spreader. This treatment decreased shoot length by 44% compared with untreated control vines. The most remarkable effect of prohexadione was on the length of the internodes of the most distal nodes. In addition, there was no prominent effect on fruit growth rate compared with the control vines (Blattmann. P, Personal communication).

According to a report from Ministry of Agriculture and Forestry's 2010, concerning the kiwifruit monitoring programme for the Bay of Plenty kiwifruit, 66% of the net cash income was spent for orchard work expenses, out which 22% was for pruning costs (maxa.maf.gov.nz/mafnet/rural-nz/statistics-and…/farm-monitoring/).

Therefore, in summary there are requirements to reduce summer pruning costs without compromising fruit quality and to understand the hormonal physiology regulating vegetative vigour of kiwifruit vines. Ideally one would want to be able to decrease vegetative vigour with a concomitant increase in fruit yield. Reducing vegetative growth is a horticulturally desirable feature as it leads to reduced pruning costs and possibly increased fruit yield.

1.8 Summary, rationale and thesis objectives

Apple and kiwifruit occupied key positions in New Zealand's economy and both are facing similar challenges in the fruit global market. Advances in a single discipline cannot solve all challenges, so Palmer (2007) suggested, the need for well-led multidisciplinary teams to bring together skills in physiology, biochemistry and molecular biology (Wollenweber et al., 2005) in order to increase fruit dry matter partitioning into fruit so as to increase both yield and quality. Apple has a higher harvest index than kiwifruit. Growth of apple trees on a dwarfing rootstock achieves over 70% of the annual dry matter production in the harvested fruit. In the case of kiwifruit, high allocation of carbohydrate to the root system and long periods of vegetative growth reduces the harvest index to 50%. With 90 or 95% light interception in both kiwifruit and apple, if efficiency of energy conversion was similar, the potential yield of kiwifruit might be expected to be increased by 20%. For example, 'Hort16A' grown on pergola in the Bay of Plenty New Zealand, which could intercept 1771 MJ m^{-2} photosynthetically active radiation (PAR), would give an estimated dry matter production of 31 t ha^{-1} if light conversion was similar to apple (Palmer, 2007). If a harvest index of 50% was assumed, this would be equivalent to 15.5 t ha^{-1} fruit yield, which is approximately equivalent to the maximum yield in kiwifruit. Therefore the difference between apples and kiwifruit is largely due to high harvest index, resulting in large part due to the fact that the apple industry has access to good vigour reducing rootstocks. Use of suitable rootstocks or major changes to the growth habit of scion cultivar in kiwifruit may increase its harvest index and fruit dry matter content by exposing the fruits to sunlight in an open canopy. In order to increase the fruit dry matter content, reducing vegetative vigour in kiwifruit is essential. Understanding the hormonal physiology in rootstock dwarfing of apple trees, and identifying major locus (*DWI*) involved in the dwarfing trait of 'M.9' rootstock (Rusholme Pilcher et al., 2008) and other genes important for traits like disease resistance, will greatly improve rootstock breeding and may assist development of rootstocks for kiwifruit vines.

1.8.1 Thesis rationale and major objectives

In apples, the effect of 'M.9' dwarfing rootstock on the scion architectural changes is similar to those that are imposed by the application of NPA on the stem part of the rootstock. Both treatments decreased the mean length and node number of the primary shoot, reduced the formation of SAS on the primary shoot and promoted early termination of the primary and SAS. The changes that are brought about by dwarfing rootstocks and NPA could be reversed by the application of the cytokinin benzylaminopurine (BAP) and gibberellins (GA_{4+7}). Therefore, it is hypothesised that dwarfing of the scion by rootstocks may result from modified transport of growth hormones. Reduction in indole-3-acetic acid (IAA), transported through the stem tissue of a dwarfing rootstock to the root, may reduce export of cytokinins and gibberellins from root to shoot in the xylem. This evidence suggests that dwarfing of the scion may be controlled by shoot-root-shoot signalling of endogenous hormones. Although, an important role for gibberellins in rootstock induced dwarfing of the scion grafted onto a dwarfing rootstock has been questioned by scientists at East Malling (Webster, 2002; East Malling, 2005), there is evidence to suggest that gibberellins may be important in rootstock induced dwarfing of the scions.

A major problem that kiwifruit growers face today is excessive vegetative vigour resulting in a top-heavy canopy that reduces production and quality of fruit. Thus there is a strong need to breed rootstocks that reduce vegetative vigour in kiwifruit vines. Understanding the physiology of how plant hormones control scion vigour will help in the development of improved rootstocks, or the development of alternative vigour controlling strategies.

Therefore, the aim of the present study was to improve the present understanding of hormonal shoot-root-shoot signalling in composite apple trees and to elucidate whether a generalised signalling mechanism exists in other economically important fruit crops such as kiwifruit vines.

Specific objectives were:

- To elucidate the dwarfing effect of rootstock and 'M.9' scion on vegetative growth of composite trees on 'M.9' and 'RG' rootstocks grafted reciprocally and also to quantify what aspects of growth can be reversed by applying gibberellins repeatedly to the scions (chapter 2)
- To study the effect of gibberellins on internode length at the cellular level to determine the extent to which hormonal stimulation of cell division and cell elongation control final length (chapter 3).
- To study the effect of auxin supply and root restriction on vegetative growth of newly propagated kiwifruit vines to understand endogenous hormonal shoot-root-shoot signalling mechanism of kiwifruit vines (chapter 4).
- To study the effect of Naphthalene acetic acid (NAA), BAP, gibberellins on the vegetative growth of potted *Actinidia deliciosa* and *Actinidia chinensis;* NPA and anti-gibberellins on the vegetative growth of potted *Actinidia deliciosa* (Chapter 5), and the effect of gibberellins and anti-gibberellins on *Actinidia deliciosa* in the orchard (Chapter 6).

Chapter 2
An improved understanding of shoot-root-shoot signalling and vigour control by rootstock and scion using reciprocal grafting and gibberellins foliar sprays to apple trees

2.1 Introduction

The physiological mechanisms by which dwarfing apple rootstocks reduce scion vigour are not yet fully understood. Lockard and Schneider (1981) postulated that in the dwarfing mechanism in composite apple trees, that amount of auxin indole-3-acetic acid (IAA) produced in the shoot apical bud moving basipetally to the root system was reduced by the rootstock, which limited root growth and the amount/type of cytokinins moving to the shoot in the xylem. In support of this hypothesis, it was reported that the concentration of IAA was lower in the stem tissue of the dwarfing rootstock 'M.9' compared with the more vigorous rootstock (Soumelidou et al., 1994a; Kamboj et al., 1999b), which might have contributed to less root growth (van Hooijdonk et al., 2011) and altered the amount and type of root-produced cytokinins that were transported to shoot in the xylem sap (Kamboj et al., 1999a; van Hooijdonk et al., 2011). However, restricting basipetal IAA transport from shoot to root using decapitation and/or 1-N-naphthylphthalamic acid (NPA) increased cytokinin concentration in xylem sap of bean plants (Bangerth, 1994) and kiwifruit rooted stem cuttings (Currie, 1997). Similarly, van Hooijdonk et al., (2011) reported that zeatin riboside (ZR) in xylem sap of apple scions increased and this response appeared to coincide with declining rates of IAA diffusion from the apex of 'Royal Gala' primary shoots grafted onto a dwarfing rootstock. Although IAA diffusion from the shoot apex does not represent the amount of IAA transported from shoot to root in the rootstock stem, the decreased IAA transport from the shoot correlated with root synthesised ZR (van Hooijdonk et al., 2011) from February onwards. The scion on 'M.9' rootstock formed fewer sylleptic axillary shoots (SAS) in January and from February onwards there were trends for the mean number of SAS growing points on the primary shoot to increase, which appeared to coincide with increasing ZR in the xylem sap of the scion. However, xylem exudates could not be

extract during January and therefore there were no data on the concentration of cytokinin just before the increased shoot formation from February onwards, to ascertain that there was limited endogenous cytokinin supply for less secondary shoot formation during January (van Hooijdonk et al., 2011).

In addition to stimulating SAS formation (branching), cytokinins were said to be involved in shoot extension growth (Jones, 1973; Lockard and Schneider, 1981), however, Wertheim and Estabrooks, (1994) found that exogenous cytokinins hardly affected the growth extension of primary shoots. Thus, cytokinins primarily appear to activate branching by stimulating axillary buds (Williams and Stahly, 1968; van Hooijdonk et al., 2010). Therefore, it seems unlikely that the reduction in the mean length of primary shoot and node number of 'RG' scion on 'M.9' rootstock can be attributed to cytokinins deficiency and there must be an involvement of other hormones, such as reduction in gibberellins in rootstock- induced scion dwarfing (van Hooijdonk et al., 2010).

Studies conducted at Horticultural Research Institute (HRI) on several tree fruit species suggested that shortening of internode length is usually only one of the contributing reasons for the smaller trees produced on dwarfing rootstocks (Webster, 2004), while it was found that there was no rootstock effect on the mean internode length of 'RG' scions grafted on dwarfing rootstocks (van Hooijdonk, 2009). The severe shortening of the internode length and the close overlapping of leaves associated with the use of growth retardants such as gibberellin biosynthesis inhibitors were not characteristics of trees grafted on most dwarfing rootstocks (Webster, 2004). In addition, he also mentioned that gibberellins are synthesised in both root and shoot tips but their effects, if any, on the natural dwarfing of fruit trees is still unclear. The involvement of rootstock in the conversion of active to inactive gibberellins may be possible, but there is no evidence that shoot growth of the scion depends upon gibberellin formed in the roots (Webster, 2004). Moreover, studies at East Malling also reported that gibberellin differences were not detected in the xylem sap of scions grown on different size controlling rootstocks, even though the scion on dwarfing rootstock terminated active

shoot extension early and rates of extension growth were also less (East Malling, 2005). Although, an important role for gibberellins in rootstock-induced dwarfing of the scion grafted onto a dwarfing rootstock has been questioned by scientists at East Malling, Webster (2004), and others such as Kende and Sitton (1967) and Carr et al. (1964), it was considered important to investigate the possible role of gibberellins as many researchers have implicated them in rootstock-induced dwarfing mechanism for example, Robitaille (1971). In their experiments, injections of 10 mg L^{-1} of gibberellic acid (GA_3) increased the terminal shoot growth of 'Red Princes Delicious' on dwarfing rootstock 'M.9'. The response increased as the vigour of the rootstock decreased. It was suggested that the deferential dwarfing effect of fruit tree rootstocks was explained on the basis of GA transported from the roots to the shoots (Carr et al., 1964; Jones and Lacey, 1968) as more vigorous trees contained greater amounts of gibberellins in the transpiration stream. In addition, the concentrations of gibberellins were found to be less in the roots of dwarfing rootstock (Ibrahim and Dana, 1971; Yadava and Lockard, 1977) compared with vigorous rootstocks. Motosugi et al., (1996) reported gibberellins were present in the xylem sap of apple trees indicating gibberellins are synthesised in the roots. In particular van Hooijdonk et al. (2010) found that the 'M.9' rootstock, or the application of 1-N-naphthylphthalamic acid (NPA) an auxin transport inhibitor onto the stem of different size-controlling rootstocks, reduced the node number and the length of 'Royal Gala' primary shoots, while GA_{4+7} applications onto the scion reversed the effect of NPA or 'M.9'. In their work, it was assumed that the dwarfing rootstock or NPA limited the amount of IAA transported to roots, thereby decreasing root-produced gibberellins that limited node neoformation of the primary shoot. Moreover, van Hooijdonk, (2011), reported significantly lower levels of GA_{19} in xylem sap of scions on 'M.9' compared with that of scions on 'Royal Gala' rootstock ['RG' (control)]. In Chapter 1, (see section 1.3.4.2) it was explained how shoot synthesised GAs might be translocated to roots, where they are converted to different gibberellins and then re-circulated to the stem tip (Crozier and Reid, 1971). From all available sources of information regarding gibberellins, it can be hypothesised that their role in reducing the size of scions depends on the synthesis, transport and metabolism of gibberellins, in root as well as in the shoot.

The activation and inactivation of gibberellin biosynthesis pathways during the life cycle of a plant are different in different species and even in different organs of the same species (Graebe, 1987). In GA biosynthesis, *GA_{20} and GA_3 oxidase* enzymes are required for activation and *GA_2 oxidase* enzyme for inactivation of gibberellins (Figure 1.3 and 1.6). *GA_{20} oxidase* activity is the major determinant for the production of bio-active gibberellins in plants (Xu et al., 1995; Fagoaga et al., 2007). Among many gibberellins so far identified from plants, only a few of them such as GA_1, GA_3, GA_4 and GA_7 are thought to function as bioactive forms, whereas many other non bioactive GAs exist as precursors for the bioactive forms or exist as deactivated metabolites (Yamaguchi, 2008). The bioactive gibberellins play a major role in promoting the shift from meristem identity to organ differentiation. The ability of GA_3 to normalise dwarf phenotypes of certain mutants provided the first indication that GAs might be endogenous growth regulators in higher plants (Hedden, 2006) and this was supported by the identification of GAs in seeds of running bean almost 50 years ago. The GA deficiency not only decreased height but also increased the over expression of *GA_2 oxidase* the GA-catabolising enzyme, which decreased bioactive gibberellin levels, height and internode length in *Arabidopsis* (Schomburg et al., 2003), rice (Sakamoto et al., 2003). This emphasises the importance of GAs in regulating height and yet work at East Malling indicated that involvement of GAs in rootstock dwarfing is still doubtful (East Malling, 2005; Webster, 2004).

Physiological studies revealed that compared to standard 'McIntosh', shoot tips of 'McIntosh wijcik' mutant trees are low in polar gibberellin levels (Looney and Lane, 1983) and therefore, it is clear that gibberellins do influence elongation growth in plants and one must suspect a role for gibberellins with regard to the compact growth habit of apple. In addition transgenic plants of 'Gravenstein' and 'McIntosh' apple cultivars, with integration of the *gai* gene, a DELLA protein, which repress growth (Richards et al., 2001), resulted in a significantly reduced stem length, internode length and node number (Zhu et al., 2008). Likewise, 'M.9' apple shoots may be deficient in GA as indicated by its short shoots with short internodes.

In an apple scion variety 'Greensleeves' grown on its own roots, the gene encoding *GA_{20} oxidase* was suppressed, which resulted in a significant reduction in height of the primary shoot. When such a scion was grafted onto normal invigorating rootstocks 'MM.106' and 'M.25', the scion remained dwarfed (Bulley et al., 2005). There was an increase in the concentration of GA_{19} in the shoot apex and lower concentrations of GA_{20} and GA_1. Hence, GA_{19} from root was not activated to GA_{20} due to the absence of *GA_{20} oxidase*. This may suggest the inability of the scion to convert the GA precursor (GA_{19}) from root into bioactive gibberellins (GA_1) in the shoot due to the lack of *GA_{20} oxidase*. This condition was reversed by GA_3 application.

Therefore, the metabolism of gibberellins depends not only on the rootstock, but also on the transportation and conversion of root-produced GA precursors into bio-active gibberellins by the shoot system of the scion. Thus, there may be two types of dwarfing caused by gibberellins in composite apple trees: one due to rootstock effect because of limited synthesis of root-produced gibberellin and subsequent transport to the shoot, and the other type due to scion effect because of inability of the shoot to metabolise root-produced GA precursors to bioactive GAs. Thus two hypotheses can be stated:

Hypothesis-1: Low scion vigour caused by a dwarfing rootstock may partly result from limited synthesis of root-produced gibberellins and their subsequent transport to the shoot, thereby limiting the formation of bioactive gibberellins at the shoot (rootstock effect).

Hypothesis-2: Vigour and size of a composite tree may be influenced by the shoot genotype and its inherent capacity to metabolise root-produced gibberellins to bioactive gibberellins (scion effect).

Therefore the present experiment was conducted to test the proposed hypotheses and elucidate the role of gibberellins in reduced vigour of the scion on a dwarfing rootstock.

2.2 Aim: To test hypothesis-1 and hypothesis-2

(a) ***Objectives for hypothesis-1 (Relating to 'M.9' rootstock dwarfing effect on the 'Royal Gala' ('RG') scion***

- To elucidate the architectural changes that a dwarfing rootstock 'M.9' imposes on 'RG' scions and the timing of these changes following tree grafting.
- To quantify what aspects of growth could be reversed by applying gibberellin (GA_3+GA_{4+7}) foliar sprays repeatedly to the scion.

(b) Objectives for hypothesis-2 (Relating to the shoot growth of 'M.9' scion)

- To determine whether a vigorous rootstock, such as 'Royal Gala' can stimulate the growth of an 'M.9' scion.
- To stimulate internode elongation and node neoformation of 'M.9' shoots using exogenous gibberellins (GA_3+GA_{4+7}) to ascertain whether the compact nature of the 'M.9' shoot is a result of its inability to synthesise bioactive gibberellins

Therefore, the over-all aim was to observe the role of rootstock on the rootstock-induced reduction in the size of scion grafted on dwarfing rootstock (rootstock effect: Hypothesis-1) and, also to observe whether the vigour and size of the tree was also influenced by scion genotype (scion effect: Hypothesis-2) in terms of gibberellins.

2.3 Materials and methods

2.3.1 Experimental plant material

Reciprocal grafting was conducted during the 2007-2008 growing season at the Plant Growth Unit, Massey University, Palmerston North (lat. 40.2^0S, long 175.4^0E), New Zealand. For grafting, rooted stools (8-10 mm in diameter) of 'Malling Nine' ('M.9') and ''Royal Gala' ('RG') were taken from stool beds set up at the Plant Growth Unit, Palmerston North. The dwarfing rootstock 'M.9' was grafted with a 'RG' scion and reciprocal grafts were carried out in which 'M.9' was the 'scion' and 'RG' was the 'rootstock'. In addition, 'M.9' was grafted on 'M.9' and 'RG' was grafted on 'RG' as

controls (Figure 2.1). The root system size was similar for all rooted stools selected for grafting. Each scion was cleft grafted at a height of 350 mm onto rooted stools of 'M.9' and 'RG' rootstocks. Graft unions were taped and the tips of scions were sealed with pruning paste. Grafting was completed in August, 2007 and the resulting composite trees were planted into black 20 L polythene bags. Planting height was standardised for each rootstock to leave 150 mm of rootstock stem above the surface of the growing medium.

2.3.2 Maintenance of plant material

The growing medium used was a bark/pumice mix (70:30) containing 50 g super-phosphate, 50 g lime, 150 g dolomite and 300 g controlled-released fertiliser containing trace elements (14 month slow release, Osmocote, Scotts, USA) per 100 L. The grafted composite trees were established in a tunnel house and the scions were debudded to a single shoot during October. In late October, trees were moved to a standing out area to harden off and the plants remained there for the duration of the experiment. Supplementary liquid fertiliser (Peters Professional, Scotts, USA) with trace elements was supplied (2g/tree) at 14-day intervals. A 19 mm polytube line was placed under the tree row to provide irrigation. A single pressure compensator 4 L hr^{-1} dripper fitted into the 19 mm polytube was placed in each polythene growing bag with an integrated system providing the supplementary liquid fertiliser. Irrigation was scheduled daily for 1 hr in the morning and 1 hr in the evening using an automated irrigation controller (Hunter, Smart Valve Controller, USA).

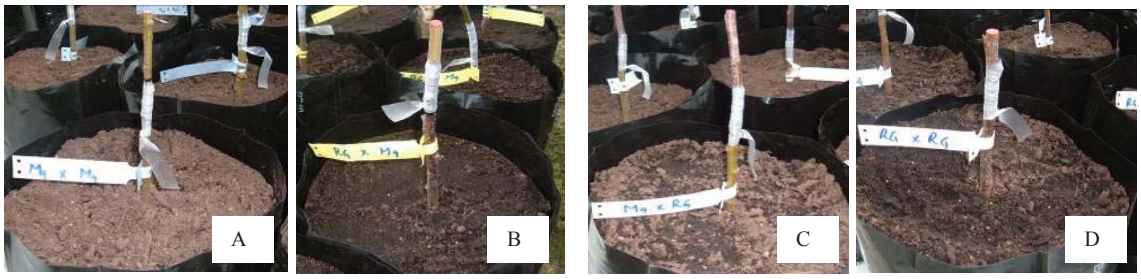

Figure 2.1. Reciprocal grafts of dwarfing rootstock 'M.9' and vigorous rootstock 'Royal Gala' ('RG') with 'M.9' and 'RG' scions. The combinations are always written with rootstock/scion: M9M9 (A), RGM9 (B), M9RG (C), and RGRG (D).

2.3.3 Foliar sprays of the gibberellins GA_3 and GA_{4+7}

After reciprocal grafting, the composite trees were divided into three groups of 24 plants with six plants for each rootstock and scion combination. A mixture of GA_4+GA_7 (Novagib®, Fine Agrochemicals Ltd., Worcester, UK) and GA_3 (Sigma, product number 48880 5G-F) as ammonium salt were sprayed together. Control plants were sprayed with distilled water. Gibberellins were sprayed onto the other two groups of trees at two different timings i.e., starting in November or starting in February; both GA foliar spray treatments were applied every two weeks until the end of the growing season. Gibberellin foliar sprays beginning in November were designated as GA (Nov) and those beginning in February were designated as GA (Feb). Shoot tips and the subtending meter of shoot (stem and leaves) were sprayed with 200 mg L^{-1} of each gibberellin (GA_3+GA_{4+7}) to the point of runoff using a 15 L knapsack sprayer. The GA foliar spray was carried out at 7:00 pm or later after sunset to avoid photo-toxic reaction. Adjacent trees were covered during spraying to prevent contamination from spray drift.

2.3.4 Measurements of scion growth

Scions were debudded to a single shoot during October, 2007, which was left to develop into the primary shoot. The length and node number of the primary shoot and sylleptic axillary shoot (SAS) were measured fortnightly throughout the growing season and the final measurement of scion growth was conducted after growth cessation in the 1st week

of June, 2008. The SAS produced from axillary buds on the primary shoot were further distinguished as either a spur (minimal internode extension <25 mm) or a sylleptic shoot (SS) (≥25 mm with internode extension). In this thesis, both spurs and sylleptic shoots are collectively called SAS. A measuring tape was used to determine shoot length, with measurements starting from the base of the shoot and ending at the first unfurled leaf at the shoot apex. Measurements were conducted for each scion until shoot growth had terminated in a dormant apical bud. Throughout the growing season, shoots that grew out from the rootstock stem were removed as required. Mean internode length was calculated by dividing the length of the shoot by its number of nodes. The growing period during the spring and early summer (16th October, 2007 to 12th January, 2008) is termed "early growth", whilst growth occurring from Jan 12th 2008 to June 5th 2008 (from midsummer till termination) is termed "late growth". During the spring of the second growing season, root systems of six plants with and without gibberellin treatment for each rootstock and scion were taken and the roots were categorised into hard, medium and soft roots based on their structure. The brown roots were classified as hard roots, white transparent roots as soft and roots in between were called medium roots. Each root type was oven dried separately at 80°C to a constant mass and obtained its dry weight on a four-decimal place balance (METTLER AE200, Switzerland). Flower cluster count was taken during the early spring of the second growing season. Total flower cluster number on each scion was expressed as a proportion to the total bud number of that scion. The total bud per scion is defined as the total number of nodes and terminal bud(s) on the primary and sylleptic shoots plus one terminal bud per spur (each spur is considered as a single bud). The diameters of primary shoot bases were measured using digital calipers and shoot cross-sectional area (SCA) was calculated as $\pi D^2/4$.

2.3.5 Statistical analysis

There were three GA treatments (control, and gibberellins applied commencing in either November or February), two scions ('M.9' and 'RG') and two rootstocks ('M.9' and

'RG'). Each treatment was replicated six times. The experiment was a completely randomised design with a factorial arrangement of treatments (3×2×2). Data were analysed using GLM procedure of SAS. Mean separations for the main effects were made using the Duncan's Multiple Range test at $P<0.05$. When there was a significant main effect much greater than that of any significant interactions involving that main effect, both main effect and interactions were reported for interpretation. On the contrary, only interaction data were reported for interpretation when main effect was similar or smaller than an interaction involving that main effect. For significant interactions, pre-planned comparisons of some treatment means were made using least square means (lsmeans test). For all treatment effects the ANOVA F-values are significant at $P\leq0.05$, 0.01, 0.001 unless otherwise stated.

2.4 Results

In this Chapter, firstly, the results section will deal with the length, node number and internode length of primary shoot during the early growth to elucidate the timing at which the dwarfing rootstock initiated the first changes in the scion architecture and, secondly it deals with the length, node number and internode length of later growth and termination of growth of primary shoot. Next, the time and termination of SAS, the final number and length of SAS were reported. Finally the overview of growth over the entire growing season was summarised to elucidate hypothesis 1 and 2.

2.4.1 Treatment effects on primary shoot growth

The primary shoot consisted of a single growth unit; therefore the primary shoot was formed as result of a season-long growth flush by the shoot apical meristem (SAM). As one of the objectives of this experiment was to elucidate the timing when the dwarfing of the scion occurred on a dwarfing rootstock, the crucial period of time when the effect of dwarfing rootstock actually started was carefully observed. The following section deals with the growth pattern of the scion during the spring and early summer (early

growth) for the reciprocal graft treatments between 'M9' and 'RG' with or without gibberellins applied to the scion.

2.4.1.1 *Treatment effects on the 'early growth' of the primary shoot*

2.4.1.1.1 Shoot length, node number and internode length

The main effects of rootstock and scion modified the mean length (*P=0.0001*), node number (*P=0.04* and *0.0002* respectively) and internode length (*P=0.0005* and *0.0001* respectively) of the primary shoot during the early period of growth. The 'M.9' rootstock produced a longer primary shoot with more nodes compared with 'RG' rootstock during early growth (Table 2.1). The 'RG' scion produced a longer primary shoot with more nodes compared with 'M.9' scion.

Table 2.1. Main effects of scion and rootstock on the mean length, node number and internode length of primary shoots of 'M.9' and 'RG' scions on 'M.9' and 'RG' rootstocks during the spring and early summer (from October, 2007 to January 2nd week) growing period following grafting (August, 2007). Means for scion main effect are averaged over rootstock and GA treatments; means for rootstock are averaged over scion and GA treatments.

Main effects		Primary shoot		
		Length (mm)	Node umber	Mean internode length (mm)
Scion	'M.9'	722.6 b	36.9 b	19.6 b
Scion	'RG'	870.4 a	39.3 a	22.1 a
Rootstock	'M.9'	852.5 a	38.7 a	21.9 a
Rootstock	'RG'	740.5 b	37.5 b	19.8 b

Within each main effect only, means within a column sharing the same letter are not significantly different at *P=0.05* using the Duncan's Multiple Range test.

During early growth, the rootstock scion interactions were significant at *P=0.08* for primary shoot length (Figure 2.2 A). The 'RG' scions on 'M.9' rootstocks ('M9RG') grew faster than 'RG' rootstock control ('RGRG') during the early period of growth and were longer for the first 55 days (10th Dec, 2007), which was statistically significant (Figure 2.2 A). The 'RG' primary shoot on 'M.9' remained longer than 'RG' rootstock control for the next 33 days (*P=0.04* at Day 88 or 12th Jan, 2008). Therefore, until 2nd

week of January i.e., 88 days after bud break, the 'M.9' rootstock stimulated a longer RG primary shoot than the RG rootstock (Figure 2.2 A).

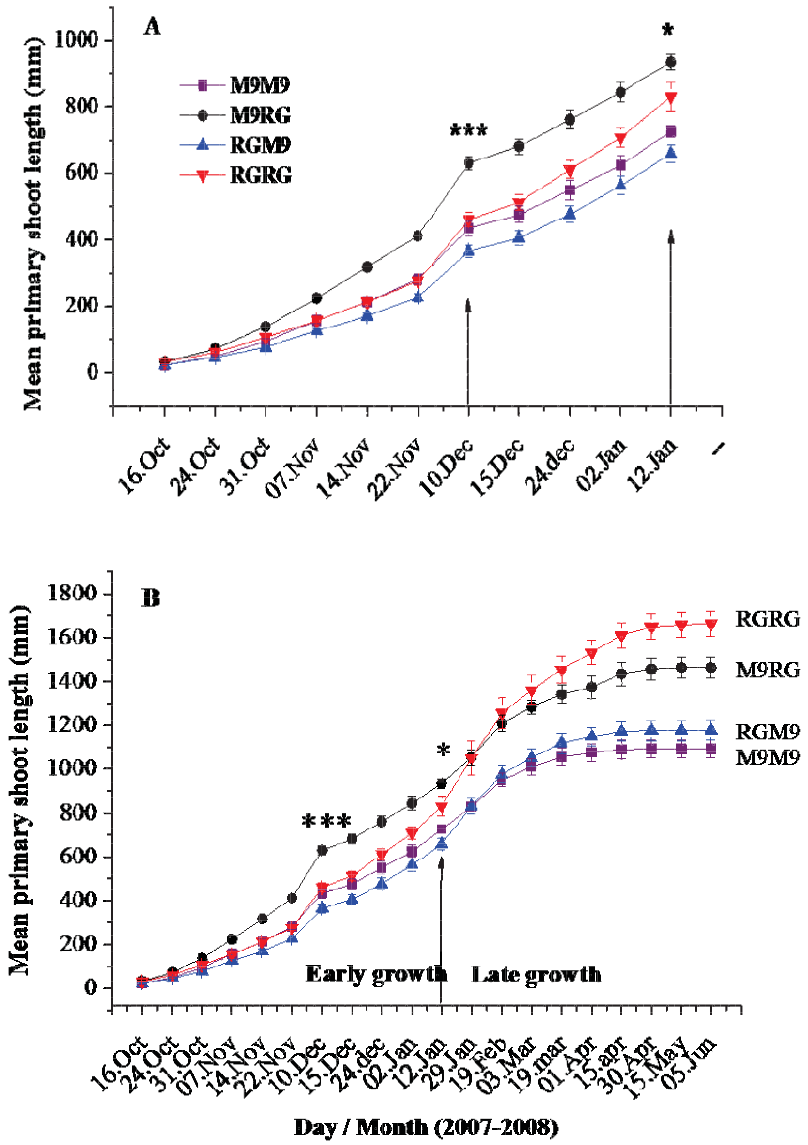

Figure 2.2. Rootstock × scion interactions on the mean length of primary shoots of 'RG' and 'M.9' scions during the spring to early summer growing period: A (early growth: from 16th Oct-12th Jan) and B; during the full growth period (early + late) following grafting onto 'M.9' and 'RG' rootstocks in August, 2007. *, * represent the significance level at P≤0.001, 0.04 on 10th December and 12th January respectively. The arrows on 'A' are pointing to the dates of significant differences Arrow on 'B' separates the early growth from late growth. Means are averaged over GA treatments. Bars represent the standard error.**

The 'M.9' primary shoots on 'M.9' rootstocks were longer (*P=0.06*) than 'M.9' primary shoots on 'RG' rootstocks until the 2nd week of January (Figure 2.2 A). Interestingly, 'RG' scion on 'RG' rootstock ['RG' rootstock (control)] and 'M.9' scion on 'M.9' rootstock ['M.9'rootstock (control)] had similar primary shoot lengths until 2nd week of December. From mid-December, there was a gradual increase in the primary shoot length of all treatments however, the growth rate of 'RGRG' was greater and so began catching up to 'M9RG', which became highly significantly longer compared with that of 'M9M9' during the late growth and by the end of the growing season (Figure 2.2 B and Table 2.4).

During early growth, there were also significant rootstock GA interactions *P=0.01* for primary shoot length (Figure 2.3). Gibberellin decreased primary shoot length for scions on RG rootstock only. The primary shoots of GA-treated scions on 'RG' rootstocks were shorter on the 12th of January (695.42 mm) compared with the untreated scions (785.62 mm) on the same rootstock.

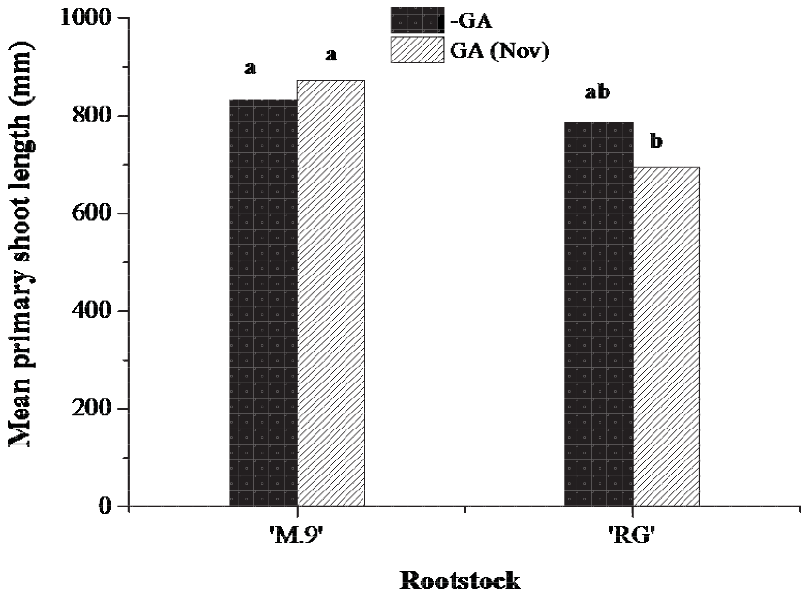

Figure 2.3. Rootstock × GA interaction on the mean length of the primary shoot of 'RG' and 'M.9' scions on 'M.9' and 'RG' rootstocks during early growth (16th Oct, 2007-12th Jan, 2008). -GA and GA (Nov) represent no GA or fortnightly GA foliar spray from November onward until growth cessation. Means sharing the same letter are not different significantly at *P=0.05* using the Duncan's Multiple Range test. Data are averaged over scions.

There were also significant rootstock × scion interactions (*P=0.05*) for the mean internode length of the primary shoot during early growth (Figure 2.4). The effect of rootstock depended upon the type of scion thereon. The 'M.9' rootstock produced longer internodes for 'RG' scions than 'RG' rootstock with 'RG' scions (Figure 2.5A). There was no significant difference between mean internode lengths of 'M.9' scions on 'M.9' and/or M.9 scions on 'RG' rootstocks (Figure 2.4).

As the rootstock × scion interactions were not significant (*P=0.9*) for node number of primary shoot, the mean node number for 'RG' scion on 'M.9' rootstock ('M9RG') was similar compared with 'RG' rootstock control ('RGRG') during early growth (Figure 2.4) but, the primary shoots were significantly longer for 'M9RG' compared with 'RGRG' composite trees (Figure 2.2). Hence, the mean internode length of primary shoot was greater for 'M9RG composite tree compared with 'RG' scion on 'RG' rootstocks (Figure 2.4).

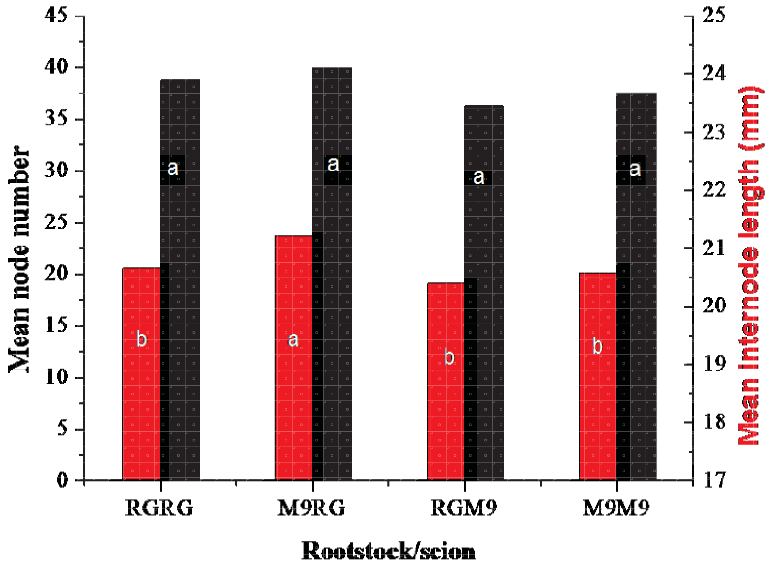

Figure 2.4. Rootstock × scion interaction on the mean node number (black columns) and mean internode length (red columns) of primary shoot on the 12th of January, 2008 during the spring and early summer of 'RG' and 'M.9' scions on 'M.9' and 'RG' rootstocks. Columns sharing the same letter for each attribute (mean node number and mean internode length) are not significantly different at *P=0.05* using Duncan's Multiple Range test. Data are pooled over GA treatments.

The mean internode length of 'RG' caught up to 'M.9' by February due to higher rate of extension growth between December, 2007 and February, 2008 (Figure 2.2B). However, the internode length gradually decreased during the later stages of the growth for primary shoots on both the rootstocks (Figure 2.5).

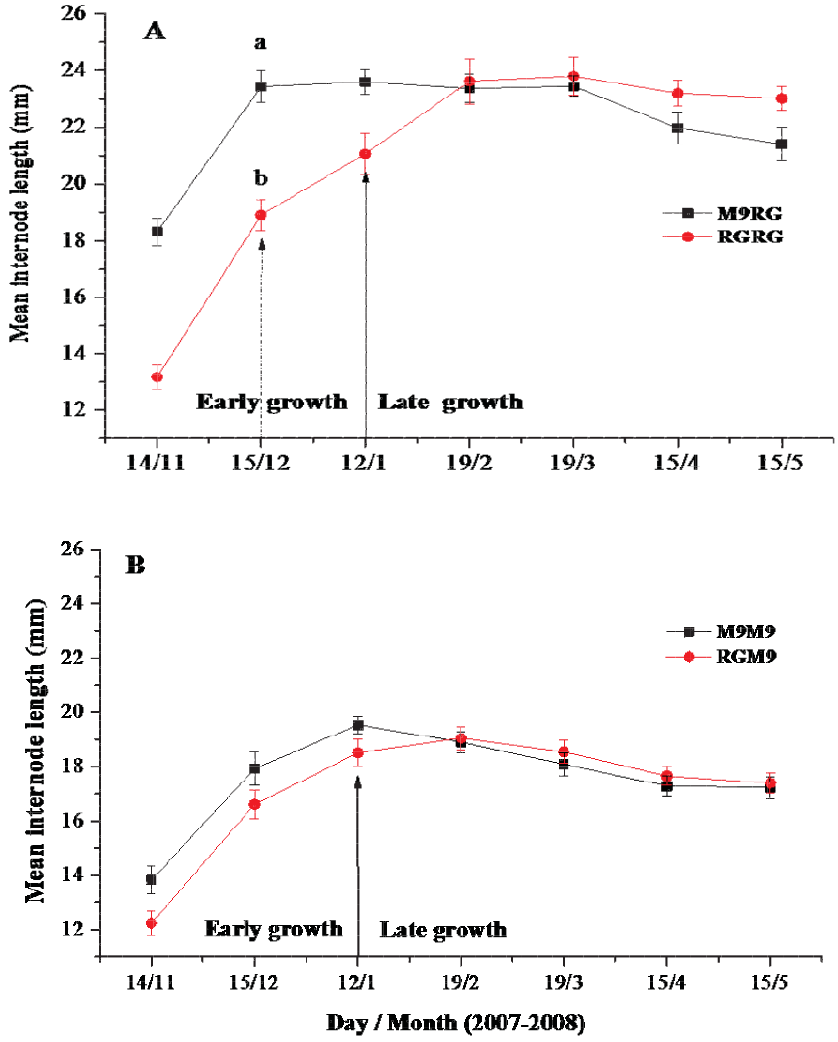

Figure 2.5. Rootstock × scion interaction on the mean internode length of 'RG' (A) and 'M.9' scions (B) on 'M.9' and 'RG' rootstocks over the entire growing season. The dotted arrow in 'A' indicates the date on which there was a significant (P=0.006) rootstock effect on the internode length. Bars represent standard errors. The vertical solid arrow in both A and B separates the early growth from late growth. Data are pooled over GA treatments.

The composite trees with 'RG' scion on 'M.9' rootstock ('M9RG') started exhibiting the dwarfing nature of 'M.9' rootstock after 12th January, 2008. These trees ('M9RG') gradually started decreasing the growth rate and the primary shoot was shorter compared with 'RG' rootstock control ('RGRG') (Figure 2.2 B), which was a rootstock effect (Hypothesis I). On the contrary, towards the end of the growth period (from 12th Jan to 15th May, 2008), the 'RG' rootstock did not change the growth patterns of the 'M.9' scion. The scion 'M.9' appeared to dominate the effect of the vigorous 'RG' rootstock and resulted in a shorter primary shoot compared with 'RG' scion on 'M.9' rootstock. This revealed the intrinsic nature of 'M.9' scion (Hypothesis II) (Figure 2.2 B). Therefore, the architectural changes that a dwarfing rootstock 'M.9' imposed on 'RG' scions and the timing of these changes following tree grafting appeared to start from the middle (12th Jan) of the first growing season and continued to the late growth i.e., until the end of the growing period (from 12th Jan to 15th May, 2008). The following section deals with the growth pattern during the late period.

2.4.1.2 *Treatment effects on the 'late growth' of the primary shoot*

2.4.1.2.1 Shoot length, node number and internode length

During late growth from midsummer to the end of the growing season (from January to May, 2008), there were meaningful highly significant main effects of scion and gibberellins for primary shoot length, node number and internode length. Irrespective of the type of rootstock, 'M.9' scions were ($P \leq 0.001$) shorter, compared with 'RG' scion (Table 2.2). Exogenous GA ($P \leq 0.001$) increased the final mean length, node number and internode length of the primary shoot (Table 2.2).

Table 2.2. Main effect of rootstock, scion and gibberellin (GA_3+GA_{4+7}) foliar spray on the final mean length, node number, internode length and shoot cross-sectional area (SCA) of the primary shoot at the end of the first growing season (June, 2008) after grafting (August, 2007). –GA, GA (Nov) and GA (Feb) represents no GA, GA sprays from November and February, respectively.

		Primary shoot			
Main effect		length (mm)	Node number	Internode length (mm)	SCA (mm^2)
Scion	'M.9'	1315.9 b	69.6 b	18.8 b	173.4 b
Scion	'RG'	1839.9 a***	76.4 a***	23.9 a***	440.4 a***
GA	-GA	1352. 1 b	67.8 b	19.7 b	298.7 ns
GA	GA (Nov)	1729.6 a***	76.0 a ***	22.5 a***	292.6
GA	GA (Feb)	1675.0 b	75.6 b	21.9 b	329.4
Rootstock	'M.9'	1587.1 ns	73.1 ns	19.3 ns	272.6 b
Rootstock	'RG'	1575.8	73.0	20.2	341.2 a***

Mean sharing the same letter within each main effect (i.e. Scion, GA and Rootstock) are not significant at *P=0.05* using Duncan's Multiple Range test. ns, *** represent non-significant or significant at *P≤0.001*.

In addition to highly significant main effects, there were two-way interactions for primary shoot length and node number, but interpreted bearing in mind that there were three-way interactions (*P=0.03)* at least for shoot length. The rootstock GA interactions for the final mean length and node number of the primary shoot were at *P=0.02* and *0.01* respectively (Table 2.3). Without GA, the rootstock 'M.9' did not reduce the final mean length and node number of the primary shoot compared with 'RG' rootstock significantly. In response to both GA timings, for 'M.9' rootstocks, the mean length and node number of the primary shoot increased more markedly but only the GA (Nov) increased length and node number for 'RG'. Although there were significant rootstock × GA interactions for final mean length and node number of the primary shoot, the rootstock × GA interaction was not significant (*P=0.1*) for internode length (Table 2.3).

Table 2.3. Rootstock × GA treatment interactions for the final mean primary shoot length, node number and internode length for the scions at the end of their first growing season (June, 2008) from grafting. –GA, GA (Nov) and GA (Feb) represents no GA, GA (GA_3+GA_{4+7}) sprays from November and February, respectively. Data are pooled over scions.

		Primary shoot		
Rootstock	GA foliar sprays	*Length (mm)*	*Node number*	Internode length (mm)
'M.9'	-GA	1277.5 c	65.8c	19.3 b
'M.9'	GA (Nov)	1748.8 a***	76.5 a***	22.6 a
'M.9'	GA (Feb)	1742.8 a	78.1 a	22.2 a
RG'	-GA	1426.7 bc	69.8 bc	20.2 ab
RG'	GA (Nov)	1710.4 a	75.5 a	22.5 a
RG'	GA (Feb)	1624.2 ab	73.8 ab	21.7 ab

Means sharing the same letter within each column are not significantly different at *P=0.05* using Duncan's Multiple range test *** represents significant ≤ *0.001*.

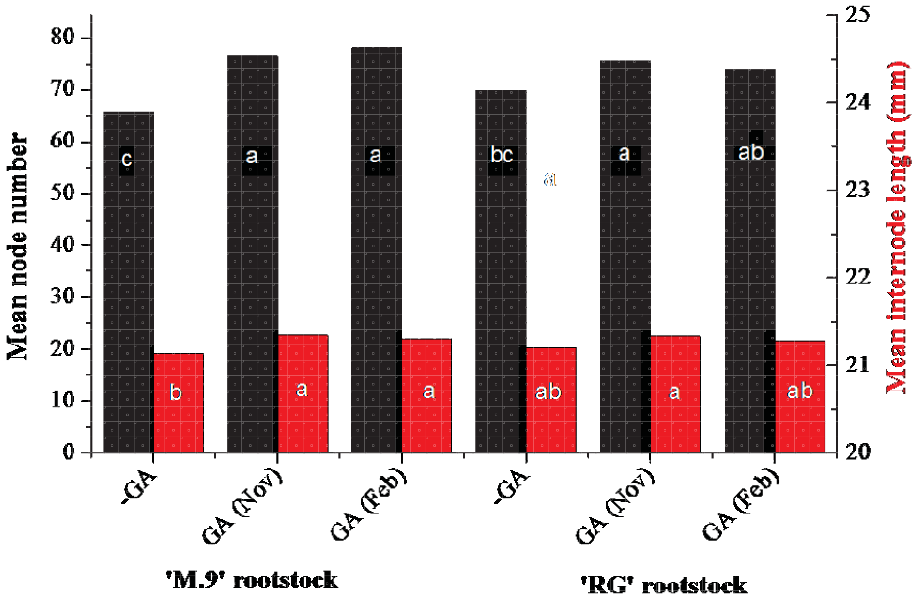

Figure 2.6. Rootstock × GA interaction on the mean node number (black columns) and internode length (red columns) of the primary shoot of 'M.9' and 'RG' scions grafted onto 'M.9' and 'RG' rootstocks. Means sharing the same letter for nodes number and internode length are not significantly different at *P=0.05* using Duncan's Multiple Range test. Data are averaged over scions. –GA, GA (Nov) and GA (Feb) represents no GA, GA (GA_3+GA_{4+7}) foliar sprays from November and February, respectively. Data are pooled over scions.

The rootstock × scion × GA treatment interactions were significant (*P=0.03*) in June for the final length of the primary shoot (Table 2.4 and Figure 2.11). In contrast to 'RG' scion on 'RG' rootstock, the 'RG' scion on 'M.9' rootstock showed a larger response to GA (Nov) and (Feb) treatments and the 'RG' scion was significantly longer compared with the untreated scions. The effect of gibberellins appeared markedly higher for 'RG' scion on 'M.9' rootstock than any other combination (Table 2.4). The final mean length of 'RG' scions on 'M.9' rootstocks was increased by 41 and 31% with GA when applied in Nov and Feb respectively; but only 17 and 19% increase for those of 'RG' scions on 'RG' rootstocks. However, both were longer compared with their respective untreated scions. The 'M.9' scion on 'M.9' and 'RG' rootstocks showed a similar response to GA (Nov) and, increased the length compared with untreated scions. Although GA increased the length of both 'M.9' and 'RG' scions, the increase in the length of 'M.9' scions was 50% less compared with those of 'RG' scions (Table 2.4). Moreover, the 'M.9' scion on 'RG' rootstock with GA (Feb) was equal in length with the untreated scions (Table 2.4).

Table 2.4. Interactions of rootstock, scion and gibberellins (GA_3+GA_{4+7}) foliar spray on the final mean primary shoot length of 'M.9' and 'RG' scions on 'M.9' and 'RG' rootstocks at the end of the growing period (June, 2008) after grafting. – GA, GA (Nov) and GA (Feb) represents no GA, GA sprays from November and February, respectively.

		Final mean primary shoot length (mm)		
Rootstock	**Scion**	**-GA**	**GA (Nov)**	**GA (Feb)**
'M.9'	'M.9'	1091.6 e	1430.8 dc	1517.5 bc
'M.9'	'RG'	1463.3 dc	2066.6 a	1923.0 a
'RG'	'M.9'	1165.8 e	1480.8 c	1275.8 de
'RG'	'RG'	1687.5 b	1940.0 a	1972.5 a

All means in columns and rows sharing the same letter are not significantly different *at P=0.05* using Duncan's Multiple Range test.

The response of 'RG' scion on 'M.9' rootstock to gibberellins was the greatest as this combination responded strongly to both times of application. The 'M.9' scion on 'RG' rootstock responded mostly to the GA (Nov) and the 'RG' scion on 'RG' rootstock and

'M.9' scion on 'M.9' rootstock also responded as well to the GA (Feb) as to a continuous supply of gibberellins (i.e. applied in Nov). Therefore, the major result is that the dwarfing effect of 'M.9' rootstock on the 'RG' scion was overcome with GA because the 'RG' scion on 'M.9' rootstock with GA was similar to 'RG' scion on 'RG' rootstock with GA and greater than 'RG' scion on 'RG' rootstock without GA (Table 2.4).

It was observed that the longer primary shoots had more nodes with longer internodes (Figures 2.7 A&B). In these figures as the number of nodes on 'x-axis is increased the length of primary shoot on y-axis increased. The internode lengths can be compared from the regression line of data on each graph by dividing y-value by its corresponding value of x. The decreased final mean length of the primary shoot of 'RG' scion on 'M.9' rootstock compared with 'RG' scion on 'RG' rootstock and of 'M.9' scions on 'M.9' and 'RG' rootstocks (Figure 2.7) was therefore, attributed to a fewer neo-formed nodes. The rate of node production was reduced from 12th Jan, 2008 (Figure 2.9) prior to shoot termination that first began between 15/4/08 - 30/4/08 and 1/4/08 - 15/4/08 respectively This slow rate of plastochron was combined with a greater proportion of shoot apical meristems terminating early during and/or after the first half of April (Section 2.4.1.3). With GA treatment for 'RG' scions on 'M.9' and 'RG' rootstocks the node number and length of primary shoots increased (Figure 2.7A), which increased internode length. Therefore, for 'RG' scions longer primary shoots had more neo-formed nodes. In contrast, for 'M.9' scions a few of the longer primary shoots with GA treatment had similar number of nodes compared with untreated scions and thus GA increased internode length (Figure 2.7B). There were also longer shoots of GA treated 'M9' scions with fewer neo-formed nodes compared with the untreated scion. Therefore, GA foliar sprays for 'RG' scion increased node number and thus increased primary shoot length (Figure 2.7A), whereas for 'M.9' scions GA increased node number and internode length (Figure 2.7B).

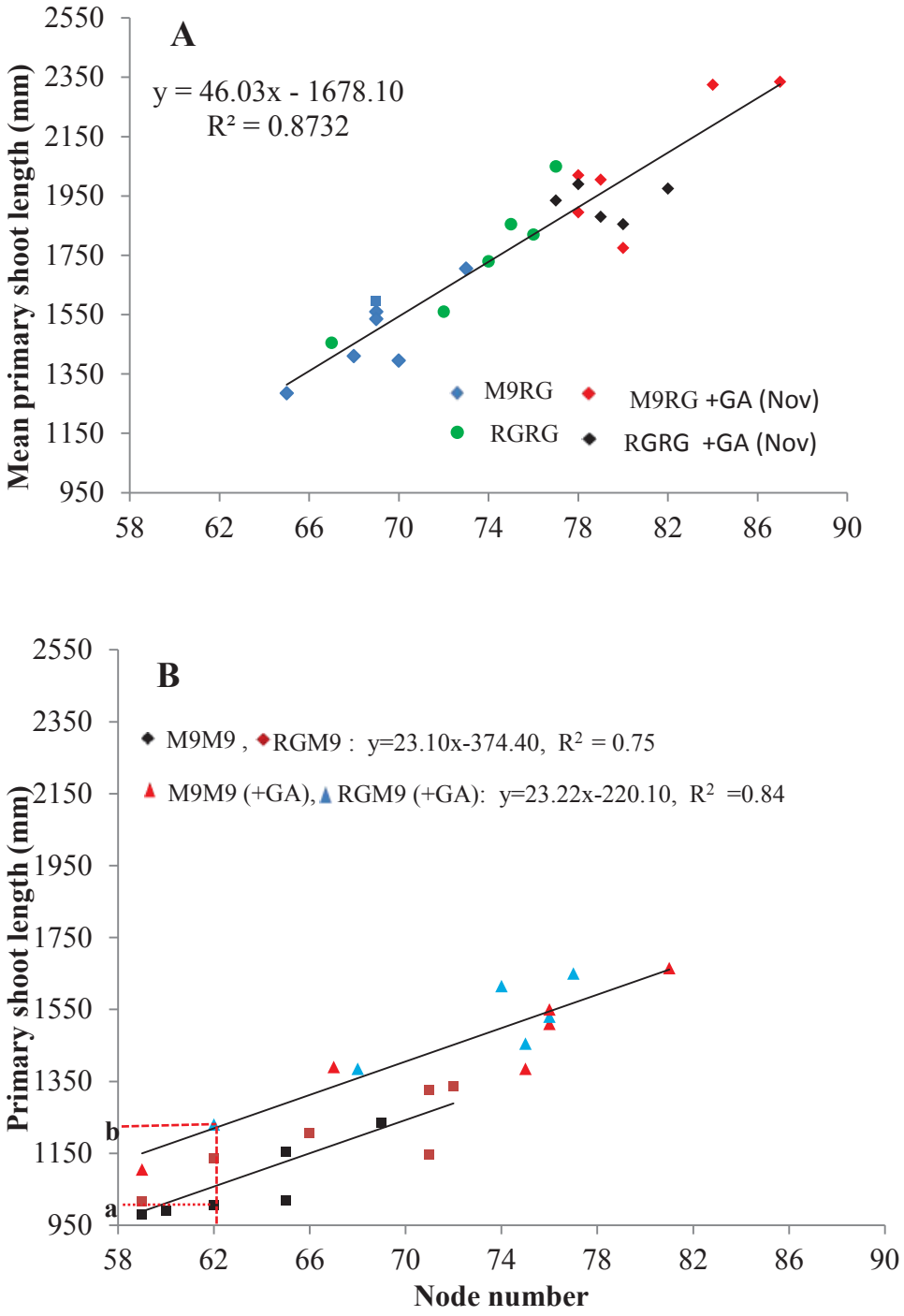

Figure 2.7. The relationship between the node number and length of the primary shoot of 'RG' (A) and 'M.9' (B) scions on 'M9' and 'RG' rootstocks with GA_3+GA_{4+7} (+GA Nov) and without foliar sprays. The internode lengths can be compared from the regression line of data on each graph by dividing a y-value by its corresponding value of x in the graph. In figure B the dotted line from x-axis to y-axis from node number 62 shows the internode lengths - a (shorter) and - b (longer) for the same node number.

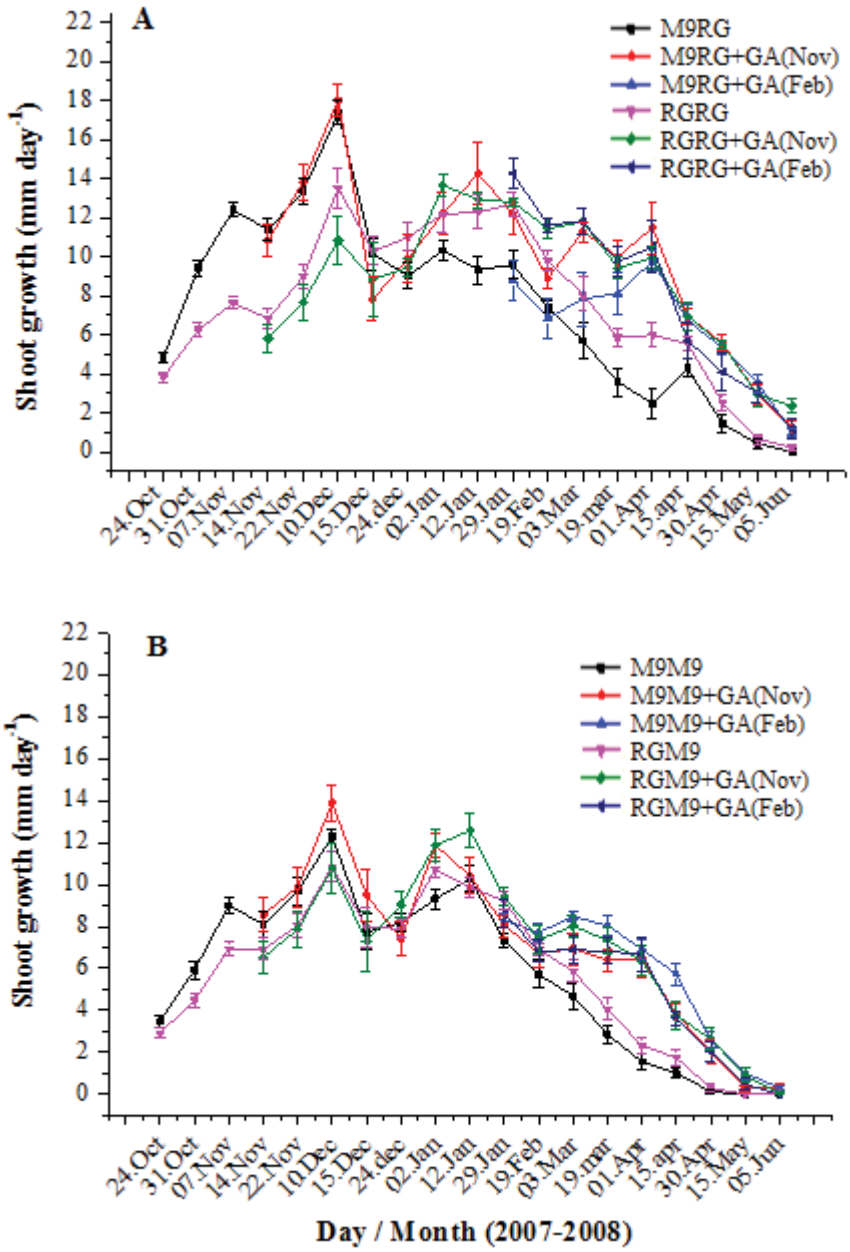

Figure 2.8. Effect of 'M.9' and 'RG' rootstock on 'RG' scion (A) and on 'M.9' scion (B) on the rate of shoot growth (mm day^{-1}) throughout the growing season; - GA, GA (Nov), GA (Feb) represent no GA and GA (GA_3+GA_{4+7}) foliar sprays from November and February respectively that continued till the end of the growing season. Bars represent the standard error.

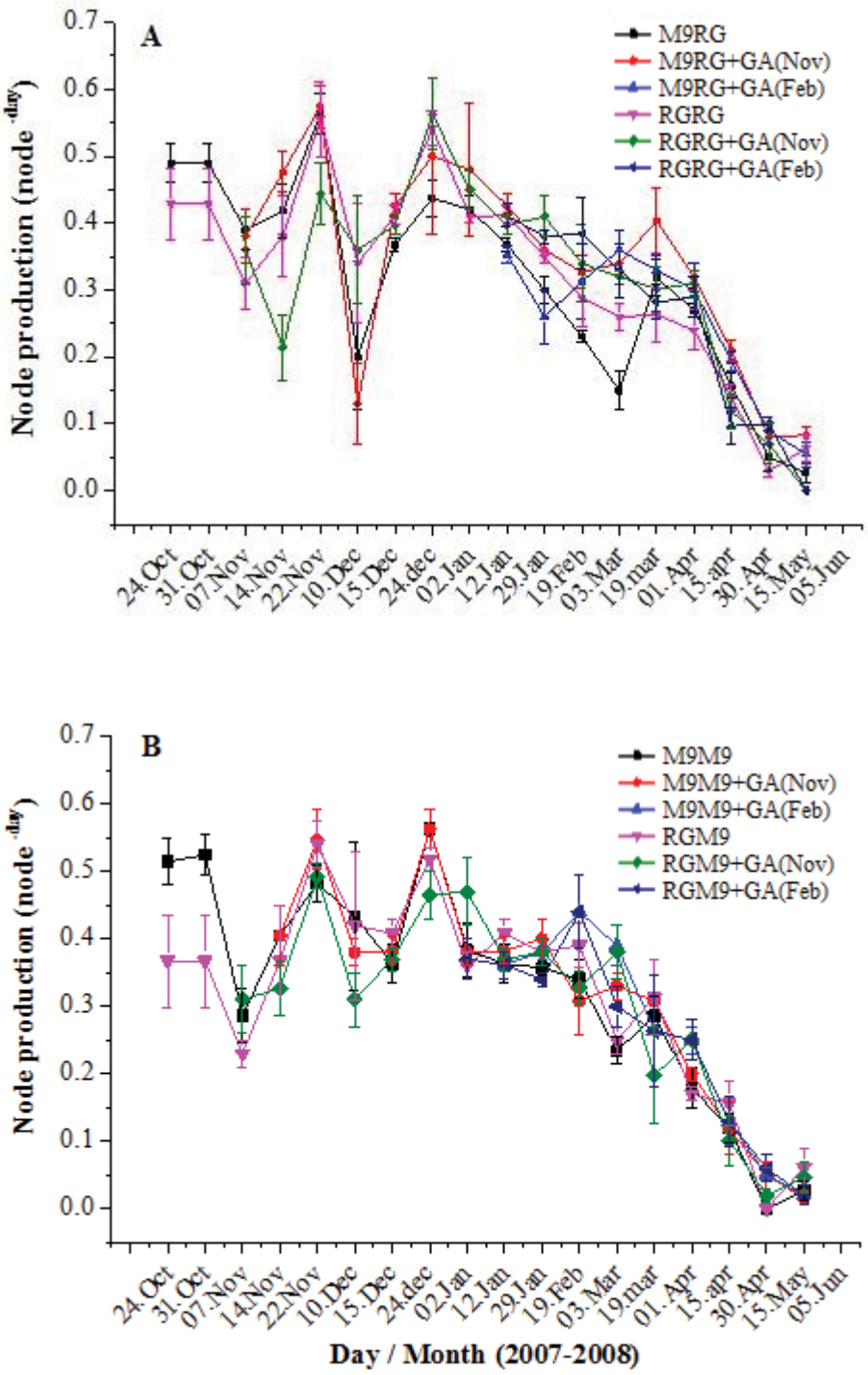

Figure 2.9. Effect of 'M.9' and 'RG' rootstock on 'RG' scion (A) and on 'M.9' scion (B) on the rate of node production (nodes day^{-1}); -GA, GA (Nov), GA (Feb) represent no GA and GA(GA_3+GA_{4+7}) foliar sprays from November and February respectively that continued till the end of the growing season. Bars represent the standard error.

Therefore, in summary, the final mean length and node number and internode length of 'M.9' scions was lower compared to 'RG' scions (Table 2.2). Although, 'RG' scions were longer compared with 'M.9' scions, the 'RG' scions on 'M.9' were shorter (Figure 2.10A) with reduced node number compared with 'RG' on 'RG' rootstock (Table 2.5). This showed the dwarfing effect of 'M.9' rootstock (Hypothesis I). The rootstock effect on node number of 'RG' scion on 'M.9' rootstock was significant only at *P=0.09* (Table 2.5; Figure 2.12). In contrast, the vigorous rootstock 'RG' had no significant effect on increasing the primary shoot length of 'M.9' scion (Figure 2.10B). This revealed the significant scion effect and the intrinsic nature of the 'M.9' scion (Hypothesis II) as the nature of 'M.9' scion was not altered by the vigorous rootstock. Interestingly, the effect of GA for both (Nov) and (Feb) increased the length (Figure 2.10A) and node number (Table 2.5) of 'RG' scion on 'M.9' rootstock compared with 'RG' scion on 'RG' rootstock. For 'RG' scions, with GA increased node number increased the length of the primary shoot thus increasing the internode length (Figure 2.7A). In contrast, the effect of GA on 'M.9' scion was on both node number and internode length. For 'M.9' scion with GA, there was an increase in the primary shoot length with similar number of nodes (62), thus increasing the internode length (Figure 2.7B). Therefore, for 'M.9' scion, the node number and mean internode length were greater with GA treatment (Table 2.5, 2.6). From 12th Jan, 2008 there was an increase in the rate of shoot growth and node production of primary shoot for 'RG' scions on 'RG' rootstock compared with that on 'M.9' rootstock (Figure 2.8A, and 2.9A). Gibberellin foliar spray increased the rate of node production and shoot growth from 12th Jan for 'RG' scions on both the rootstocks compared with untreated scions. Significant dwarfing of primary shoot occurred for 'RG' scions on 'M.9' rootstock even before any signs of shoot termination, which started from 15th April, 2008 compared with 'RG' scion on 'RG' rootstock.

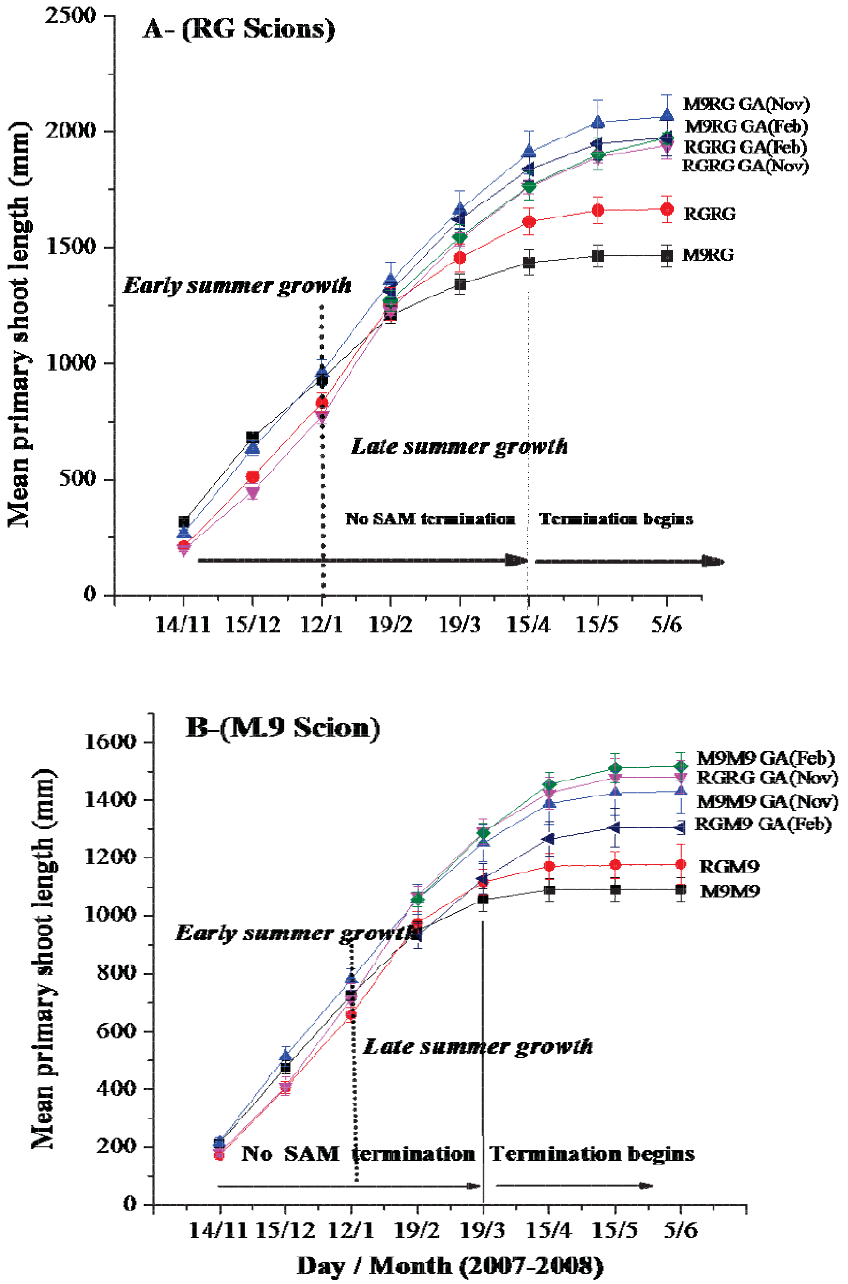

Figure 2.10. Rootstock × GA interactions on primary shoot length during the first growing season from grafting for 'Royal Gala' (A) and M.9 (B) scions grafted onto M.9 and 'Royal Gala' rootstocks. Bars represent standard errors; GA (Nov) and GA (Feb) represent the foliar spray of GA_3+GA_{4+7} from November and February, respectively, that continued until the end of the growing season. Vertical dotted line separates early growth from late growth. The early growth represent the growth during spring and early summer (from 14th Nov to 12th Jan on this graph) and late growth represents the growth from mid-summer to the end of the growth season (from 12th Jan to 5th June). The solid vertical line along the x-axis denotes the time after which shoot termination first began for all treatments (15th April, 2008). Bars represent the standard error.

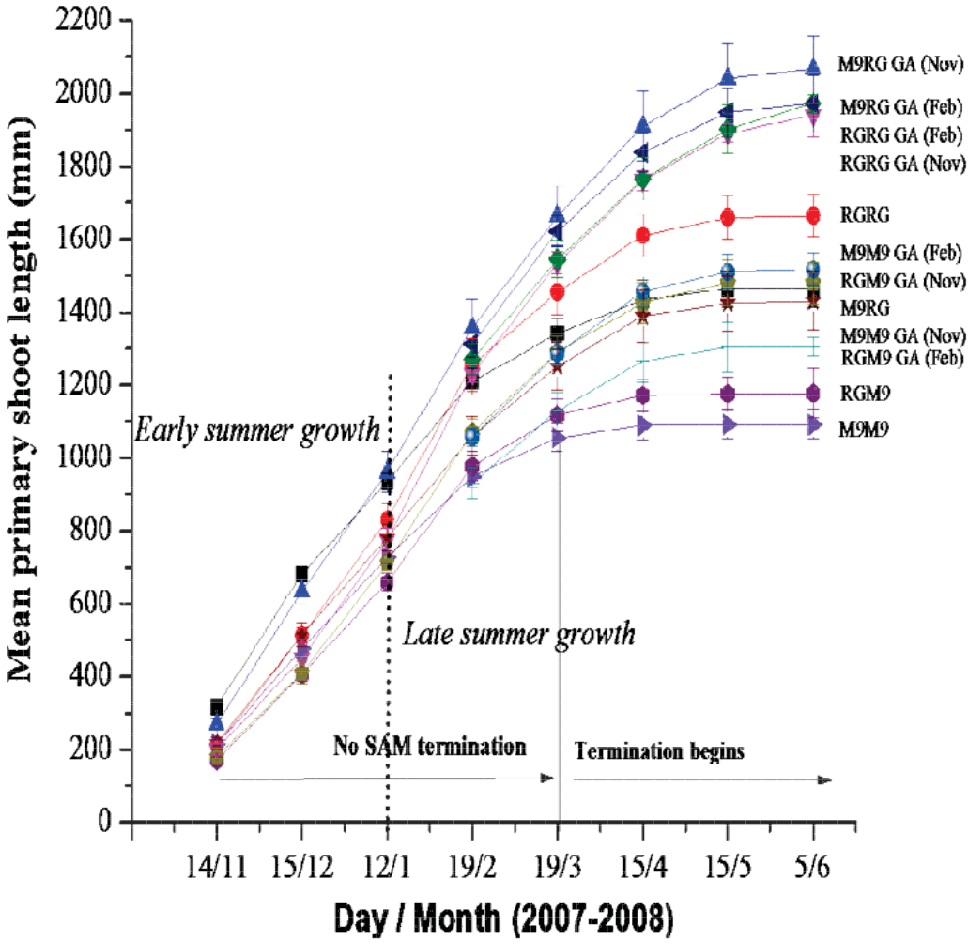

Figure 2.11. Rootstock × scion × GA interactions on primary shoot length during the first growing season from grafting for 'Royal Gala' and M.9 scions grafted onto M.9 and 'Royal Gala' rootstocks. Bars represent standard errors; GA (Nov) and GA (Feb) represent the foliar spray of GA_3+GA_{4+7} from November and February respectively, that continued till the end of the growing season. Vertical dotted line separates early growth from late growth. The early growth represents the growth during spring and early summer from 14th Nov to 12th Jan. on this graph and late growth represents the growth from mid-summer to the end of the growth season (from 12th Jan to 5th June). The vertical line along the x-axis denotes the time after which shoot termination first began for 'M.9' rootstock and scion without GA. Bars represent the standard error.

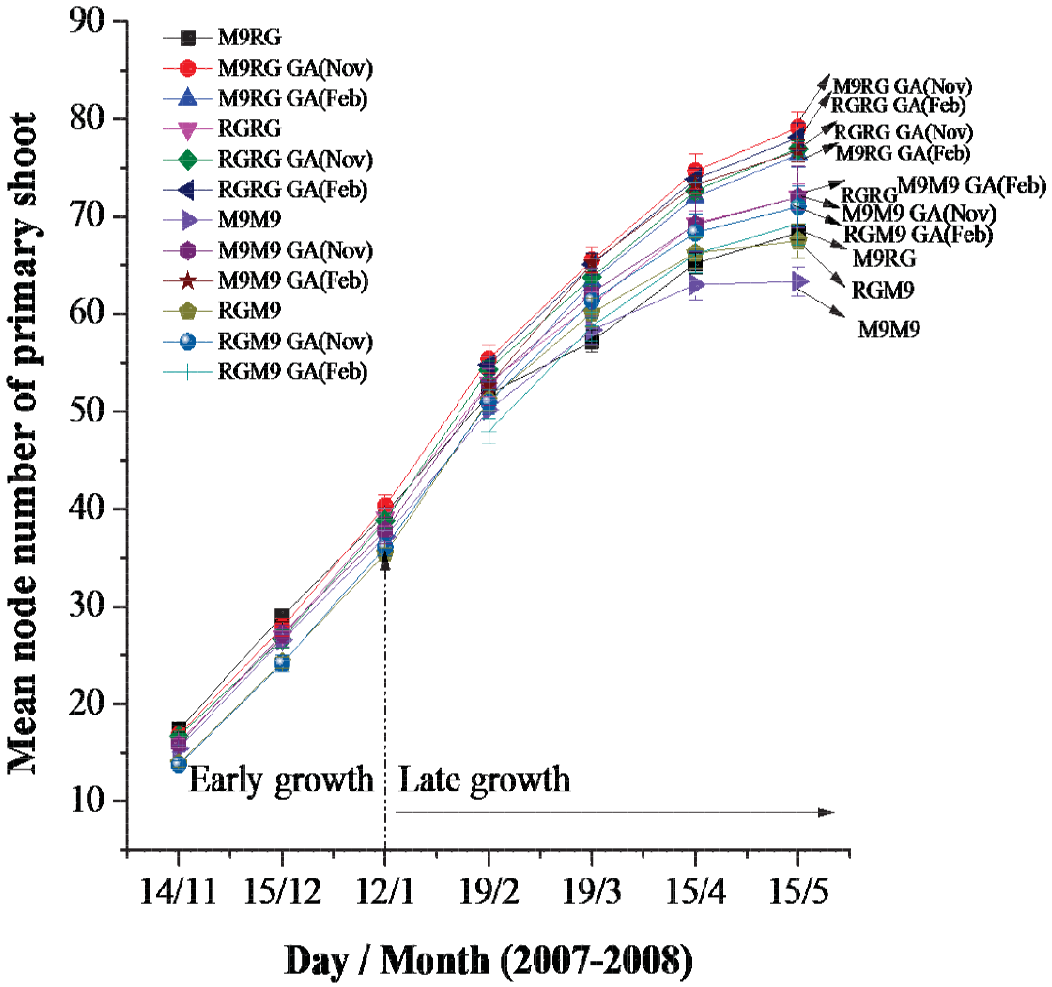

Figure 2.12. Rootstock × scion × GA interactions on mean node number of primary shoot during the first growing season from grafting for 'Royal Gala' and M.9 scions grafted onto M.9 and 'Royal Gala' rootstocks. Bars represent standard errors; GA (Nov) and GA (Feb) represent the foliar spray of GA_3+GA_{4+7} from November and February, respectively, that continued till the end of the growing season. Vertical dotted line separates early growth from late growth. The early growth represent the growth during the spring and early summer from 14th Nov to 12th Jan. on this graph and late growth represents the growth from mid-summer to the end of the growth season from 12th Jan to 5th June. Bars represent the standard error.

Table 2.5. Interactions of rootstock, scion and gibberellins (GA_3+GA_{4+7}) foliar spray on the final mean node number of 'M.9' and 'RG' scions on 'M.9' and 'RG' rootstocks at the end of the growing period (June, 2008) after grafting. –GA, GA (Nov) and GA (Feb) represents no GA, GA sprays from November and February, respectively.

Rootstock	Scion	- GA	+ GA (Nov)	+ GA (Feb)
		Node number		
'M.9'	'M.9'	63.3 c	72.3abc	77.7 bc
'RG'	'M.9'	66.8 bc	72.0 abc	68.3 a
'M.9'	RG	68.3 bc	80.6 a	78.4 a
'RG'	RG	72.8 ab	79.1 a	79.3 a

All means sharing the same letter in columns and rows are not significantly different *at P=0.05* using the Duncan's Multiple Range test.

Table 2.6. Interactions of rootstock, scion and gibberellins (GA_3+GA_{4+7}) foliar spray on the final mean internode length of 'M.9' and 'RG' scions on 'M.9' and 'RG' rootstocks at the end of the growing period (June, 2008) after grafting. –GA, GA (Nov) and GA (Feb) represents no GA, GA sprays from November and February, respectively.

Rootstock	Scion	- GA	+ GA (Nov)	+ GA (Feb)
		Internode length (mm)		
'M.9'	'M.9'	17.2 e	19.7 cd	19.5 cd
'RG'	'M.9'	17.4 e	20.5 bcd	18.6 de
'M.9'	RG	21.7 bc	25.2 a	24.1 a
'RG'	RG	23.1 ab	24.5 a	24.8 a

All means sharing the same letter in columns and rows are not significantly different *at P=0.05* using the Duncan's Multiple Range test. .

2.4.1.3 Treatment effect on termination of the shoot apical meristem on the primary shoot

For all treatments, 100% of primary shoots were actively growing on the 15/3/08 (Figure 2.11). However, 70-85% 'M.9' scions had fully terminated on the 15/4/08, approximately one month later and, 100% had terminated on the 30/4/08. Thus, termination for 'M.9' scions started at some point in time between the growth measurements conducted from 15/3/08 to 15/4/08 and the termination completed by the 30/4/08. While 100% 'M.9' scions had completely terminated on (30/4/08), 80% of the

'RG' scions were actively growing and, the termination of 'RG' scions started approximately from 15/4/08 i.e., one month after 'M.9' scions started terminating (15/3/08). The termination of 'M.9' scions was similar whether grafted onto dwarfing ('M.9') or vigorous ('RG') rootstocks (Table 2.7) but 'RG' scions behaved differently on different rootstocks. The 'RG' scions on 'M.9' started termination earlier than those on RG rootstock; termination started from 15/4/08 onward and 100% shoots had terminated by 15/5/08, whereas only 70% shoots of RG scions on the 'RG' rootstock had terminated on 15/5/08 (Table 2.7).

The termination of the shoot apical meristems on the primary shoots was delayed with GA foliar sprays. For 'RG' scions on both rootstocks, growth continued until 5/6/08 when scions were fully dormant. For 'M.9' scions on 'M.9' rootstock, the GA treatment prolonged the activity of SAM and only 50% shoots had terminated for GA (Nov), and had no termination for GA (Feb) treatment while 100% untreated shoots had terminated by 30/4/08 (Table 2.7). For 'M.9' scions on 'RG' rootstock, there was a higher percentage (67%) of termination for GA (Feb) compared with GA (Nov) treatment (25%) on 30/4/08 compared with untreated scions (Table 2.7). Gibberellin foliar sprays prolonged the growth of primary shoots until the end of the growing season. However, for 'RG' scions on both the rootstocks, 100% primary shoots growth was prolonged, while for the 'M.9' scions irrespective of the rootstock the GA foliar sprays prolonged growth of only 15-25% of the primary shoots (Table 2.7).

Table 2.7. Effect of rootstock and gibberellins (GA_3+GA_{4+7}) foliar sprays on the percentage of primary shoots that were fully terminated in April, May and June for 'Royal Gala' and 'M.9' scions during their first growing season after grafting (August, 2007).

			Initial	Percentage (%) shoot termination			
Rootstock	scion	GA_3+GA_{4+7}	application time	15/4/08	30/4/08	15/5/08	5/6/08
'M.9'	'RG'	-	-	0	20	100	100
'M.9'	'RG'	+	Nov	0	0	0	100
'M.9'	'RG'	+	Feb	0	0	0	100
'RG'	'RG'	-	-	0	0	70	100
'RG'	'RG'	+	Nov	0	0	0	100
'RG'	'RG'	+	Feb	0	0	0	100
'M.9'	'M.9'	-	-	85	100	100	100
'M.9'	'M.9'	+	Nov	50	50	85	100
'M.9'	'M.9'	+	Feb	0	0	100	100
'RG'	'M.9'	-	-	70	100	100	100
'RG'	'M.9'	+	Nov	0	25	75	100
'RG'	'M.9'	+	Feb	0	67	100	100

2.4.1.4 Final shoot cross-sectional area (SCA) of the primary shoot

Rootstock and scion affected the final mean SCA of the primary shoot (Table 2.2). However, interpretation of these main effects required consideration of interactions present in the data. The rootstock × scion, rootstock × GA and scion × GA were significant at *P=0.01, 0.004, 0.001*, respectively (Appendix.1). The interactions between rootstock × scion × GA approached significance (*P=0.06*) and are presented to compare some three-way interaction means, particularly the effects of rootstock × GA at different levels of scion (Figure 2.13).

Without GA, the 'RG' rootstock had a greater mean SCA for the 'RG' scion compared with the 'M.9' rootstock (Figure 2.13). In addition, SCA was similar amongst 'M.9' scions grafted on 'M.9' or 'RG', irrespective of whether GA was sprayed or not (Figure 2.13). For 'RG' scion, the SCA was lower when grafted onto the 'M.9' dwarfing rootstock compared to that when grafted onto 'RG' rootstock. For 'RG' scions on 'RG'

rootstocks, the GA (Nov) foliar sprays reduced the mean SCA more markedly than GA (Feb). GA (Nov) had lower (*P=0.001*) SCA for 'RG' scion on 'RG' rootstock when compared with 'RG' scion on 'RG' rootstock without GA (Figure 2.13), but for the 'RG' scion on the 'M.9' rootstock the SCA with GA (Nov) and without gibberellin foliar sprays were not different but significantly less compared with GA (Feb). However, in addition, SCA of 'RG' scion on 'M.9' rootstock with GA (Feb) increased significantly (*P=0.0001*) and was similar to 'RG' scion on 'RG' rootstock with or without GA.

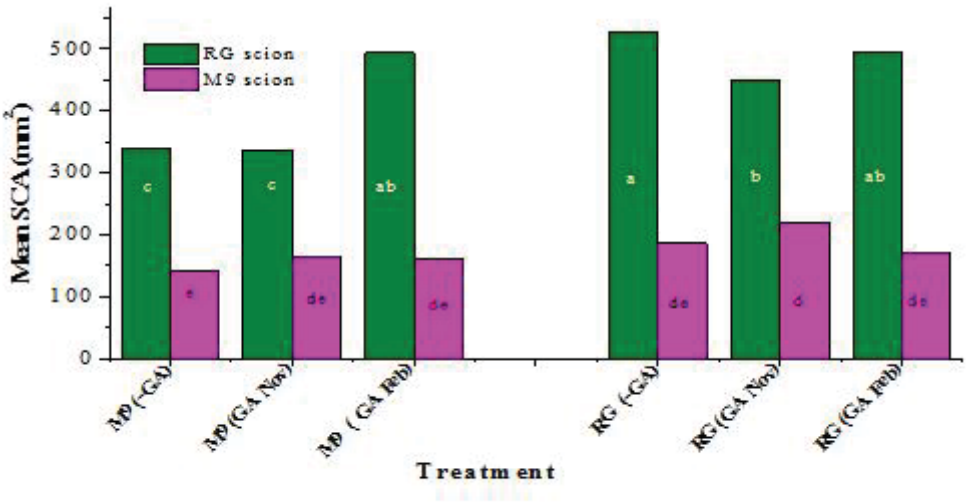

Figure 2.13. Rootstock × scion × GA interactions on the final mean shoot cross-sectional area (SCA) of primary shoot of 'RG' and 'M9' scions on 'M.9' and 'RG' rootstocks at growth cessation in June, 2008. –GA, GA (Nov) and GA (Feb) represent no GA (GA_3+GA_{4+7}), GA foliar sprays from November and February respectively and continued until the end of the growing season. Means sharing the same letter are not significant at *P=0.05*.

2.4.2 Treatment effects on the formation of sylleptic axillary shoot formation on the primary shoot

The sylleptic shoots produced by the axillary bud activity on the primary shoot were further distinguished as either a spur (minimal internode extension <25 mm) or a

sylleptic shoot (SS) (≥25 mm with internode extension). In this thesis, both spurs and sylleptic shoots are collectively called "sylleptic axillary shoots" (SAS).

2.4.2.1 *Treatment effects on the number of sylleptic axillary shoots formed per scion*

Out of the total number of SAS formed on the primary shoot, the 'M.9' scions increased the number of spurs compared with 'RG' scion on 'M.9' and 'RG rootstocks (Table 2.8). The 'M.9' rootstock and 'M.9' scion reduced the total number of sylleptic shoots compared with 'RG' rootstock and 'RG' scion. The GA foliar sprays at both timings (Nov) and (Feb) increased the number of sylleptic shoots for all scions on both rootstocks (Table 2.8).

The rootstock × scion × GA interactions for the final total number of sylleptic shoots approached significance (*P=0.09*) and are presented to compare treatment influence on SAS formation (Table 2.9). The untreated 'RG' scion on 'M.9' rootstock had fewer Sylleptic shoots (SS) compared with 'RG' scions on 'RG' rootstocks. The number of SS for 'RG' scion on 'M.9' rootstock increased (*P=0.02*) with GA. The number of SS formed for 'RG' scion on 'M.9' rootstock with GA foliar sprays was similar to that formed for untreated 'RG' scion on 'RG' rootstock. There was no significant difference between GA treated and an untreated 'RG' scion on 'RG' rootstock in the number of SS (Table 2.9). The effect of GA on the number of SS for 'RG' scions on 'RG' rootstock appeared to be less than that for 'RG' scions on 'M9' rootstock.

Table 2.8. Main effect of scions ('M.9' or 'R.G'), gibberellin (GA_3+ GA_{4+7}) foliar sprays to the scion commencing in November GA (Nov) or February GA (Feb) and rootstocks ('M.9' or 'R.G') on the mean total number, length of SAS [spurs + sylleptic shoots (SS)] and total shoot length of the scion at the end of the first growing season (June, 2008) after grafting (August, 2007). SS represent sylleptic shoots.

Main effect		Number of SAS			SAS total length (mm)	Total shoot length (mm) (primary plus SAS)
		Spurs	SS	Total(spurs+SS)		
Scion	M.9	23.3 a***	6.8 b***	30.2 a***	1613.8 b***	2929.7 b***
Scion	RG	4.8 b	9.5 a	14.6 b	6091.3 a	7924.6 a
GA	-GA	14.4 a	6.3 b***	21.1 a	2379.7 c	3722.3 c
GA	GA (Nov)	12.8 a	9.7 a	22.6 a	5137.9 a***	6867.5 a***
GA	GA (Feb)	14.6 a	8.4 a	23.2 a	4173.3 b	5848.3 b
Rootstock	M.9	14.3 a	6.7 b***	21.1 a	3116.2 b	4691.1 b***
Rootstock	RG	13.5 a	9.6 a	23.3 a	4589.7 a***	6171.3 a

Means sharing the same letter within a single main effect (i.e. Scion, GA and Rootstock) in each column are not significantly different at *P=0.05* using the Duncan's Multiple Range test and, *** represent s significant at *P=0.001*.

Table 2.9. Rootstock × scion × GA interactions on the mean number of sylleptic shoots formed on primary shoots of 'M.9' and 'RG' scions on 'M.9' or 'R.G' rootstocks at the end of the first growing season (June, 2008) after tree grafting (August, 2007). Gibberellin (GA_3+ GA_{4+7}) foliar sprays applied to the scion commencing in November GA (Nov) or February GA (Feb),) and –GA represents without GA foliar sprays.

Rootstock	Scion	-GA	GA (Nov)	GA (Feb)
'M.9'	'M.9'	3.3 b	6.3 ab	6.5 ab
'RG'	'M.9'	6.3 ab	11.6 a	6.8 ab
'M9'	'RG'	5.6 b	10.3 a	8.0 ab
'RG'	'RG'	10.6 a	10.8 a	11.8 a

Means sharing the same letter within a single growth attribute (sylleptic shoots) only are not significantly different at P=0.05 using Duncan's Multiple Range test.

Although, 'M.9' scion on 'RG' rootstock produced less number of SS, still the effect of 'RG' root system on SAS formation was observed. With 'RG' rootstock, the number of SS was more (*P=0.1*) for 'M.9' scion compared with 'M.9' on 'M.9' rootstock (Table 2.9). The GA (Nov) foliar spray increased the number of SS for 'M.9' scions on both the rootstocks, but GA (Feb) sprays increased the number of SS only for scions on 'M.9' rootstocks (Table 2.9).

2.4.2.2 Treatment effects on the termination of sylleptic shoots

The sylleptic shoots of untreated scions had terminated very early during January even before primary shoots had terminated (Table 2.10) and, with gibberellin foliar sprays the growth duration of the apical meristem of sylleptic shoots was increased, thus longer sylleptic shoots with more nodes were produced as the growth continued until the end of April.

Among the sylleptic shoots (SS) of 'M.9' scions on 'M.9' rootstocks, more than 50% had terminated by the 2nd week of January, a time when the primary shoots had not begun to terminate (Table 2.7 & 2.10). However, by the end of February almost 90% SS had terminated while primary shoots were still growing. About 80% of SS of 'M.9' scions on both rootstocks with GA foliar sprays had terminated by the 2nd week of March. Very few secondary shoots continued growth to the end of the season (Table 2.10).

For 'RG' scions on 'M.9' rootstocks, 69% and 76% SS had terminated in January and February when there were no traces of primary shoot termination and, 80% SS had terminated by the 15th of April without GA treatment, while termination for primary shoots was just 20% and, 100% by middle of May for both primary and sylleptic shoots (Table 2.7 and 2.10). The GA foliar sprays prolonged the growth of sylleptic shoots of 'RG' scions on both rootstocks compared with the untreated scions.

Table 2.10. Effect of rootstock and GA (GA_3+GA_{4+7}) foliar sprays on the mean proportion (%) of sylleptic shoots that were fully terminated for 'M.9' and 'RG' scions during their first growing season after grafting. No GA, Nov and Feb indicate no GA sprays and sprays from November, 2007 and February, 2008 until the end of the growing season (June, 2008)

			Percentage of sylleptic shoots terminated						
Rootstock	GA_3+GA_{4+7}	Timing	**12/1/08**	**19/2/08**	**19/3/08**	**15/4/08**	**30/4/08**	**15/5/08**	**5/6/08**
Scion 'M.9'									
'M.9'	-	No GA	56	92	90	98	100		
'M.9'	+	Nov	58	82	83	89	100		
'M.9'	+	Feb	64	86	84	88	100		
'RG'	-	No GA	50	89	94	97	100		
'RG'	+	Nov	40	74	71	84	100		
'RG'	+	Feb	46	89	73	81	100		
Scion 'RG									
M.9'	-	No GA	0	69	76	80	92	100	100
M.9'	+	Nov	0	41	39	76	82	83	100
M.9'	+	Feb	0	41	43	67	81	95	100
RG'	-	No GA	0		65	64	72	99	100
RG'	+	Nov	0	0	33	34	72	78	100
RG'	+	Feb	0	0	36	36	83	83	100

2.4.2.3 Treatment effects on the final mean total length of sylleptic axillary shoots (spurs + Sylleptic shoots)

The rootstock, scion and GA main effects for the mean total length of SAS were significant, each at *P=0.0001*. The mean total length of the SAS for 'M.9' scion was reduced compared with 'RG' scions (Table 2.8), and 'M.9' rootstock reduced the mean total length of SAS compared with the 'RG' rootstock. Foliar sprays of gibberellins significantly increased their mean total length (Table 2.8).

There were rootstock × scion (*P=0.001*) and scion × GA (*P=0.02*) interactions for the mean total length of SAS. For 'RG' scion on 'RG' rootstock, the mean total length of SAS was significantly higher compared with 'RG scion on the 'M.9' rootstock (Figure 2.14). On the contrary, the 'M.9' scion on 'RG' and 'M.9' rootstocks had a similar mean total length of SAS, but significantly less compared with 'RG' scions on 'RG' and 'M.9' rootstocks (Figure 2.14).

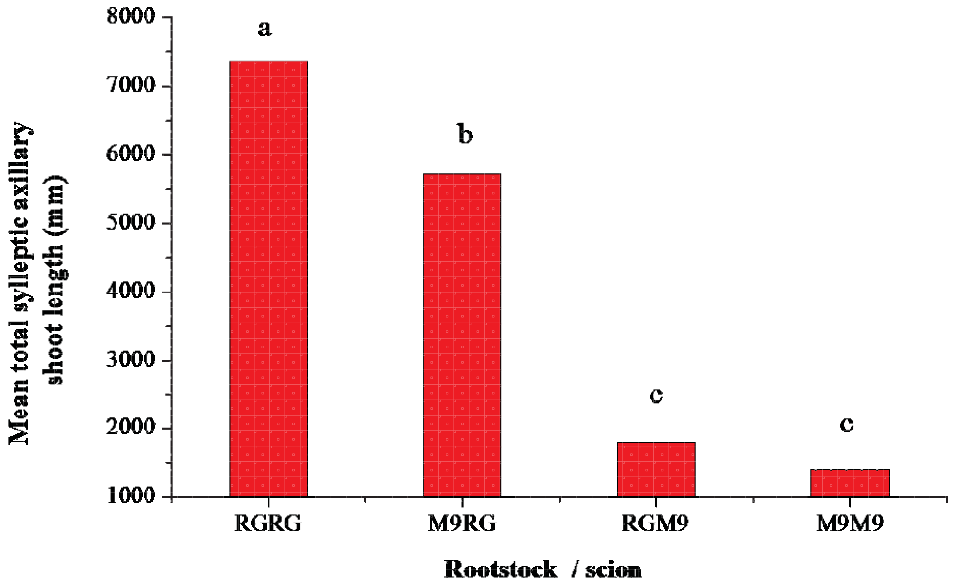

Figure 2.14. Rootstock × scion interaction on mean total length of sylleptic axillary shoots of composite trees 'RGRG' and 'M9RG' with 'RG' scion; 'RGM9' and 'M9M9' with 'M.9' scions (rootstock/scion combination). Means sharing the same letter are not different at *P=0.05* using Duncan's Multiple Range test. Means are averaged over GA treatment.

In addition, for the scion × GA interactions, the 'RG' scion with GA (Nov) and (Feb) had a significantly greater mean total length of SAS compared with 'M.9' scion. For 'M.9' scions, there was an increase with GA (Nov) foliar sprays, whilst the GA (Feb) treatment was statistically similar with untreated scions (Table 2.11).

Table 2.11. Scion × gibberellin interaction on the final mean total length of sylleptic axillary shoot (SAS) (*P=0.02*) and mean total shoot length (primary shoot plus SAS length) of 'M.9' and 'RG' scions (*P=0.02*) at the end of their first growing season (June, 2008) from grafting (August, 2007). –GA, GA (Nov) and GA (Feb) represent no gibberellin (GA_3+GA_{4+7}) foliar sprays and foliar sprays from November and February and continued till the end of the growing period. Means are averaged over rootstocks.

Scion	-GA	GA (Nov)	GA (Feb)
Mean total SAS length (mm)			
M.9	813.3 d	2518.3 c	1489.0 dc
RG	3946.3 b	7757.5 a	6613.6 a
Mean total shoot length of scion (mm)			
M.9	1942.1 d	3974.2 c	2861.5 dc
RG	5502.0 b	9760.8 a	8563.6 a

Means sharing the same letter within a single growth attribute are not significantly different at *P=0.05* using Duncan's Multiple Range test.

2.4.2.4 Treatment effects on the length and node number of Sylleptic shoots for 'RG' scions

The length of sylleptic shoots for 'RG' scions on 'M.9' and 'RG' rootstocks increased as the node number increased. Thus the internode length also increased. There was a strong positive correlation between length and node number of sylleptic shoots (Figure 2.15).

For untreated 'RG' scion on 'M.9' rootstock the length of each SS increased as the node number increased with 94% correlation between node number and SS length (Figure 2.15A), whereas for 'RG' scions on 'RG' rootstocks, there were more SS (0.6-1.0m) and the relationship between node number and length was 77% (Figure 2.15B). Gibberellin foliar sprays from November increased the number as well as the length of SS for 'RG' scions on 'M.9' which was equal with that on 'RG' rootstock (Figures 2.15C&D) and, the correlation was more than 90%. Similar correlation was observed among GA treated SS from February onwards (Figures 2.15E&F). Compared with untreated scions, GA significantly increased the mean total length of sylleptic shoots for 'RG' scions on 'M.9' rootstock (Table 2.8). The internode lengths can be compared from the regression line of data on each graph by dividing a y-value by its corresponding value of × from figure 2.15. Therefore, for sylleptic shoots the length increased as the node number increased, increasing internode length.

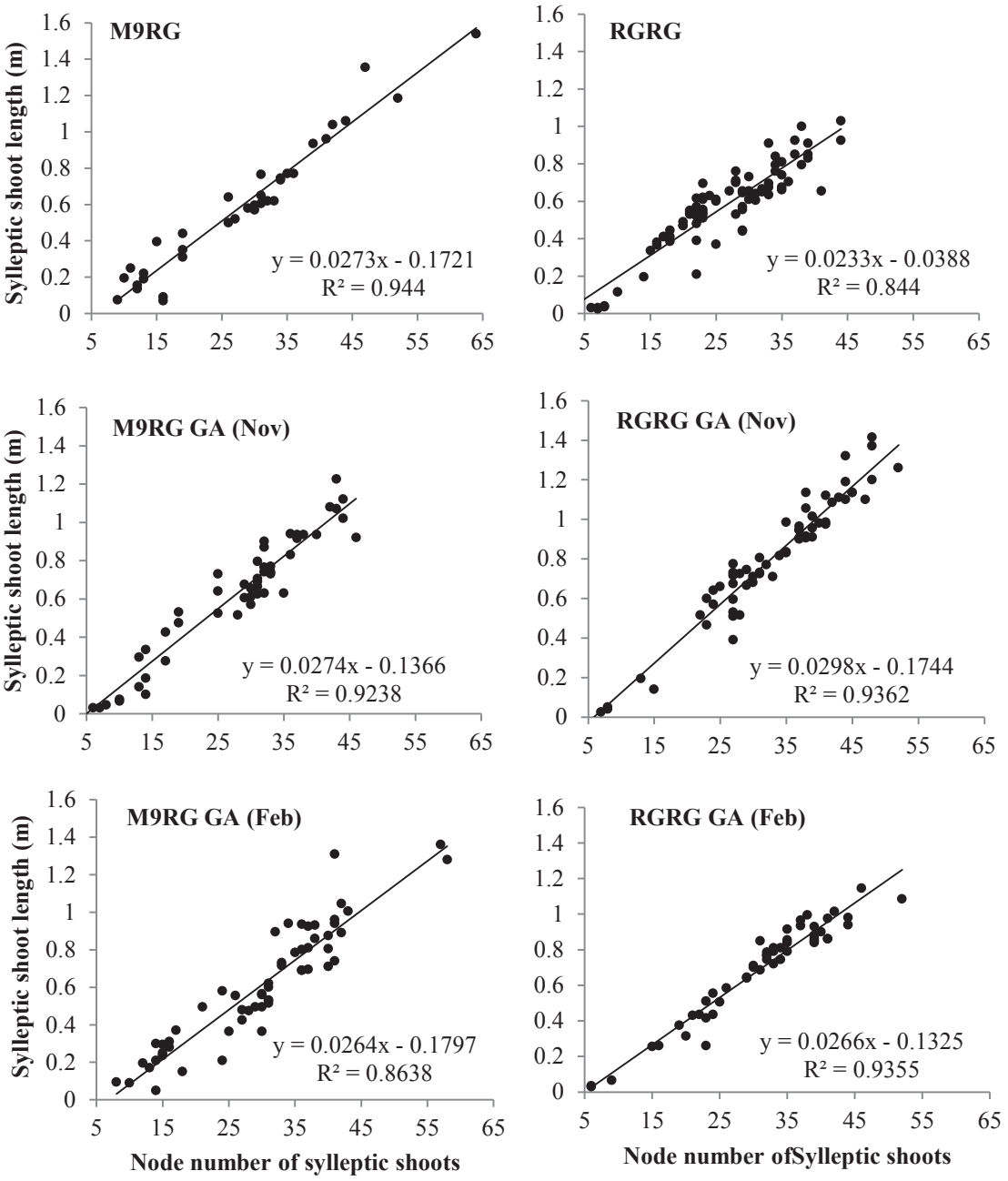

Figure 2.15. Effect of 'M9' and 'RG' rootstocks ('M9RG' and 'RGRG') , GA (Nov) foliar sprays ('M9RG (Nov)' and 'RGRG' (Nov) and GA (Feb) ('M9RG' (Feb) and 'RGRG' (Feb) foliar sprays on the relationship between SS length and node number for 'RG' scion on 'M.9' (left) and 'RG' (right) rootstocks. GA (Nov) and GA (Feb) represent the GA (GA_3+GA_{4+7}) foliar sprays from November and February, respectively and continued till the end of the growing season (May, 2008).

2.4.2.5 *Treatment effects on the total length and node number of sylleptic axillary shoots (spurs + Sylleptic shoots) for 'M.9' scions*

The total length and node number of untreated 'M.9' scions on 'M.9' and 'RG' rootstocks was less compared with those of treated scions (Figure 2.16). However, there was a strong positive correlation (80 and 90%) between the total node number and total length of SAS for 'M.9' scions on 'M.9' and 'RG' rootstocks (Figure 2.16 A&B). As total node number increased, total length of SS increased. Therefore, the GA effect for 'M.9' scions was both on node number and extension growth of SS.

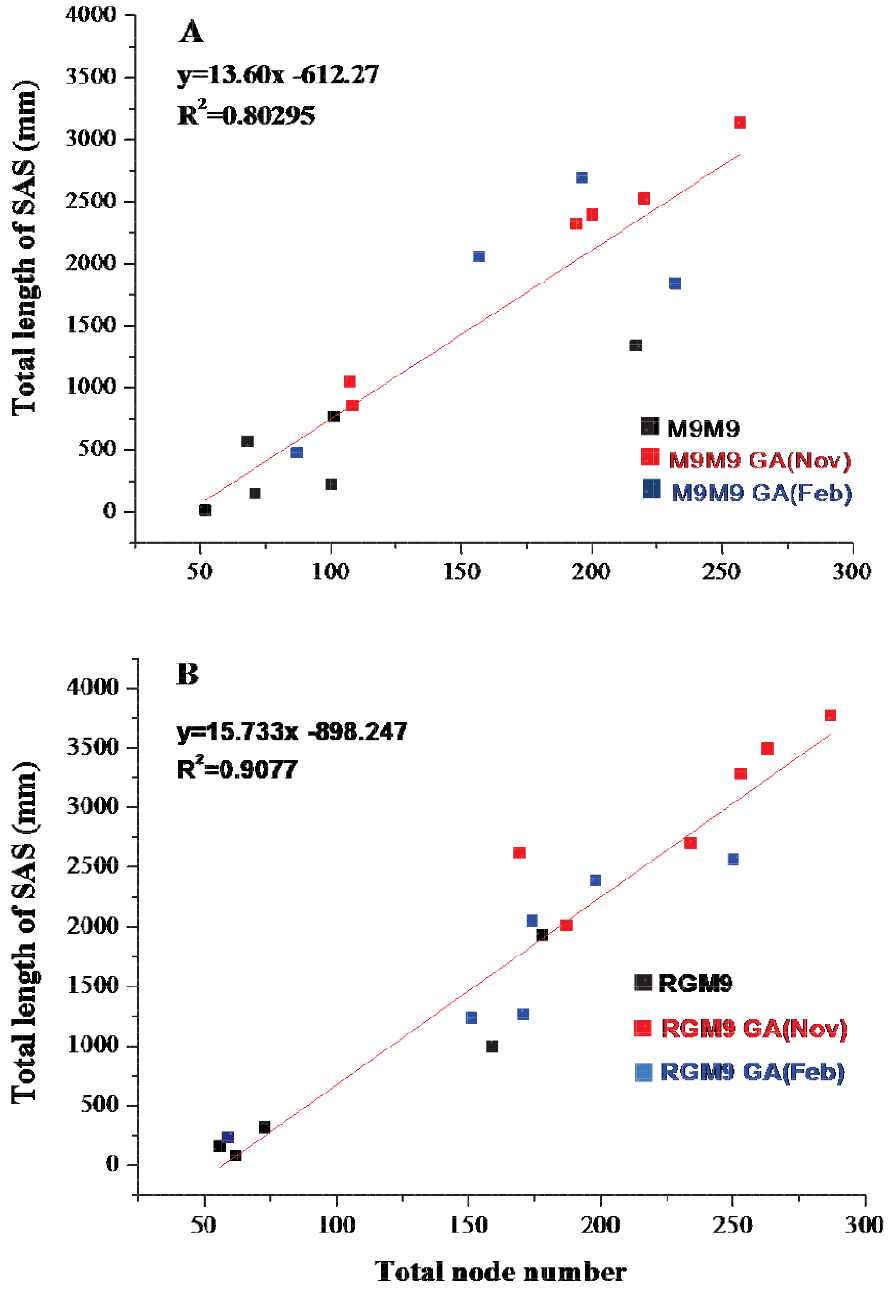

Figure 2.16. Effect of rootstock 'M.9' (A) and 'RG' (B) each without GA (GA_3+GA_{4+7}) and with GA (Nov) and GA (Feb) foliar sprays on the relationship between mean total node number and mean total length of SAS of individual tree replicates of 'M.9' scions at the end of the growing season (June,2008). GA (Nov) and GA (Feb.) are the gibberellin foliar sprays from November and February continued till the end of the growing season.

2.4.3 Treatment effect on the total scion growth

2.4.3.1 *Treatment effect on the mean total shoot length of scion (Primary shoot + SAS)*

For mean total shoot length of scion there were rootstock, scion and GA treatment effects (Table 2.8). Therefore, 'RG' scions grew greater than 'M.9' scions irrespective of the rootstock, 'M.9' rootstock significantly reduced the total shoot growth compared with 'RG' rootstock and, with gibberellin foliar sprays the scions were longer than untreated scions. In addition, for the mean total shoot length i.e., length of primary shoot and SAS together, there were rootstock × scion interactions (*P=0.001*). The rootstock effect on vigour depended on scion genotype. With 'RG' scion, the total length was greater for 'RG' rootstock than 'M.9' (Figure 2.17). However, the total shoot length was similar for 'M.9' and 'RG' rootstock when 'M.9' was the scion (Figure 2.17). The scion GA interactions were also significant for the total scion length at *P=0.02* (Table 2.11).

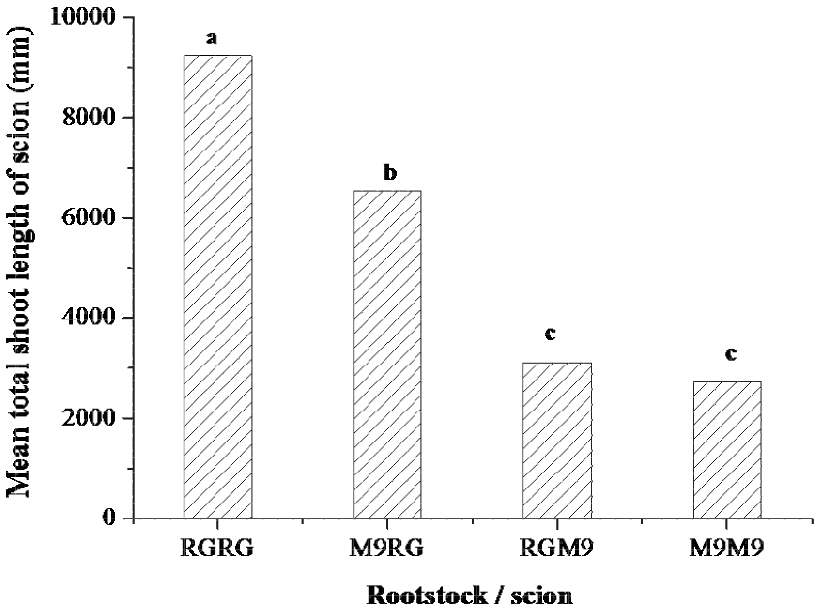

Figure 2.17. Effect of rootstock scion interaction on mean total shoot length (mm) of 'M9' or 'RG' scions on 'M9' or 'RG' rootstocks at the end of the growing season. The composite trees 'RGRG' and 'M9RG' are with 'RG' scion; 'RGM9' and 'M9M9' are with 'M.9' scion. Means sharing the same letter are not different at *P=0.05* using the Duncan's Multiple Range test. Means are averaged over GA treatments.

Therefore, the total shoot growth of 'RG' scion on 'RG' was significantly greater compared with that of 'RG' scion on 'M.9' rootstock (Figure 2.17). The gibberellin foliar sprays to the scion clearly revealed the fact that GA foliar sprays made 'M9RG' composite tree behave more like 'RGRG'. The composite trees 'RGRG' without GA, 'M9RG' and 'RGRG' with GA looked similar compared to 'M9RG' without GA.

The size of 'M.9' was similar whether scion grown on dwarfing or vigorous rootstock (Hypothesis II: Scion effect). There was no difference in mean total length of SAS (Figure 2.14) and mean total shoot length for 'M.9' scion on 'M.9' and 'RG' rootstocks (Figure 2.17). The number of sylleptic shoots (Table 2.9) and the mean total length of SAS for 'M.9' scion on both rootstocks were significantly less (Table 2.14) compared with 'RG' scions.

The Primary shoots length for 'M.9' scions on "M.9' and 'RG' rootstocks were almost similar (1091.66 mm and 1165.83 mm respectively) and, node number (63.33 and 66.83 respectively) was not significantly different (Table 2.4 and 2.5). There was no rootstock effect on primary shoot termination. It was not delayed by 'RG' rootstock for 'M.9' scion as it delayed one month for 'RG' scions (Table 2.7). Shoot cross sectional area was also significantly less compared with 'RG' scions but for 'M.9' scions, it was similar on both rootstocks (Figure 2.13). Although sylleptic shoots formed were more for 'M.9' scions on 'RG' rootstocks, they were not significantly different (Table 2.9). Mean total SAS length and mean total shoot length of 'M.9' scions on 'M.9' and 'RG' rootstocks were also not significantly different (Figure 2.14 and 2.17). Therefore, there was no difference between the total shoot length of untreated 'M9M9' and RGM9' composite trees. The 'M.9' scion behaved similarly with dwarfing as well as vigorous rootstocks.

2.4.4 Overview of growth response

2.4.4.1 *Overview of 'RG' scion growth in response to rootstock and GA foliar sprays (Rootstock effect: Hypothesis I)*

The mean primary shoot length of 'RG' scion on 'M.9' rootstock without GA was shorter *(P=0.05)* compared with 'RG' scion on 'RG' rootstock (Table 2.12). The dwarfing rootstock promoted termination of primary shoots of 'RG' scions one month earlier (during May) compared with 'RG' rootstocks (Table 2.12). While 100% primary shoots of 'RG' scions on 'M.9' rootstocks had terminated, 30% of 'RG' scions were still growing on 'RG' rootstocks (Table 2.12). The GA foliar sprays from November as well as February delayed termination and prolonged growth of primary shoot with higher shoot growth rate and rate of node formation (Figure 2.8A & 2.9A) from 12th January to 19th March, during the period at which untreated scions delayed the extension growth and plastochron. As a result, 'RG' scions on 'M.9' rootstock with GA continued growth and reached statistically similar length with 'RG' scion on 'RG' rootstock (Table 2.12). Therefore, the reduction in the final length and node number of primary shoot of 'RG' scion on the dwarfing rootstock ('M.9') was reversed by GA foliar sprays and this was a bigger effect of GA for 'RG' scions on 'M.9' rootstocks. Although, primary shoot termination of 'RG' scions on 'M.9' rootstocks started from 15/4/08, the actual dwarfing effect could be noticed from Jan 12th with decreased rate of node production and growth rate (Figure 2.8A and 2.9A).

For 'RG' scions, the number of spurs was reduced compared with 'M.9' scions and there was neither rootstock nor GA treatment effect on the formation of spurs. However, the number and mean total length of sylleptic shoots was reduced for 'RG' scion on dwarfing rootstock 'M.9' (Table 2.12) compared with 'RG' rootstock control. All sylleptic shoots of 'RG' scions on both rootstocks had terminated by April 30th, two weeks earlier than their primary shoots. The total shoot growth (primary shoot and SAS together) for 'RG' scions on 'M.9' rootstocks was reduced compared with 'RG' scion on 'RG' rootstock. The GA (Nov) foliar sprays increased the total shoot growth of all 'RG' scions on 'M.9' rootstocks by increasing primary shoot length, node number and

internode length (Table 2.12). Thus, the total shoot growth of 'RG' scion on 'M.9' rootstock with GA was similar with 'RG' scion on 'RG' rootstock with GA (Nov) treatment and greater than 'RGRG' without GA (Table 2.12).

Thus, the dwarfing effect of 'M.9' rootstock expressed on 'RG' scion was reversed by GA foliar sprays by increasing the total shoot growth with more nodes and longer internodes and delaying the termination of both primary and sylleptic shoots.

2.4.4.2 *Overview of 'M.9'scion growth in response to rootstock and GA foliar sprays (Scion effect: Hypothesis II)*

For 'M.9' scions, whether grafted to a dwarfing ('M.9') or vigorous ('RG') rootstock, the growth was similar (Table 2.13). The final mean length of 'M.9' scion on 'M.9' rootstock was not significantly different compared with 'M.9' scion on 'RG' rootstock (Table 2.13). There was no 'RG' rootstock effect on prolonging shoot growth as 70-85% of 'M.9' scions on both rootstocks had terminated by 15th April and 100% by 30th April, 2008. Irrespective of the rootstock, the 'M.9' scions produced similar node number and internode length (Table 2.5 and 2.6). However, the GA foliar sprays partially reversed 'M.9, scion effect and increased the mean final length, node number and internode length of primary shoots significantly (Table 2.5, 2.6 and 2.13) compared with untreated 'M.9' scions. Although there was significant GA effect on 'M.9' scions on both rootstocks, they were still compact relative to 'RG' scion.

The 'M.9' scion significantly increased the total number of spurs compared with 'RG' scions (Table 2.9). The mean total number of sylleptic shoots was less compared with 'RG' scions. However, the number of sylleptic shoots produced for 'M.9' scion on 'RG' rootstock was more compared with those that were on 'M.9' rootstocks (Table 2.13). The GA foliar sprays had no effect on the mean number of spurs on 'M.9' scions. For 'M.9' scions on 'RG' rootstocks the GA (Nov) increased sylleptic shoots. The mean total length of SAS for M.9 scions on 'M.9' and RG rootstocks was significantly (*P=0.0001*) reduced compared with RG scions on 'RG' and M9 rootstocks (Scion

effect: Hypothesis II) (Table 2.12 and 2.13). Approximately 90% of sylleptic shoots had terminated by 15/2/08 for 'M.9' scions on both dwarfing and vigorous rootstocks (Table 2.10). Therefore there was no rootstock effect on the termination of sylleptic shoots of 'M.9' scions. The total shoot growth (primary shoot + SAS) was less for M.9 scions on both dwarfing and vigorous rootstock (Table 2.13) compared with RG scions. Although, there was significant GA treatment effect for 'M.9' scions compared with the untreated scions, the GA effect did not change the size of the composite trees similar to the changes that occurred for 'RG' scions on 'M.9' rootstocks. The architecture of composite apple trees with 'M.9' scions ('M9M9' and 'RGM9') did not change to that of 'RGRG' with gibberellin treatment.

Therefore, the growth of 'M.9' scion irrespective of the vigour of the rootstock remained dwarfed, and the gibberellin foliar sprays significantly increased the total shoot length, but did not modify the architecture of 'M.9' scion compared with 'RG' scion.

Table 2.12. Rootstock and GA treatment effects on the final length of primary shoot, number and length of sylleptic shoots, total shoot growth, termination dates and % of shoots terminated on given dates of 'RG' scions (Hypothesis I).

Treatment	Primary shoot (A)		Sylleptic axillary shoots (B)				Primary + SAS (A+B)
	Length (mm)	Termination date (%)	No. of spurs <25mm	Sylleptic shoot ≥25mm			
				Mean number	Mean Total length(mm)	Termination date (%)	
M9RG (-GA)	1463.3 c	May 15th (100%)	4.2ns	5.6 b	2386.6 c	April 30th (100%)	3850 a
M9RG (GA Nov)	2066.6 a	June 5th	4.0	10.3 a	6979.1 ab	June 5th (83%)	9045 d
M9RG (GA Feb)	1923.0 a	June 5th	5.8	8.0 ab	4839.0 b	June 5th (95%)	6762 c
RGRG (-GA)	1654.1 b	May 15th (70%)	4.7	10.6 a	6400.8 ab	April 30th (100%)	8055 b
RGRG (GA Nov)	1940.0 a	June 5th	4.8	10.8 a	8535.8 a	June 5th (78%)	10475 d
RGRG (GA Feb)	1972.5 a	June 5th	5.7	11.8 a	8092.5 a	June 5th (84%)	10065 d

Means sharing the same letter within the same column are not significantly different at *P=0.05* using the Duncan's Multiple Range test. ns represents non-significant

Table 2.13 Rootstock and GA treatment effects on the final length of primary shoot, number and length of sylleptic shoots, total shoot growth (A+B), termination dates and % of shoots terminated on given dates of 'M.9' scions (Hypothesis II).

Treatment	Primary shoot (A)			Secondary axes (B)			Primary + SAS (A+B)
				Sylleptic shoot ≥ 25mm			
	Length (mm)	Termination date (%)	No. of spurs <25mm	Mean number	Mean total length (mm)	Termination date (%)	
M9M9	1091.6 c	April 30th (100%)	25.3 ab	3.3 b	509.0 a	April 15h (98%)	1600 c
M9M9 (GA Nov)	1430.8 ab	June 5th	23.1 ab	6.3 ab	2048.3 b	May 15th (93%)	3479 ab
M9M9 (GA Feb)	1517.5 b	June 5th	27.2 a	6.5 ab	1775.0 ab	May 15th (95%)	3292 ab
RGM9	1165.8 c	April 30th (70%)	23.8 ab	5.7 ab	1117.5 ab	April 15h (97%)	2283 bc
RGM9(GA Nov)	1480.8 a	June 5th	19.6 b	11.6 a	3044.0 c	May 15th (96%)	4575 a
RGM9 (GA Feb)	1275.8 bc	June 5th	20.6 ab	6.5 ab	1298.3 ab	May15th (100%)	2569 bc

Means sharing the same letter within the same column are not significantly different at P=0.05 using the Duncan's Multiple Range test.

2.4.5 Treatment effects on the mean root dry weight at the end of the first growing season

2.4.5.1 *Treatment effects on the total dry weight*

There were significant rootstock, scion and GA main effects on the mean total dry weight of the root system (Table 2.14). The 'M.9' rootstock, 'M.9' scion and GA foliar sprays reduced the mean total dry weight of the root system.

Table 2.14 Main effects of rootstock ('M.9' and 'RG'), scion ('M.9' and 'RG') and gibberellin (±GA) foliar sprays on the dry weight of hard, medium and soft roots (different categories) of 'RG' and 'M.9' root systems at the end of the first season of growth (June 2008) after grafting.

Main effect		Root dry weight			
		Hard	Medium	Soft	Total
Rootstock	M9	14.59 a ***	17.48 b	31.87 a**	63.96 b***
	RG	32.51 b	21.49 a *	44.44 b	98.45 a
Scion	M9	18.31 b***	15.79 a	25.82 a***	59.93 b***
	RG	28.79 a	23.18 b***	50.50 b	102.48 a
GA	-GA	24.74 ns	20.79 ns	42.71 a*	88.25 a**
	+GA	22.35 ns	18.19 ns	33.60 b	74.15 b

Within each main effect only, means within a column sharing the same letter are not significantly different at $P=0.05$ using Duncan's Multiple range test. ns, *, **, *** represent non significant or significant at $P \leqslant 0.05$, *0.01*, and *0.001*, respectively.

The total dry weight of the 'M9' root system was significantly less than 'RGRG' when 'M.9' or 'RG' was the scion (Figure 2.18). The dry weight of the 'RG' root system was significantly greater with 'RG' than the 'M.9' scion. Therefore, the rootstock was influenced by the type of scion grafted thereon. The mean total dry weight of 'M9M9', 'RGM9' (rootstocks/scion combination) and 'M9RG' were statistically similar and all these differed significantly compared with 'RGRG' at $P=0.0001$ (Figure 2.18).

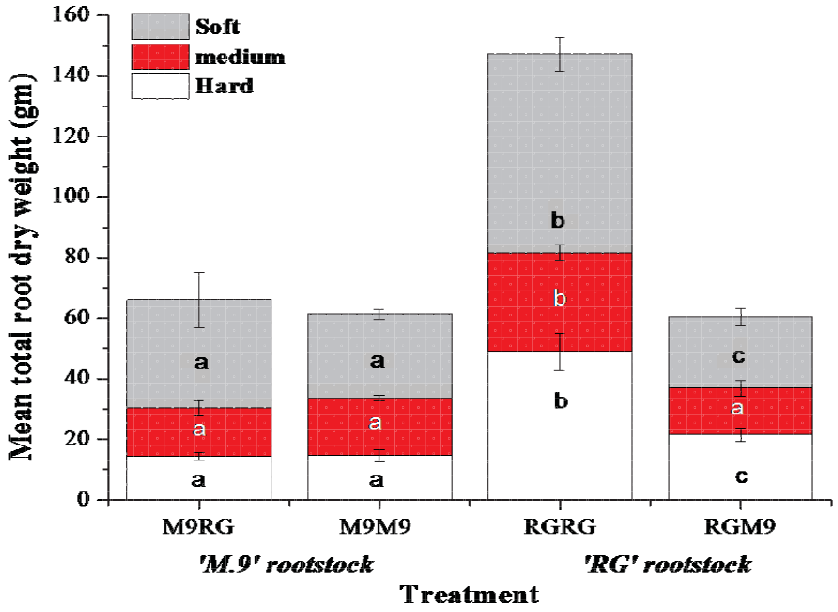

Figure 2.18. Rootstock × scion interactions on the mean dry weight of soft, medium and hard roots for composite trees of 'M9RG', 'RGRG'; M9M9, RGM9 (rootstock/scion combination). Vertical bars are the standard error. For the same category of roots, columns with the same letter are not significantly different at *P=0.05* using Duncan's Multiple Range test. Data are averaged over GA treatment. Bars represent the standard error.

The scion × GA interactions for mean total dry weight of the root system were also significant (*P=0.03*). The mean dry weight of the root system was less when treated with GA for 'RG' scions. There was no GA treatment effect on the root system for 'M.9' scions (Figure 2.19).

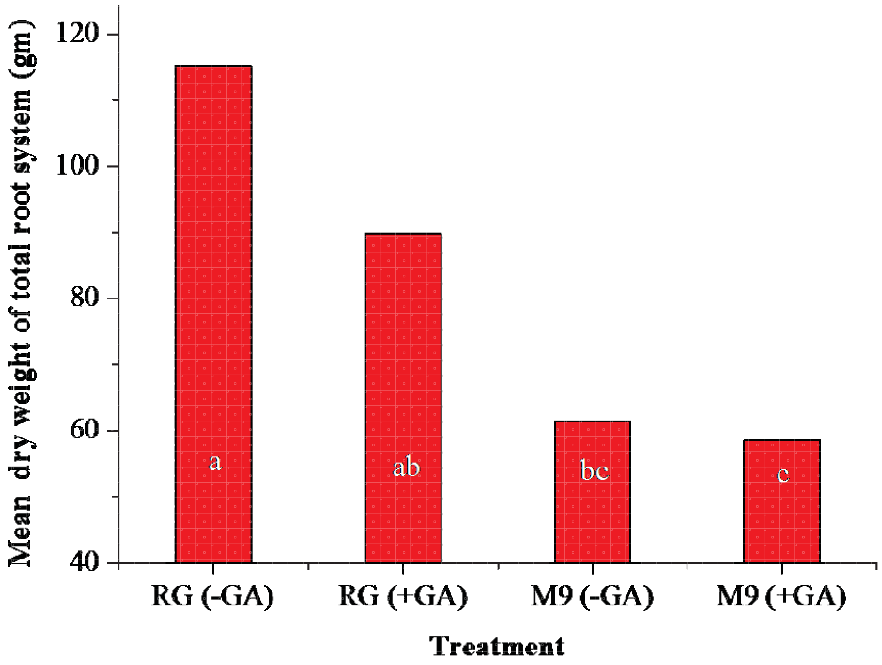

Figure 2.19. Effect of scion × GA interactions, on the mean dry weight of the total root system of 'M.9' and 'RG' rootstocks with (+GA) and without GA (-GA) foliar sprays (GA_3+GA_{4+7}). The vertical bars sharing the same letter are not significantly different at *P=0.05* using Duncan's Multiple Range test. Means are averaged over rootstocks.

2.4.5.2 *Treatment effects on dry weight of hard roots*

The total root system was categorised into three groups such as hard, medium and soft roots and analysed separately for treatment effects. The interactions between scion × rootstock were significant at *P=0.0004* and, therefore, 'M.9' scion when grafted with the rootstock 'RG' had a lower mean dry weight of hard roots than RGRG (Figure 2.18).

2.4.5.3 *Treatment effects on dry weight of medium roots*

There was a significant rootstock, scion main effect (Table 2.14) and rootstock × scion interactions (Figure 2.18) on the dry weight of medium roots. Although 'RG' rootstock had a greater medium root dry weight compared with 'M.9' rootstock, the 'M.9' scion effect influenced 'RG' rootstock and reduced the dry weight of 'RG' medium roots.

Therefore, for all the composite trees where the rootstock or scion was 'M.9' there was a significant reduction in the mean dry weight of medium roots (Figure 2.18).

2.4.5.4 *Treatment effects on dry weight of soft roots*

There were rootstock × scion interactions (*P=0.0003*) on the dry weight of the soft root. The 'RG' rootstock (*P=0.0001*) increased the mean dry weight of the soft root compared with 'M.9' rootstock (Table 2.19). However, it was reduced (*P=0.04*) when 'M.9' scion was grafted with 'RG' (Figure 2.18). Therefore the effect of rootstock depended upon the type of scion with which it was grafted.

Therefore, in summary, the GA foliar sprays reduced the mean dry weight of all categories of roots for 'RG' root system. For 'M.9' root system, only soft root dry weight was reduced with GA (Table 2.14) and for other categories of 'M.9' roots there was no effect of GA treatment (Table 2.14).

2.4.6 Treatment effects on the first occurrence of flowering in the second spring after tree grafting

In the second spring from grafting, flower buds on the scion began to emerge on 10/9/08. Total flower cluster number on each scion was expressed as a proportion to the total bud number of that scion. Total bud number is equal to buds on primary shoot plus one terminal bud and, buds on sylleptic shoots plus one terminal bud per SS and each spur considered as one bud. All these buds together gave the total number of buds per scion.

The mean number of flower clusters per scion in the spring of the second year (October, 2008) after grafting (August, 2007) was greater for 'RG' scions on 'RG' rootstocks compared to 'RG' scion on 'M.9' rootstocks (Figure 2.20). For 'M.9' scions, the 'M.9' rootstock promoted more flowering compared with 'RG' rootstock (Figure 2.20). The flower cluster emergence on the scion depended upon the type of scion-rootstock combination (Figure 2.21). Only when 'RG' was grafted with 'RG' there was a higher number of flower clusters (15.2%) than when it was grafted to 'M.9' scion (6.57%). The

'M.9' scion produced more flower cluster (7.8%) when grafted to 'M.9 than when 'RG' scion was grafted (0.2%). In addition, GA foliar sprays completely inhibited flower cluster formation (data not shown).

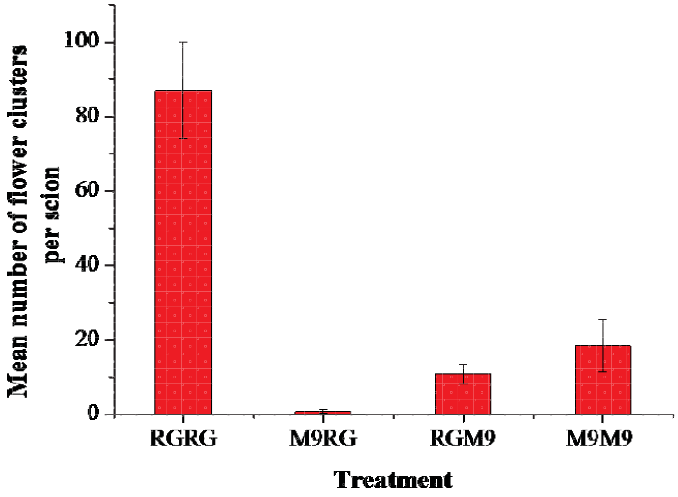

Figure 2.20. Mean number of flower cluster per scion for the composite trees RGRG, M9RG (with 'RG' scion); RGM9, M9M9 (with 'M.9' scion) during the second spring (October, 2008) from grafting (August, 2007) values are means with standard error bars. GA treatments are excluded because no flowers were present.

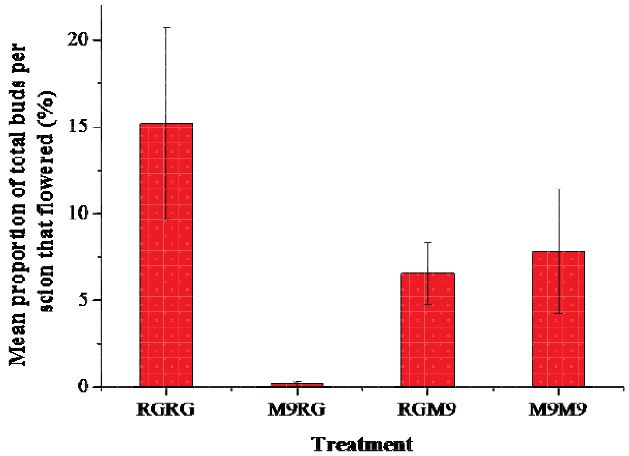

Figure 2.21. The mean proportion of total buds per scion that were floral for the composite trees RGRG, M9RG (with 'RG' scion); RGM9, M9M9 (with 'M.9' scion) during the second spring (October, 2008) from grafting (August, 2007) values are means with standard error bars. GA treatments are excluded because no flowers were present.

2.5 Discussion

The most important objectives of this chapter were:

- To elucidate the architectural changes that a dwarfing rootstock 'M.9' imposed on 'RG' scions and the timing that these changes modified scion architecture following tree grafting.
- To quantify what aspects of growth could be reversed by spraying gibberellins (GA_3 + GA_{4+7}) repeatedly to the scion.
- To determine whether a vigorous rootstock, such as 'Royal Gala' can stimulate the growth of an 'M.9' scion.
- To stimulate internode elongation and node neoformation of 'M.9' shoots using exogenous gibberellins to ascertain whether the compact nature of the 'M.9' shoot is a result of its inability to synthesise bioactive gibberellins.

In this experiment reciprocal grafting resulted in two groups of composite trees: one with 'RG' as scion and the other with 'M.9' as scion on 'M.9' and 'RG' rootstocks ('M9RG', 'RGRG'; 'M9M9', 'RGM9' represented in rootstock/scion combination) to elucidate the rootstock and scion dwarfing types i.e., Hypothesis I and 2 respectively. Both types of dwarfing involved gibberellins; where the 'RG' scion was dwarfed may be because of lack of gibberellin precursors produced by the rootstock (rootstock dwarfing: Hypothesis 1) and the other, where the 'M.9' shoot may be putatively unable to convert root produced gibberellins to bioactive GA (scion dwarfing: Hypothesis 2).

One of the objectives of this chapter was to determine when a dwarfing rootstock induced dwarfing of scion after grafting of the composite tree. In this experiment, two different growth patterns were observed during the first growing season from grafting (August, 2007). During spring and early summer (early growth) i.e., from October, 2007 to January, 2008, the growth of scions on dwarfing rootstocks was more vigorous compared to the later stages of the growth i.e., from mid-summer to the end of the growing season (late growth). Therefore, in the following sections, early growth and late growth patterns will be discussed separately.

2.5.1 Early growth of the primary shoot and effect of gibberellin foliar sprays

The longer primary shoots of 'RG' scion on 'M.9' than those on 'RG' rootstock, similarly, the longer primary shoots of 'M.9' scion on 'M.9' rootstock than those on 'RG' rootstock until the 2nd week of January (Figure 2.2A) during the early spring and midsummer (Oct, 2007- Jan, 2008) showed that the shoot may not be gibberellin deficient. Additionally, during this early period of growth, there was no GA effect on increasing the length of the scion (Figure 2.3). This also suggested that during the early growth period the dwarfing rootstocks were not deficient in GA.

The slow growth for scions on dwarfing rootstocks became apparent from January 2nd week onwards to the end of the growing season (June, 2008) by decreasing the shoot length (Figure 2.2B), shoot growth rate (Figure 2.8) and rate of node neoformation (Figure 2.9) of scions, finally leading to early termination of active growth. Thus, the dwarfing rootstock effect became evident from the middle of the first growing season (from 12th of Jan, 2008). The rootstock and/or scion effect on the final stages of the growth will be discussed later in this chapter (see section 2.5.2). The following paragraph discusses the possible reasons for a dwarfing rootstock to make the scions grow faster during early growth.

The growth rate of an apple cultivar 'McIntosh' and 'RG' on 'M.9' was much higher in early summer than those on a vigorous rootstock, but the former slowed down later in the season while the cultivars on vigorous rootstock grew rapidly (Colby, 1935; van Hooijdonk, 2009). Colby, (1935) observed the roots of 'M.9' rootstock started growth earlier than the vigorous rootstock and the small roots at the back of the root tips did not seem to be heavily suberised and probably they were not entirely dormant during winter. Therefore, it could be assumed that the faster growth of the scion on dwarfing rootstock, early in the growing season, may be due to its active root system at the beginning of the growing season. However, rootstocks do not differ in the mean total length and dry weight of the roots system from December to February (van Hooijdonk et al., 2011). Thus, the reason for faster growth of shoots on dwarfing rootstocks may be

attributable to its interaction with environment, which gives a better understanding of the physiological mechanisms involved.

In addition, when endogenous auxin levels in phloem and gibberellins in xylem, in low and high vigour apple seedlings were analysed, it was found that their levels were higher in low vigour than in the high vigour trees in the beginning of the growing season and decreased dramatically in low vigour trees and increased dramatically in high vigour trees later in the season (Crochowska et al., 1984) and, the high levels of auxin coincided with swelling of the terminal shoots. High levels of gibberellins were assumed to be produced in roots and the root system supplied GA to the aerial parts of the tree at the beginning of the growing season (Carr et al., 1964; Skene, 1967; Jones and Lacey, 1968). As the growth rate of the scion on the dwarfing rootstock was higher in the beginning of the season, it may be assumed that the levels of auxin in the shoot and gibberellins in roots were higher in the beginning of the growing season. As the GA foliar sprays during this early period of growth were not as effective in increasing the shoot length as they were in later stages of the growth, it supports the findings of Crochowska that the levels of GA were higher during the spring and early summer (October, 2007 – January, 2008) for dwarfing rootstocks. Growth of 'RG' scion on dwarfing rootstock and, 'M.9' scion on dwarfing and vigorous rootstocks began to slow gradually from the 2nd week of January (Figure 2.2B). The evidence of dwarfing effect of 'M.9' rootstock in the first growing season was supported by other studies that reported similar effect of dwarfing rootstock on the extension growth and final length of the primary shoot (Rao and Berry, 1941; van Hooijdonk et al., 2010).

2.5.2 Final growth of the primary shoot

Further objectives of this chapter were to elucidate the architectural changes imposed by a dwarfing rootstock (rootstock dwarfing) and/or an 'M.9' scion (scion dwarfing). All untreated 'M.9' scions were shorter than untreated 'RG' scions whether grafted onto dwarfing or vigorous rootstocks. This may partly be attributed to the 'M.9' scions beginning shoot termination one month earlier than 'RG' scions (Table 2.7). For 'RG'

scions on 'M.9' rootstock, the shoot extension growth on primary shoot was decreased from 19/2/08 onwards (Figure 2.5A). Notably, these decreases in primary shoot length preceded the shoot termination, which began at some point in time between 15/4/08 and 15/5/08. On the 15/5/08, 100% 'RG' scions on 'M.9' rootstocks had completed termination while, 30% 'RG' rootstock control shoots were still growing very slowly with growth rate of 0.24 mm^{-day}. The untreated 'RG' scion on 'M.9' rootstock was shorter (Table 2.4) compared with 'RG' rootstock control. Because rootstocks did not affect the mean internode length (Table 2.6) of the primary shoot, the decreased length of 'RG' primary shoot on 'M.9' rootstock resulted from slower growth of primary shoot and slower rate of node production from 12th Jan, 2008 (Figure 2.8 and 2.9) and early termination from 15th April (Table 2.1).

The primary shoots of untreated 'M.9' scions on 'M.9' rootstock ('M.9' control) were the shortest (Figure 2.11) and 'RG' scions on 'RG' rootstocks ('RG' control) were the longest of all the treatments. In the reciprocal grafting of 'M.9' scion on 'RG' rootstock and 'RG' scion on 'M.9' rootstock, the 'M.9' rootstock reduced the final mean length of 'RG' scion (Rootstock effect: Hypothesis I) and, on the contrary, the vigorous rootstock 'RG' had no effect on increasing the length of 'M.9' scion (Figure 2.11). This means that the length of the primary shoots of 'M.9' scion was not significantly affected by the vigorous rootstocks 'RG' as 'RG' scion was affected by 'M.9' rootstock. This reveals the significant scion effect and the intrinsic nature of the 'M.9' scion (Scion effect: Hypothesis II).

The final mean length for 'RG' scion on 'M.9' rootstock (Figure 2.10A) was reduced (Rootstock effect: Hypothesis I) compared with 'RG' rootstock control. Although there was no significant rootstock effect on node number and internode length, in the present work, it was clear that there was a strong correlation between node number and length of the shoot. The longer shoots had more nodes with longer internodes (Figure 2.7) whether 'M.9' or 'RG' scions. The fairly short primary shoots of 'RG' scions had similar internode length to that of 'M.9' shoots. Therefore, it was clear that shorter shoots on dwarfing rootstocks were short, not due to reduced internode length, but due

to reduced primary shoot length, which was mainly due to reduced node number and early termination. The dwarfing rootstock did not affect the internode length, but slowed down the rate of node production from 12th Jan, 2008 and promoted early termination. Therefore, the reason for dwarfing rootstocks to dwarf 'RG' scions may be attributed to a decrease in node number and to early termination. In contrast, there was no significant rootstock effect on the length and node number of the primary shoot of 'M.9' scions grafted onto 'M.9' and 'RG' rootstocks, although with 'RG' rootstock there was an increase in the average values. The growth of 'M.9' scion seemed to be independent of the vigorous rootstock 'RG' as 'M.9' scion maintained low vigour even on the vigorous 'RG' rootstock (Scion effect: Hypothesis II).

Therefore, dwarfing of 'RG' scion by 'M.9' rootstock occurred within the first year of growth. It actually started from 2nd week of January, even before the symptoms of termination were noticed. This occurred as change in vegetative growth. The primary shoot on 'M.9' had a slower rate of growth from January 12th towards the end of the growing season (15th May, 2008) with reduced rate of node neoformation (Figure 2.8B and 2.9B) and proceeded with termination of shoots from 15th April resulted in a primary shoot on 'M.9' that was shorter at growth cessation because of fewer neoformed nodes. Although, the growth of 'M.9' scion on both 'RG' and 'M.9' rootstock was reduced, there is limitation of one year assessment, which was not sufficient to draw conclusions over the growth of 'M.9' scion on 'RG' the vigorous rootstock.

2.5.3 Gibberellin effect on the final growth of primary shoot

Another objective of this chapter was to quantify what aspects of dwarfing effect on growth can be reversed by spraying gibberellins repeatedly to the scion. Gibberellin foliar sprays (GA_3+GA_{4+7}) given to the scions on 'M.9' and 'RG' rootstocks started at two different timings i.e., November and February and continued until the end of the season.

The GA foliar sprays increased the length and node number of both 'M.9' and 'RG' scions, but the increase in the length and node number of 'M.9' scions was less compared with 'RG' scions (Table 2.4, 2.5 and 2.6). The primary shoots of 'RG' scions on 'M.9' rootstock with GA were significantly longer with more neoformed nodes compared with those of untreated scions on 'M.9' rootstock (Table 2.5). The GA (Nov) sprays for 'RG' scions were effective from the time when the growth of the untreated scion was reduced (12th Jan, 2008). This shows that 'RG' scions probably were not deficient in GA from the beginning (October) till the middle of the growing season i.e., till 12th January (Figure 2.5A&B). From mid January, presumably when endogenous GA levels were reduced, the exogenous GA became effective in supplementing what was limited by the 'M9' rootstock. The GA (Nov) application was very effective for the 'RG' scion on 'M.9' rootstock compared to the 'RG' rootstock (control). This indicates that 'RG' scion on 'M.9' was deficient in GA from 12th Jan, 2008. The GA treatment kept the meristem active, prolonged the period of growth and increased the number of neoformed nodes with significantly longer internodes. There was significant GA treatment effect on the node neoformation, as the 'RG' scion on 'M.9' rootstocks produced more nodes with GA treatment than untreated scions (Table 2.5). For 'RG' scion, from January 12th to March 19th 'M.9' rootstock reduced the rate of node production (Figure 2.9A) compared to 'RG' rootstock and GA foliar sprays increased the rate of node production. In April and May both rootstocks reduced the plastochron rate at the same time, GA prolonged the meristematic activity and produced more nodes and longer shoots. The nature of dwarfing rootstock in producing a fewer node from 12th Jan, 2008 was reversed by GA application. There is extensive recent research on the involvement of GAs in induction of growth cessation (Eriksson et al., 2000; Eriksson and Moritz, 2002; Olsen, 2010); reduced levels of bio-active GA is a prerequisite for growth cessation, and the down regulation of *GA_{20} oxidase* enzyme for the rate limiting step in GA biosynthesis (Cooke et al., 2012). It was also found that reduction of bio-active GAs rather than the sensitivity towards GAs plays a major role in growth cessation (Hoffman, 2011).

The dwarfing rootstock stem reduces polar IAA transport from scion to root (Lockard and Schneider, 1981; Soumelidou et al., 1994a; Kamboj et al., 1997), which influenced the levels of root-produced hormones such as gibberellins and cytokinins (Kamboj et al., 1999a; van Hooijdonk et al., 2010) (see Chapter 1; Section 1.3.4.1). The significant response of 'RG' scion on 'M.9' rootstock to GA may be attributed to its lack of GA from rootstock. As gibberellins from roots have an effect on stem elongation (Crozier and Reid, 1971), the reason for shorter primary shoots can be attributed to GA deficiency in the scions on dwarfing rootstock. 'M.9' rootstock decreased endogenous gibberellin concentration in roots (Yadava and Lockard, 1977) and in xylem sap (Ibrahim and Dana, 1971). As meristematic activity of scion on 'M.9' rootstock was stimulated by exogenous GA, it would be reasonable to suggest the decreased meristematic activity of the scion on dwarfing rootstock resulted from reduced supply of gibberellins from dwarfing rootstock.

Until the middle of the growing season i.e., till 2nd week of January, the internode elongation was more for 'RG' scion on 'M.9' rootstock (Figure 2.5 A&B) compared with 'RG' rootstock (control). After this period, the extension growth rate of primary shoot for 'RG' scion on 'RG' rootstock increased. After the node emergence the extension of growth occurs below apical meristem (sub apical meristem), extending an internode. The internode elongation basically occurs in two ways: cell division and cell elongation. GA promoted cell division and increased cell elongation in sub apical meristem (Sachs et al., 1959; Kamijima, 1981; Jupe et al., 1988). The endogenous auxin and gibberellins are viewed as hormones that promote cell division (Kende and Zeevert, 1997). Exogenous gibberellin can increase sub-apical meristem activity, thereby increasing internode length during node neo-formation (Little and MacDonald, 2003). There was a difference of opinion regarding the activity of GA on the internode elongation. GAs may contribute to elongation of internode by promoting cell division (Sachs, 1965; Brown et al., 1994; Ripetti et al., 2008), or by cell elongation (Sachs, 1965; Ripetti et al., 2008). However, in this experiment the mean internode length of primary shoot for 'RG' and 'M.9' scions was significantly increased by GA application compared with those of untreated shoots. The shoot length increased as node number

increased which increased internode length. The Figure 2.7A suggests shoots with GA developed more neoformed nodes and as a result had longer internodes. Therefore, to find out whether the GA signalling in apple trees acted through cell division and/or cell elongation, histological studies were performed using confocal microscopy (see Chapter 3). Thus, gibberellin foliar sprays to 'RG' scion on 'M.9' rootstock could reverse the dwarfing effect of 'M.9' rootstock for 'RG' scion. Now, in the following paragraph, the growth of 'M.9' scion on a vigorous rootstock and the effect of GA will be discussed.

The 'M.9' scion on 'RG' rootstock maintained its natural growth habit and was unchanged by the vigorous rootstock. The effect of GA on primary shoot was similar for 'M.9' scions on both rootstocks (Table 2.13) and less compared with 'RG' scions. Conversely, the effect of GA foliar sprays was almost 50% more for 'M.9' scions on 'RG' rootstock compared with 'M.9' scion on 'M.9' rootstock (Table 2.13). Endogenous gibberellins (GA_{19}), are transported through the xylem sap of apple trees (Motosugi et al., 1996), which indicates that gibberellins are produced in roots and are supplied to shoots (Jones and Lacey, 1968). The untreated 'M.9' scion on 'M.9' and 'RG' rootstocks were almost similar in size (Table 2.13). Although vigorous rootstocks increased endogenous concentration of gibberellin-like substances in the root (Yadava and Lockard, 1977), the 'M.9' scion may be less efficient in utilising gibberellin precursors coming from 'RG' rootstock. As a result, 'M.9' scion even after being grafted onto a vigorous rootstock remained dwarfed. This observation supports the work of Bulley, (2005) where the gene encoding *GA-20 oxidase* (*GA20ox*), the gibberellin biosynthetic enzyme, was suppressed and reduced the levels of bioactive gibberellins in the dessert apple variety 'Greensleeves', resulted in significant reductions in stem height. When such a scion, with suppressed gene encoding *GA20ox* was grafted onto an invigorating 'MM.106' and 'M.25' rootstocks it remained dwarfed. This reduction in the stem height may be due to the inability of the scion to convert the GA precursors (GA_{19}) from root to bioactive gibberellins (GA_1) in the shoot as the biosynthetic enzyme *GA20ox* was suppressed. The same reason may be applicable to 'M.9' scion which remained dwarfed on 'RG' rootstock, that the 'M.9' shoot had no capacity to activate GA precursors (GA_{19}) to bioactive GA_1 in order to stimulate growth. Although

for dwarfed 'Greensleeves' scion the effect was reversed with the application of GA_3 (Bulley et al., 2005), for 'M.9' scion the compact growth habit was not fully reverse with gibberellin foliar sprays. If 'M.9' shoot was deficient in *GA20ox* as 'Greensleeves' shoot, it would have responded to GA as the shoot system's inability to make bioactive GA had been overridden by applying GA foliar sprays to the scion. For 'RG' on 'M.9' rootstock the response was great and the dwarfing effect was reversed. Therefore, there may be other possible reasons other than being deficient in GA for the compact nature of 'M.9' scion.

The vigorous rootstock 'RG' could not change the growth of 'M.9' scion. This may suggest that the intrinsic properties of 'M.9' scion were interfering with vigour modifying signals from the rootstock. This observation once again supports the statement that the rootstock is not an important source of bioactive GAs (Bulley et al., 2005). The reason for 'M.9' scion making the 'RG' rootstock effect not significant or ineffective opens several hypotheses to be studied in the future. At this point the successful work done at Massey University, New Zealand (van Hooijdonk et al., 2010) explains clearly that the IAA hormonal signal from shoot to root is required for root growth and to produce GA precursors and cytokinins that are translocated to the shoot. Therefore in this experiment, it is highly likely that the 'M.9' scion reduced the amount of IAA from shoot to root, which reduced the root signalling to influence the shoot growth. As the composite tree with 'M.9' scion on 'RG' rootstock had a similar size root system to the composite tree with 'RG' scion on 'M.9' rootstock (Figure 2.18), this further suggests that auxin flow from 'M.9' was limiting growth of 'RG' roots.

Other possible reasons for 'M.9' scion to maintain its growth habit on a vigorous rootstock may be that the genetic expression in shoot apical meristem might have repressed gibberellin signalling by synthesising *GA2 oxidase* at the base of the apical meristem which prevented GA transport from the nearest young leaves to apical meristem or by the suppression of *GA 20 oxidase* which prevent the conversion of GA precursors (GA_{19}) from roots to bioactive GAs (GA_1) in the shoot (Sakamoto et al., 2003).

In addition, auxins in the apical meristem play an essential role in the initiation of leaf primordial and phyllotaxy (Reinhardt et al., 2000; 2003). For node initiation and elongation of internode, apical and sub apical meristem are involved respectively. The IAA concentration may be reduced if GA was deficient. as gibberellins are involved in the increase of the biosynthesis of IAA (Law, 1987),Although GA foliar sprays increased the shoot length (Table 2.4) and node emergence (Table 2.5) of 'M.9' shoots on 'M.9' and 'RG' rootstocks, the size of 'M.9' scion on both rootstocks was not changed as it was changed for 'RG' scion on 'M.9' rootstock because the total shoot length of 'M.9' scion on both rootstocks was significantly less compared with 'RG' scions (Table 2.12 and 2.13). Therefore, it may be assumed that 'M.9' scion on both the rootstocks, reduced gibberellin supply as the auxin levels reaching roots was reduced. Although GA foliar sprays significantly affected the node emergence and elongation, the GA effect on 'M.9' scions was not as effective as it was for 'RG' scions. Perhaps gibberellins cannot act when IAA is low in the shoot. The second reason may be attributed to insufficient amounts of IAA, as the thick bark of 'M.9' stem with higher levels of IAA oxidases (Leopold and Kriedemann, 1975; Lockard and Schneider, 1981) decreased auxin levels, which are essential, as auxin and gibberellins interaction is required (Ross et al., 2000) for a node formation. The distribution of auxin may be required to down regulate KNOX gene expression to control the node production (Fleet and Sun, 2005). KNOX genes are expressed at the meristem where they exclude expression of the biosynthetic gene GA*20ox.* GA signalling could be reduced either by producing GA_2 *oxidase* or by reducing the production of GA_{20} *oxidase* as a result of which, the conversion of gibberellin precursors to bio-active gibberellins could be reduced. The activation and deactivation of gibberellins are tightly regulated by developmental hormonal and environmental signals which are consistent with the influence of GA on growth. It was reported that in intact pea plants, auxin from apical bud moves into the elongating internodes, where it regulates the production of *GA3oxidase* enzyme and deactivation of *GA2oxidase* enzyme (Ross et al., 2000). Therefore, the reduced growth of 'M.9' scion on 'RG' rootstock could be attributed to the reduced synthesis of auxin and activation of GA*2ox* at the SAM for meristematic activity or it could be due to reduced polar auxin transport (PAT) to the root system

which reduced the root produced GA precursors. Possibly the rapid destruction of active gibberellins may be due to over accumulation of GA*2ox,* a deactivating enzyme as there was no big response with exogenous gibberellins.

2.5.4 Growth of sylleptic axillary shoots

By the activity of shoot apical meristem, neoformed nodes were produced on the primary shoot throughout the growing period. Only a few of the axillary buds in the leaf axils of nodes developed into SAS and others remained as latent buds during the same growing season. Axillary bud outgrowth can be influenced by many factors such as environment and internal factors, including growth hormones.

'M.9' scions increased (main effect, $P \leq 0.001$) the mean total number of spurs compared with 'RG' scions (Table 2.8) and 'M.9' scions (main effect, $P=0.001$) and 'M.9' rootstock (main effect, $P \leq 0.007$) reduced the mean total number of sylleptic shoots. The total number of SAS and their total length was less for 'M.9' rootstock compared with 'RG' rootstock (Table 2.8). The onset of termination of sylleptic shoots on dwarfing rootstock was much earlier than those on vigorous rootstocks. The sylleptic shoots were shorter for 'RG' scions on 'M.9' rootstock and 'M.9' scions on both the rootstocks may be due to early termination (Table 2.10).

Endogenous cytokinins are thought to be involved in regulating sylleptic shoot formation, especially because BAP applied to young apple scions stimulated axillary buds along the primary shoot to break and form sylleptic axillary shoots (Williams and Stahly, 1968; Volz et al., 1994; van Hooijdonk et al., 2010). This may indicate that reduction in scion bud break was due to lack of root produced cytokinins. The 'M.9' rootstock reduced (main effect, $P=0.05$) the overall number of sylleptic shoots formed for 'RG' scion compared with 'RG' rootstock (control). However, in this experiment, it was found that during the time when growth of 'RG' scion on 'M.9' started to slow (from 12/1/08), the axillary bud break was higher and formed more sylleptic shoots. Previous studies indicated that decreased rates of IAA diffusion appeared to coincide

with the increased levels of ZR with an increase in the secondary growing points from February to April (van Hooijdonk et al., 2011). However, the number of buds broken for 'M.9' scions was more compared with 'RG' scions on 'M.9' and 'RG' rootstocks that developed into spurs (Table 2.9). The reason for more axillary bud break may be connected to a lack of apical dominance (Bangerth et al., 2000) or due to increased levels of ZR with decreased rates of IAA diffusion.

For 'M.9' scions, irrespective of the rootstock termination of sylleptic shoots started three months earlier and had a greater proportion of terminated shoots in March (Table 2.10). Furthermore, termination of all sylleptic shoots was completed one month earlier than 'RG' scion sylleptic shoots. Collectively, these changes by 'M.9' scion reduced total growth of the sylleptic shoots compared with 'RG' scions. The reason for the early termination of SS of 'M.9' scion on 'M.9' and 'RG' scion on 'M.9' may in part result from lack of bioactive GAs because GA_3+GA_{4+7} applied to young scions stimulated extension growth of SS.

2.5.5 Gibberellin effect -growth of sylleptic shoots

The increase in the length of sylleptic shoots with gibberellin treatment suggests that gibberellins were involved in the node emergence and elongation of the sylleptic shoots. This might be by activating the apical meristem, forming more nodes, and keeping the apical and sub-apical meristem active (see also Chapter 3). The sylleptic shoots of 'RG' scions responded to GA treatment and termination was delayed (Table 2.10) and the length and node number was increased, increasing the total shoot length (Table 2.12). Although, the GA treatment delayed termination of 'M.9' scions (Table 2.10) and increased total shoot growth, it was less compared with 'RG' scions. Therefore, the termination of SS of 'M.9' scions was much earlier than those of 'RG' scions (Table 2.10) suggests a different genetic capacity of 'M.9' scion to use gibberellins or GA may not be the primary mechanism limiting vigour of 'M.9' shoot.

A new mechanism controlling plant height in maize and sorghum dwarf mutants was identified (Multani et al., 2003). The mutant gene in dwarf maize encodes a protein

responsible for the auxin transport. As auxin regulates growth and developmental process in plants, its defective transport could affect the growth in plants (Salamini, 2003). When the model plant *Arabidopsis* was treated with the auxin transport inhibitor N-1-naphthylphthalamic acid (NPA) multiple morphological changes appeared and the plant resembled the PIN mutant phenotype. As the mutant gene in dwarf maize and sorghum encoded a protein for the transport of auxin now it can be suggested that the defective auxin transport through the stem part of 'M.9'dwarfing rootstock resulted in shortening shoots and dwarfism in the 'RG' scion grafted onto it.

2.5.6 Treatment effects on the first occurrence of flowering in the second spring after tree grafting

For the composite apple tree 'RGRG', the mean number of flower clusters per scion in the spring of the second year (October, 2008) after grafting (August, 2007) was greater compared with all other composite trees in this experiment (M9RG, RGM9, M9M9). The 'M.9' rootstock promoted more flowering clusters for 'M.9' scions, and, on the other hand, 'RG' rootstock promoted flowering for 'RG' scions (Figures 2.20 and 2.21). The flower cluster emergence on the scion depended upon the type of scion-rootstock combination (Figure 2.21). In addition, GA foliar sprays completely inhibited flower cluster formation.

Literature revealed that experiments published to date show that application of GA_3 and /or a mixture of GA_3 and GA_{4+7} inhibited flowering in apple trees (Guttridge, 1962; Marcelle and Sironval, 1963; Dennis Jr and Edgerton, 1966; Greenhalgh and Edgerton, 1967; Luckwill, 1970; Tromp, 1973; Luckwill, 1974; Tromp, 1982) although, this effect was influenced by timing (Tromp, 1973; Tromp, 1982) and by the type of GA applied. On the other hand there were reports that applied zeatin promoted flowering in apple (Ramirez and Hoad, 1981). Looney et al., (1985), reported that GA_4 alone or with zeatin promoted flowering of spurs of apple trees exhibiting a strong tendency to flower in alternate years. Chan and Cain (1967) found that seeded 'Spencer seedless' apples

inhibited the formation of flower buds on the same spurs, whereas seedless fruits did not.

Conversely, in this study scions treated with GA_3+ GA_{4+7} exhibited a greater inhibition of flowering in the second spring from grafting. As the inhibition of flowering due to GAs also depends upon the timing, the GA foliar sprays given from the early vegetative growth might have affected flower bud formation. When a mixture of GA_3+GA_{4+7} inhibited flowering, Looney et al., (1985) proved that GA_4 is not inhibitory. Thus, the reason for more number of flower clusters for 'RG' scions on 'RG' rootstocks may be the native apple GA, the GA_4 of 'RGRG'.

2.6 Conclusion

In conclusion the attention could be drawn towards the two types of dwarfism in this experiment. "***Hypothesis One***" suggested the dwarfism by the rootstock was because of the limitation of gibberellin precursors from root to shoot. This limitation may be due to reduced IAA transport through the stem part of dwarfing rootstock. This dwarfism was reversed by the application of gibberellins since 'Royal Gala' scion on 'M.9' dwarfing rootstock showed a very big response to GA_3+GA_{4+7} foliar sprays. Therefore the effect of gibberellins foliar sprays provides evidence to support that the 'Royal Gala' scion grafted on to 'M.9' rootstock may be deficient in bioactive gibberellins (GA_1) as the dwarfing rootstock could not supply sufficient precursors of GA_1 (GA_{19} and GA_{20}) from the root system.

"***Hypothesis Two***" suggested that the intrinsic block to growth within the 'M.9' shoot may be related to:

(a) The reduced basipetal auxin (IAA) transport through 'M.9' shoot to the root system (Soumelidou et al., 1994a; Kamboj et al., 1999b; van Hooijdonk; van Hooijdonk et al., 2010) affected root growth and increased the production of cytokinins in roots (Bangerth et al., 2000). Increased levels of cytokinins helped in increased bud burst on

the primary shoot of 'M.9' scions, most of which developed into spurs. This again confirmed that SAS terminated quickly forming spurs due to gibberellins deficiency.

(b) A poor ability of 'M.9' scion to transport root synthesised gibberellins.

(c) A poor ability of 'M.9' to convert gibberellin precursor (GA_{19}) from root to active GA_1 in the shoot.

(d) The rapid destruction of active gibberellins may be due to over accumulation of GA*2ox* a deactivating enzyme as there was no big response with exogenous gibberellins.

(e) Low levels of auxin at the apex in the 'M.9' shoot which made KNOX gene to be expressed to inactivate the gibberellins for the node initiation (Fleet and Sun, 2005).

(f) The presence of IAA oxidases and peroxidases and also the high ratio of ABA to IAA (Soumelidou et al., 1994a) in the bark of 'M.9' shoot make the levels of basipetal auxin transport to roots low.

It was made clear that decreased IAA levels from the shoot to the root through rootstock stem increased cytokinin levels that were associated with increased bud break from February onwards (except for January), and decreased GA precursors (GA_{19}) (van Hooijdonk et al., 2011) from root to shoot, which coincided with early termination of primary and secondary shoots on dwarfing rootstocks. However, in this experiment, the 'RG' scion on 'M.9' rootstock produced fewer SAS compared with 'RG' rootstock (control), which could not be explained satisfactorily by cytokinin concentration alone. This may indicate that other endogenous hormonal signals were also involved in regulating scion branching. Moreover, recent research has proposed a group of terpenoid lactones, called strigolactones, produced in root exudates and available in very minute concentration, that reduced branching in some branching mutants (Gomez-Roldan et al., 2008; Umehara et al., 2008; Vogel et al., 2010; Kapulnik et al., 2011). Therefore the reduced growth of scion on a dwarfing rootstock may also involve this novel hormone in association with IAA and CK, but, future research is needed to understand whether compounds such as strigolactones are also involved in rootstock induced dwarfing of apple.

Chapter 3
Role of gibberellins in cell division and/or cell elongation of shoot internodes for composite 'Royal Gala' apple scions grown on a dwarfing and a vigorous rootstock

3.1 Introduction and Aims

The adult body of the vascular plant is the result of meristematic activity. Plant meristems are centres of mitotic cell division and are composed of a group of undifferentiated self-renewing stem cells from which most plant structures arise. Plants grow in height by addition of metamers. Each metamer consists of a leaf, an axillary bud and an internode that are initiated by the shoot apical meristem of the plant. Morphologically, shoot growth consists of formation of leaves at nodes, axillary buds in the leaf axil and, elongation of internodes (distance between two consecutive nodes bearing leaves). In general, shoots usually have indeterminate growth, and internodes which constitute the shoot are determinate organs (Sussex and Kerk, 2001; Tsukaya, 2006; Ripetti et al., 2008).

In Chapter 2, the primary shoot length for 'RG' scion on dwarfing rootstocks showed a significant response to gibberellin treatment (Table 2.4) by increasing both node number (Table 2.5) and internode length (Table 2.6). Thus, gibberellins acted upon both apical and sub-apical meristems to increase node formation and internode length respectively. Therefore, the aim of this study was to understand whether gibberellins increased cell number or cell elongation in the elongation of internodes. Before going into details of the study, the following sections explain the structure and functions of shoot apical meristems in the plant body.

3.1.1 Shoot apical meristem (SAM)

3.1.1.1 *SAM - Structure*

The shoot apical meristem [SAM; (Lyndon, 1998)] is a group of meristematic cells located at the shoot apex (shoot tip) that lay the foundation of the primary plant body. The SAM denotes the part of the shoot lying distal to the youngest leaf primordium and mainly involved in producing metamers continuously or in some case rhythmically (bicyclic) in a regular order until the end of the growth period i.e., until cessation of growth each year (Figure 3.1A). The SAM has relatively small, non-vacuolated, densely stained and nearly iso-diametric cells and, is the place where all initials (source cells) are found and considered to be the ultimate source of all the cells of the aerial shoot. The cells in the SAM are organised in two ways: cells organised in radial zones and also in layers. The cells at the top of the meristem divide infrequently and constitute the central zone (CZ). This is the location of self-renewing undifferentiated stem cells and this zone is surrounded by the peripheral zone (PZ); leaf primordial initiation takes place in this peripheral zone (Medford, 1992). Below the CZ is another region of rapidly dividing cells, called the rib meristem (Figure 3.1B and C). Division and elongation of rib meristem cells produce ground tissue. The base of this tissue, by anticlinal divisions, produces very orderly cell files that form the pith of the stem (Figure 3.1 B) (Esau, 1965; Howell, 1998; Evert, 2006).

The sub-apical meristem is located where the shoot widens; primordia enlarge and are regions of maturation, where differentiation becomes apparent. In the sub-apical meristem, the cells divide and elongate and, promote the elongation of the internode (Wardlaw, 1957; Cutter, 1965). Thus, the apical meristem is involved in shoot morphogenesis (formation of metamers) and the sub-apical in internode elongation (Sachs, 1965). Conversely, in monocotyledonous plants, the intercalary meristems are the active tissues that have been separated from the shoot apical meristem by regions of more matured or developed tissues and are inserted between tissues that are no longer meristematic. They continue their meristematic activity at some distance from the meristem. These meristems, unlike the apical and lateral meristems, do not contain

initials (Evert, 2006). The lateral meristem is involved in increasing the diameter of plant organ, which are strips or cylinders of dividing cells located parallel to the long axis of the organ in which they occur (vascular and cork cambia). Radial enlargement of the cells derived from these meristems increases the diameter of the organ. Therefore, lateral meristems in the stem are concerned with increasing the girth or diameter of the stem.

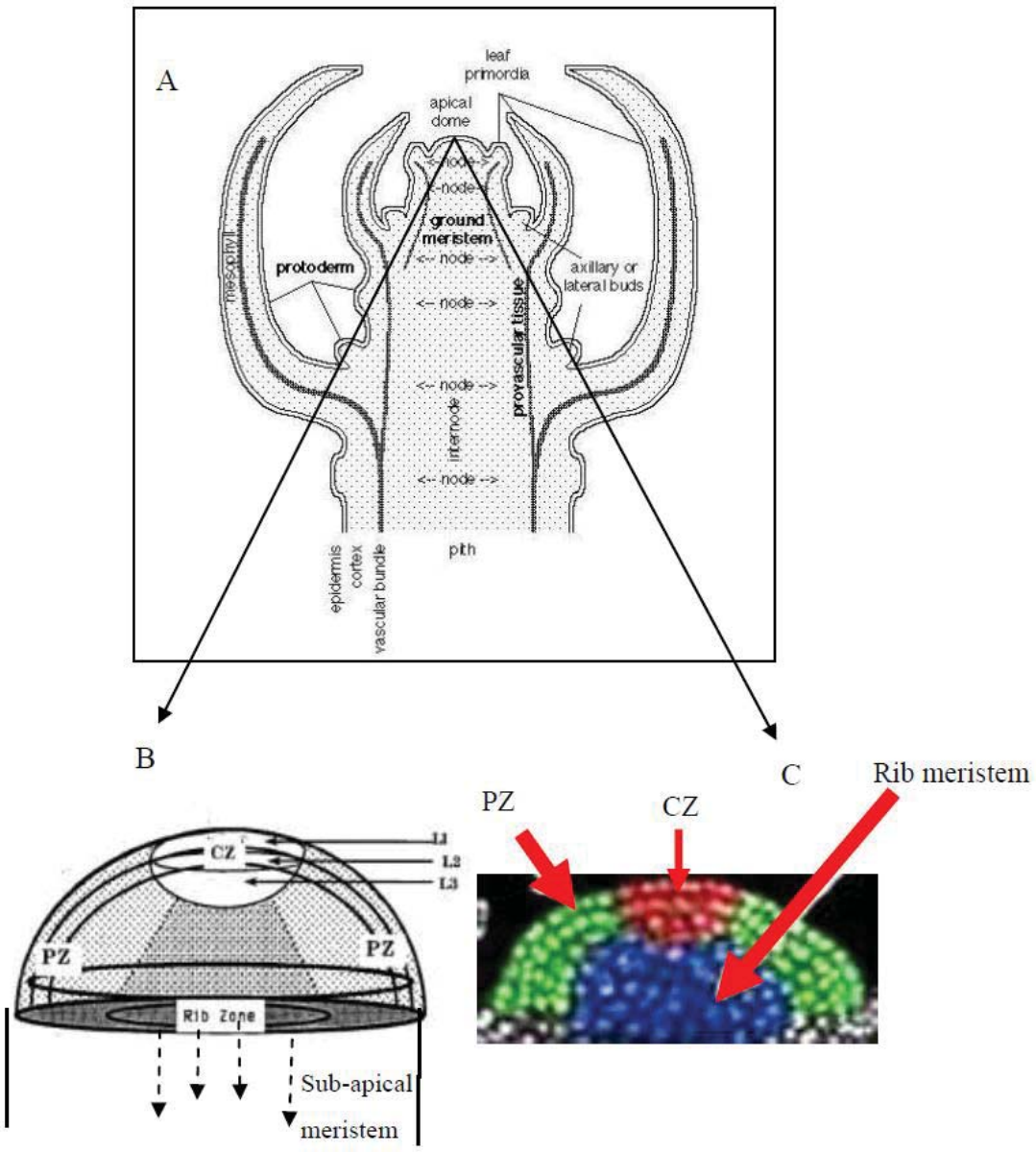

Figure 3.1. Comparison of shoot apex (A) and shoot apical meristem (B & C). A: The entire shoot apex with apical dome at the centre with leaf primordia, leaf axillary buds, immature nodes. B: Diagrammatic representation of the shoot apical meristem C: Tissue organisation of the shoot apical meristem. In shoot apical meristem (C) the central zone is shown in red, the peripheral zone in green, and the rib meristem in blue In B peripheral zone L1, L2 and L3 correspond to the genetically defined three cell layers from exterior of the meristem to the interior, respectively. Distance between two consecutive matured nodes is an internode as represented in A and B, below the central rib zone is the sub-apical meristem. (modified from http://biology.kenyon.edu/course/biol114/Chapter 12A.html;and (Medford, 1992).

3.1.1.2 *Shoot apical meristem – activity*

The shoot apical meristem is composed of initials. It becomes the source of new cells and their derivatives for the perpetuation of meristem. Derivatives also usually divide and produce one or more generations of cells before they undergo any cytological changes (Evert, 2006). The cytological zonation into different groups of cells form CZ, PZ and Rib meristem as explained in 3.1.1.1 (Figure 3.1). The change from apical meristem to adult primary tissue is gradual and involves the integration phenomena of cell division, cell enlargement and cell differentiation (Evert, 2006). In *Arabidopsis*, it was found that for initiation and maintenance of SAM function throughout the life time of the plant, the *SHOOTMERISTEMLESS* (*STM*) gene is required (Long and Barton, 1998). These *STM* genes are expressed in central and peripheral zone, but not in the developing leaf primordia (Long and Barton, 1998). The *WUSCHEl* (*WUS*) gene is necessary to maintain meristematic nature otherwise, the meristem would undergo differentiation. The *WUS* gene is restricted to a small group just below the central zone cells and persists throughout shoot development (Vernoux et al., 2000). To become an organ, cells need to go beyond the range of *WUS*, thus losing their meristematic nature and become available for differentiation (Schoof et al., 2000). The higher-plant shoot meristem is a dynamic structure whose maintenance depends upon the coordination of two antagonistic processes, organ initiation and self-renewal of stem cell population (cell identity). In *Arabidopsis*, the *WUS* gene is required for cell identity and *CLAVATA1, 2* and *3* (*CLV*) genes promote organ initiation (Schoof et al., 2000). Continuous organ formation from the SAM requires the integration of two functions: a set of undifferentiated stem cells is maintained at the tip of the meristem, while their daughter cells in the periphery initiate organ primordia (Lenhard et al., 2002). Thus both *WUS* and *STM* genes are essential for the formation and maintenance of a functional meristem. Basically, *STM* suppress the cell differentiation and *WUS* provides stem cell identity (Tooke and Battey, 2003). The genes *CLV1, CLV2* are responsible for inhibiting the expression of *WUS* (Schoof et al., 2000) to lead cells towards differentiation. *WUS* activities and its expression maintain the CZ. The expression of *CLV3* a peptide legand secreted is perceived through the *CLV1-CLV2* complex to negatively regulated *WUS*

and restricts the size of the CZ (Schoof et al., 2000). Although this pattern of gene expression maintains CZ/PZ model in the apical meristem, the radial patterning of lateral organ is auxin-dependant (Tooke and Battey, 2003).

3.1.1.3 *Shoot apical meristem-hormonal regulation*

The shoot system of a plant develops by producing lateral organs (leaves) on the flanks of the SAM, which establishes the phyllotaxy, or spatial arrangement of lateral organs around the apex (Richards, 1951). Auxin is thought to be synthesized in young apical tissue and transported downward to the maturing stem and roots by polar auxin transport (PAT) (Davies, 1995). When auxin was inhibited either by culturing vegetative tomato shoot apices on a synthetic medium containing 1-N-naphthylphthalamic acid (NPA), an auxin transport inhibitor, or genetically using mutants such as *pin-formed 1-1 (pin1-1)* of *Arabidopsis,* the tomato shoot apical meristems failed to produce new leaf primordia. However, the stem tissue continued to grow leading to a pin like structure devoid of leaves and, micro application of IAA in lanolin paste to the apices of such pins restored leaf formation (Reinhardt et al., 2000). Therefore, two independent patterning systems were proposed in the meristem: (1) the apical-basal pattern specified by genes *STM, WUS* and *CLVs* the maintenance of which does not depend on auxin (Fletcher et al., 1999); (2) the radial patterning system regulated by auxin and determining where, within PZ, organs are initiated (Reinhardt et al., 2000). Shoot apical meristem requires a balance between lateral organ initiation from its peripheral zone (PZ) (Carraro et al., 2006; Evert, 2006) and indeterminate growth at its centre (CZ). Lateral organs are produced in the meristem in a continuous but nevertheless flexible manner. At this point endogenous hormones and transcription factors cooperate to balance meristem maintenance and organ production.

The time interval between two successive leaf initiation events is called the plastochron. The latest emerged primordium is P_1, next oldest is P_2 and so on. The region within the meristem from which the next lateral organ primordium will be formed is termed P_0. High auxin levels promote the initiation of lateral organs at specific sites in the SAM.

The young leaves at the shoot apex are rich in endogenous IAA (Ljung et al., 2001), and IAA to the peripheral zone may be supplied by subtending leaves by acropetal transport (Vogler and Kuhlemeier, 2003). Although auxin normally is transported basipetally, in this case the auxin can move acropetally in response to sink demand by the surrounding primordia. Thus, IAA signal is a vital for primordia initiation. Gibberellins are also considered to be prerequisites for leaf initiation in the PZ of the SAM of *Arabidopsis* (Tooke and Battey, 2003) as high relative concentration of auxin at P_0 down regulates *KNOXI* and *GUC* genes allowing leaf initiation (Shani et al., 2006).

It has been suggested that bioactive gibberellins in leaves promotes division and elongation of leaf cells (Wheeler, 1960). A tight spatial restriction of GA accumulation is crucial to specify the boundary between the meristem and the incipient leaf primordium (Fleet and Sun, 2005; Shani et al., 2006). In fact gibberellin production and activity are down regulated in the SAM by *KNOXI* protein by repressing *GA_{20} oxidase* enzyme (Sakamoto et al., 2001; Hay et al., 2002). Jasinski et al., (2005) also showed that *STM* in shoot apical meristem positively regulate the expression of gene encoding GA_2 oxidase, which deactivates bioactive GAs and other precursors. They also mentioned that *KNOXI* maintains a delicate balance between levels of hormones, which is critical, as a combination of reduced cytokinins and increased gibberellins in the SAM lead to meristem abortion. Furthermore, ectopic expression of *KNOX* gene in the *PZ* region of the SAM lead to altered phyllotaxy with abnormal leaves (Nishimura et al., 2000). In tobacco ectopic expression of *KNOX* genes showed decreased production of *GA20 oxidase* that is at least partially responsible for a reduction in bioactive GA levels that caused abnormal leaf morphology and the application of GAs could partially rescue abnormal phenotypes (Tanaka-Ueguchi et al., 1998; Sakamoto et al., 2003) indicating that the increased GA content promoted the lateral organs. Thus both IAA and gibberellin are important in the SAM for leaf formation in *Arabidopsis* and tobacco.

In addition, Jasinski et al., (2002) found that *KNOX* genes promote meristem function partly through repression of GA biosynthesis. They also found that *KNOX* function is mediated by cytokinin (CK), a growth regulator that promotes cell division and meristem function. Their results indicated that cytokinin activity is both necessary and

sufficient for stimulating GA-catabolic gene expression, thus reinforcing the low-GA regime by *KNOX* protein in the central zone of the SAM. Therefore, *KNOX* may act as general orchestrator of growth regulators at the CZ of the shoot apex of *Arabidopsis* by simultaneously activating CK and repressing GA biosynthesis, thus, promoting meristematic activity (Jasinski et al., 2002). In the SAM, cytokinins have been thought of as regulating cell proliferation (Mok and Mok, 2001) and have been associated with the *STM* and *KNOTTED*-like genes and their levels are regulated by cytokinin levels (Rupp et al., 1999). Ectopic expression of cytokinin biosynthesis gene *ipt* delayed senescence, altered leaf shape. Ectopic meristems as the *abnormal meristem mutant 1(amp1)* in *Arabidopsis* has abnormal levels of cytokinins with an enlarged meristem, which produced leaf primordia at a higher rate than normal (Riou-Khamlichi et al., 1999). Thus, for the overall stability and maintenance of the meristem, a balance between cytokinin and KNOX gene is essential (Traas and Vernoux, 2002). Therefore, for *Arabidopsis*, there is high cytokinin and low GA in the central zone for meristematic activity and in the peripheral zone high auxin and GA for leaf development.

Attempts were made to illustrate how far the SAM models for *Arabidopsis* and pea are similar to that of apple. *Arabidopsis* hypocotyls have been extensively studied in relation to elongation. *Arabidopsis* has a rosette habit, with little stem elongation until bolting occurs, which generally is a response connected with flowering. Therefore, it was not clear whether *Arabidopsis* hypocotyls are truly representative of an elongating shoot system (Ross et al., 2011). Exogenous gibberellin promoted hypocotyl elongation only in light-grown hypocotyls. In the dark, the gibberellin response was close to saturation and there was no gibberellin effect (Cowling and Harberd, 1999). For hypocotyl elongation, it was found that only cell elongation occurred and not cell divisions (Gendreau et al., 1997) whereas both cell division and cell elongation are critical for stem elongation in most crop plants. Growth was inhibited when wild-type *Arabidopsis* seedlings were grown on media with wide range of auxin concentrations, but when mutants with reduced auxin response were grown in similar conditions, auxin promoted growth of hypocotyls. These results showed that the levels of auxin in wild-type *Arabidopsis* were optimal for elongation and that additional auxin is inhibitory (Collett et al., 2000). When auxin mutants were grown on media containing gibberellin

and gibberellin mutants on media containing auxin, the response was similar to wild-type. In addition, adding NPA to the media did not affect the gibberellin response. These results suggest that auxin and gibberellin act independently and a normal auxin response was not needed for a normal response to gibberellin for hypocotyls elongation for *Arabidopsis*. In this it differs from pea, where for internode elongation it has been reported that interaction between auxins and gibberellin was essential (Yang et al., 1996; Ross, 1998). For pea internode elongation both cell division and cell elongation, and for *Arabidopsis* hypocotyl elongation only cell elongation was required. Therefore, the interaction between auxin and gibberellin was essential for cell division, but may not be for cell division (Collett et al., 2000).

In apples, although the termination of vegetative growth was regulated by light intensity, temperatures, vernalisation and also rootstock (Johnson and Lakso, 1985; Hirst and Ferree, 1995), it has been demonstrated that the endogenous levels of highly bioactive GAs were significantly lower in the apices of spur-type shoot that terminated early in summer than in the apices of extending shoots (Looney et al., 1988) of 'McIntosh' apple strains. In support of this, Foster et al., (2007a) reported higher levels of DELLA activity in recently arrested spur tips than the growing tips of apple shoots. The role of endogenous hormones in the apical and sub apical regions of an apple SAM are largely unknown and, there have been a few studies using exogenous hormones, which are important as they provided information on how hormones like IAA, gibberellins and cytokinins may interact to regulate meristematic activity (van Hooijdonk, 2009).

A 'Royal Gala' scion on a dwarfing rootstock supplied with gibberellin continued growth for longer than the scion on a dwarfing rootstock (Chapter 2), which may be due to deficiency in GAs (van Hooijdonk, 2009). In contrast to the effect of gibberellin, cytokinins had very little effect on the time to shoot termination. For example, primary shoots of 'Royal Gala' (van Hooijdonk, 2009) terminated very early with repeated application of BAP and for 'Red Boskoop' (Wertheim and Estabrooks, 1994) the primary shoot on 'M.9' rootstock was similar in length with untreated controls. Cytokinins were said to be involved in shoot extension growth (Jones, 1973; Lockard

and Schneider, 1981), however, Wertheim and Estabrooks, (1994) found that exogenous cytokinins hardly affected the growth extension of primary shoots. Therefore, cytokinins sprayed for scions on 'M.9' rootstocks did not promote meristematic activity of SAM as the concentration of cytokinins was optimal and additional cytokinin was inhibitory

For apple scion on 'M.9' rootstock and NPA treatment of rootstock stem reduced mean node number and final length of the primary shoot and for both treatments GA_{4+7} foliar sprays increased node number and length of the primary shoot by activating apical and sub apical meristems of the SAM. Exogenous supply of GAs promoted shoot extension by delayed termination and by producing more nodes with longer internodes for 'Royal Gala' scions on 'M.9' rootstocks (refer Chapter 2; (van Hooijdonk et al., 2010). Thus GAs acted on both apical and sub-apical meristems for scions on 'M.9' rootstocks. In addition, for pea plants removal of the apical bud or blocking basipetal auxin transport, resulted in decreased endogenous GA_1 in the subtending internodes, which resulted from decreased GA_3 oxidase activity, thereby reducing the levels of bioactive GA_1 levels (Ross et al., 2000). Therefore, IAA transport from the shoot apex may regulate bioactive GAs and mediate internode elongation. In a similar way, gibberellins may also be required for IAA in the shoot apex. In the literature, it was shown that in several species such as pea and sunflower, treatment with GAs greatly increased quantities of diffusible auxin (Nitsch and Nitsch, 1959; Phillips et al., 1959; Kuraishi and Muir, 1962).

Therefore, for *Arabidopsis*, pea and apple both IAA and GA are required for leaf primordia initiation and development and, auxin and gibberellin interaction was required for pea and apple internode elongation, which promote both cell division and cell elongation. In the case of *Arabidopsis* hypocotyl elongation, auxin and gibberellin act independently to promote only cell elongation. Therefore, in order to elucidate whether cell division and/or cell elongation are required for internode elongation of apple shoot, it was decided to conduct this experiment using confocal microscopy.

3.1.2 Internode elongation

3.1.2.1 Role of cellular activity cell division and/or cell elongation in internode elongation

Growth depends on a combination of cell division and subsequent cell enlargement (Green, 1969; Hanson and Trewavas, 1982). Thus, growth may be divided into two stages: growth by cell division and limited cell elongation near the apex, and growth without cell division but with much cell elongation further down (Evert, 2006). As the derivatives of cell division move away from the initials, cell enlargement becomes dominant over cell division and, in time, replaces it completely. These cells acquire a characteristic specific shape for the tissue and eventually become mature. The apical meristem is the site of the initial cells of stem and the sub-apical region is the site of formation of most cells to constitute the mature stem. The length of plant depends upon the activity of apical meristem by the addition of metamers and sub-apical meristem by elongation of stem. Sachs (1965) mentioned that centuries ago it was pointed out by Hartings that shorter internodes contained fewer, not shorter, cells than long internodes and proposed that duration and amount of cell division activity in the sub-apical region may vary considerably during the life cycle of a plant. In addition, Sachs, (1965) while investigating stem growth in general, found that new cells were formed parallel to the main axis by transverse division, the number of cells increased tenfold and the length of each cell approximately doubled in the pith tissue, while in cortical tissue the amount of elongation was greater than that of pith cells. Therefore, he suggested that the pattern of histogenesis in the stem was different for different tissues.

Furthermore, Bland (1978) explored the differences in length between mature long shoots and vegetative short shoots (spurs) of 'McIntosh' apple shoot system and concluded that in apple trees long shoot have longer but not more internodes than short shoots and, the internode length depended upon the rate and duration of cell division rather than on the length of the pith cells. However, in previous studies and also in this study (see Chapter 2, section 2.4.2.4 and Figure 2.15) it was observed that the final length of an annual shoot is determined by the number of nodes and length of internodes

(Seleznyova et al., 2003; van Hooijdonk, 2009). As the node number increased the length of the annual shoot (sylleptic shoots) increased with increased internode length. Pith cells of long shoots divide periclinally to form vertical files of cells; in short shoots (spurs) cells divide for shorter duration and their derivatives are randomly arranged without any regular pattern (Rouffa and Gunckel, 1951). Apple pith is heterogeneous (Solereder and Scott, 1908), with small thick walled peripheral living cells and both living and dead large thin-walled central cells. The living cells in the centre frequently contain tannins.

Compared with the scions grown on 'MM.106' and 'M.793', the scions on dwarfing rootstock had reduced primary shoot length and reduced node number (van Hooijdonk, 2009), but, not internode length. For the reciprocal grafts in Chapter two, the 'RG' scion with 'M.9' dwarfing rootstock significantly reduced the length of the primary shoot compared with that on 'RG' rootstock, but mean node number and mean internode length were not significantly different (refer Chapter 2.4.1.2.1 and Table 2.5). However, node number and internode length together produced a significant difference in primary shoot length. Gibberellin treatment on both composite trees significantly increased primary shoot length by increasing both node number and internode length. Gibberellin treatment not only affected these attributes, but also increased the duration of shoot growth. Therefore, it was understood from these observations that GA acted both on apical and sub-apical meristems in composite apple trees.

3.1.2.2 Role of gibberellins on cellular activity cell division and/or cell elongation in internode elongation

GA is well known to play a role in stem elongation by promoting either or both cell division and cell elongation (Sachs, 1965; Kamijima, 1981; Potter and Fry, 1993; Brown et al., 1995; Daykin et al., 1997; Granier et al., 2000; Bulley et al., 2005; Fagoaga et al., 2007; Ripetti et al., 2008). Many studies ascribed gibberellins induced increase in stem length to increased cell division in the internode: in bean (*Phaseolus vulgaris*) (Greulach and Haesloop, 1958) and in pea plants (*Pisum sativum* L) (Arney

and Mancinelli, 1966; Yang et al., 1996). Treatment of tomato plants with gibberellins increased cell length as well as cell number (Jupe et al., 1988). Therefore, the relative contribution of the activities cell division and/or cell elongation to GA induced stem extension growth differs in different plant species.

In Chrysanthemum, sub-apical meristematic activity was completely inhibited by spraying Amo-1618 on the upper portion of the plant and GA_3 treatment reversed this effect. With Amo-1618 the apical meristem continued to function normally resulting in rosette plants (short internodes). This differential action in the apical and sub-apical meristems is the reason for the rosette habit of growth of Amo-treated chrysanthemum plant. With GA_3 treatment cell division increased in the sub-apical meristem and caused the stem to resume normal elongation enabling the plants to retain a normal growth habit. Thus GA appeared to regulate activity of sub-apical meristem and played an important role in shoot development (Sachs et al., 1960) by affecting internode length. However, in Chapter 2, for the composite apple trees on dwarfing rootstock ('M.9') the GA_3+GA_{4+7} foliar sprays increased both node number and internode length significantly (Table 2.5).

Gibberellins from excised apple shoot apices, leaves and internodes of apple trees (*Malus* × *domestica*) can be collected by diffusion into agar (Grauslund, 1972). The presence of many gibberellins like GA_1, GA_3, GA_4, GA_7, GA_8, GA_9, GA_{19}, GA_{20}, and GA_{29} in the apple shoot tips has been demonstrated (Koshioka et al., 1985; Steffens and Hedden, 1992). The largest amount of GAs was found to diffuse from the apical meristem, the two upper leaves and the two upper internodes. Removal of young leaves retarded elongation of internode. Probably GAs and auxin produced in young leaves exercised some control over this process. Although, many studies proved that GA is known to increase rates of cell division and cell elongation, it was also reported in literature that IAA is a crucial regulator of stem elongation and that a substantial presence of IAA is necessary for moderate stem elongation to occur, regardless of the level of GA (Yang et al., 1996). GA is known to enhance basipetal transport of IAA (Plich and Jankiewicz, 1970) and GA stimulated elongation of buds that had already started to develop and produce IAA in pea plants (Wickson, 1958). Part of the auxin produced in the apical bud is used for stimulating axis elongation, and the remainder

augments the auxin pool which transported basipetally towards the main axis. A part of the auxin pool transported towards the main axis is probably temporarily bound or destroyed by phenols and the remaining auxin is transported basipetally in the main axis, stimulating cambial activity, attracting nutrients towards auxin source and, converting starch to sugars. Therefore, the bud having a better connection with the vascular system of the main axis also has better access to the hormones produced in roots. Gibberellins are not only produced by the roots but also by young leaves of apple shoot (Kato and Ito, 1962; Luckwill and Whyte, 1968). Gibberellin precursor GA_{19} produced in roots is transported to shoot through xylem sap and converted to bioactive GA_1 (Yamaguchi, 2008). In addition, cytokinins and GA seem to be indispensably involved in internode elongation of apple shoots (Jones, 1967; Plich and Jankiewicz, 1970). Root apex appears to be the major site of cytokinin, gibberellin and abscisic acid synthesis (Little and Savidge, 1987) and there is evidence that cytokinins exist in roots xylem exudates (Kende and Sitton, 1967; Skene, 1972). There is also evidence for cytokinins to be present in cambium and all actively dividing tissues (Chen et al., 1985), as cultured rootless tobacco plants supplied with radioactive adenine (8-^{14}C) were capable of synthesising radioactive cytokinins. Moreover, plant tissues contain enzymes to interconvert cytokinin bases, nucleotides and nucleosides (Chen, 1981). Therefore, together with auxins, cytokinins and gibberellins act synergistically in stimulating elongation of shoot in apple trees.

The information for shoot development is summarised in figure 3.2, which illustrates how the supply of endogenous hormones play a role in plant morphogenesis and how exogenous hormones could be used to alter the same. For example, auxins from apical meristem (from young leaves) through polar auxin transport reach root system and influence root-produced hormones cytokinins and GAs. Cytokinins and gibberellins from root are transported to shoot through xylem. The GAs act on the sub-apical meristem for internode elongation and GAs, along with auxins from young leaves, act on the apical meristem for leaf initiation, thereby promoting shoot elongation. With exogenous gibberellins, the internodes are longer compared with controls suggesting that gibberellins which stimulate the apical meristem to produce more nodes also stimulate the sub-apical meristem to produce longer internodes.

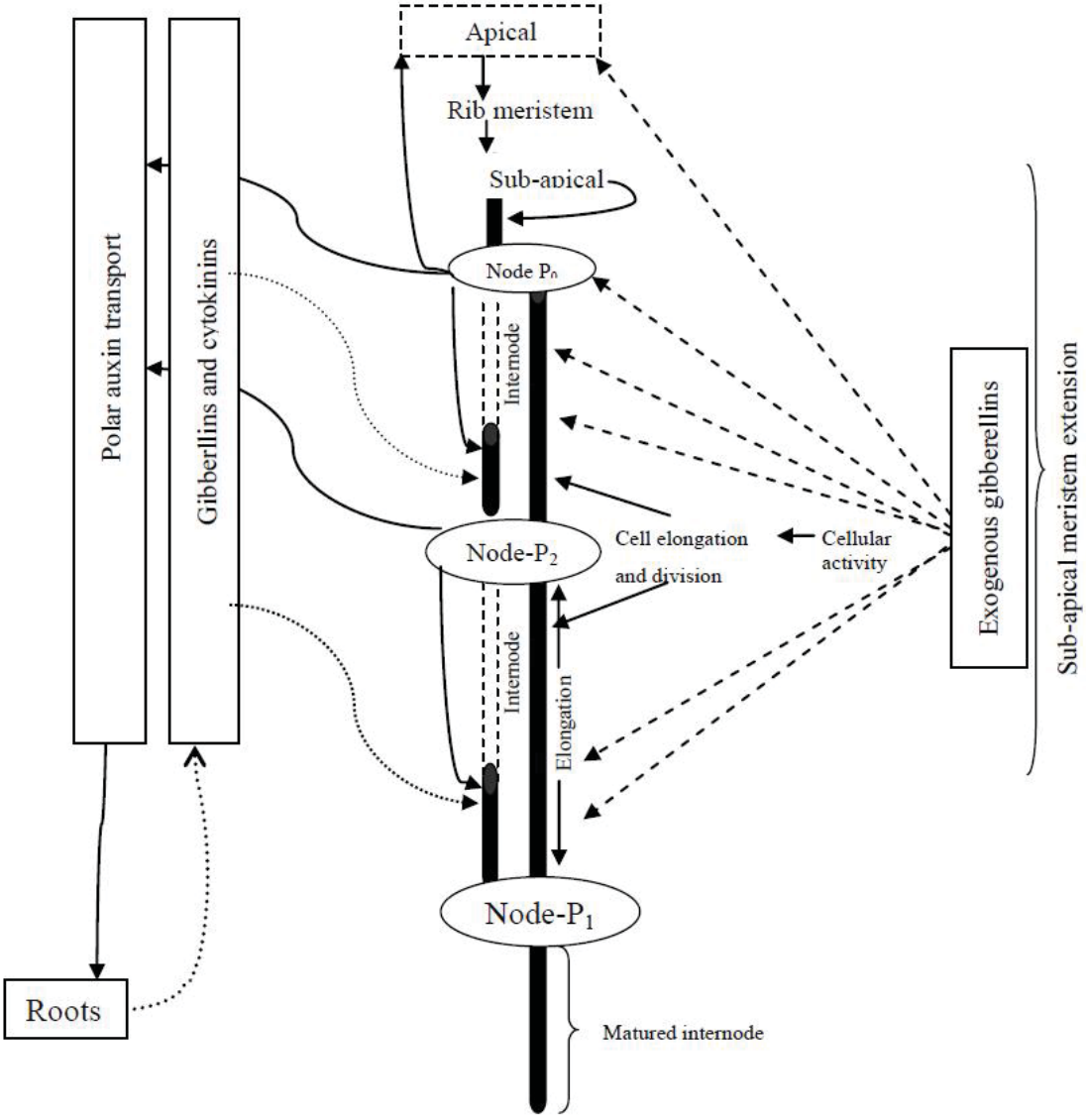

Figure 3.2. A model for apple SAM shoot development: nodes, internodes, involvement of apical, rib and sub-apical meristems. Effect of auxin, gibberellin, cytokinins, cell elongation and/or cell division on internode elongation of a plant body. The solid black column beside dotted column in internode indicates the increased elongation due to exogenous gibberellins Dotted lines on left from root indicate the supply of endogenous GAs and cytokinin through xylem sap. The black arrow from node on left to PAT indicates basipetal auxin transport to root. The black arrow on left from the youngest node (P0) to the apical meristem indicates acropetal auxin supply to the PZ and CZ of the SAM. The region between rib meristem and node P1 indicate the extension of sub-apical meristem and below this region is the region of maturation. On the right side the exogenous gibberellin supply to sub-apical and apical meristem, which increased internode length and node number by cell division and cell elongation.

In dwarf GA_1-deficient *le* mutants of light-grown pea (*Pisum sativum L.*) exogenous gibberellin and auxin (IAA) strongly stimulated elongation; the responses to both were additive (Yang et al., 1996). They mentioned that the effect of GA was mainly in very young internodes (less than 25% expanded) in increasing cell length and cell number and that of IAA was in older elongating internodes in increasing cell elongation. Several other studies also have shown that the interaction of GA with auxin enhance the effect of GA (Ross et al., 2000; Wolbang and Ross, 2001; Wolbang et al., 2004). However, GA response became ineffective when the shoot apex was removed, which assumes that for a normal response of GA an intact shoot apex is necessary in pea plant (Ross et al., 2002) and in tobacco (Wolbang and Ross, 2001). Moreover, auxin controlled the growth of *Arabidopsis* roots by modulating the cellular response to GA. The primary roots of GA-deficient *Arabidopsis gal-3* mutant were shorter than those of wild type, whereas GA-treatment increased their length, but decapitation and NPA prevented the effect of GA from restoring the normal growth to GA-deficient *gal-3* roots (Fu and Harberd, 2003); thus similar to shoot, IAA regulated root elongation by controlling *DELLA* expression.

In summary, nodes are formed by the activity of apical meristem and internode by sub-apical meristem. The internode tissue originated from the rib meristem located just below the apical dome (Figure 3.1) by anticlinal divisions forming parallel longitudinal files of cells to form cylindrical parts of plant such as cortex and pith. It was also understood that there is a synergistic action of all three hormones: gibberellins, auxins and cytokinins, in the elongation of internodes. At this point, further studies of pith development should enhance our understanding of the control mechanism important in tree architecture.

3.1.3 Pattern of Pith development

In shoot apical meristem, cells below the central zone is another region of rapidly dividing cells: the rib meristem (Figure 3.1B and C) (Medford, 1992). Division and elongation of rib meristem cells produce ground tissue, the base of which, by anticlinal

divisions produces very orderly cell files that form the pith of the stem (Figure 3.1 B) (Esau, 1965; Howell, 1998; Evert, 2006).

The mode of pith development may be correlated, in general, with the activity of pith rib meristem and its derivatives. In different species of family Rosaceae there were differences in the length of time during which the shoot apices were active and this activity was paralleled by developmental changes taking place in the apical derivatives, changes which correlated with growth habit (Rouffa and Gunckel, 1951). Therefore, pith rib meristem is considered here under pith development as well as under apical meristem, as the behaviour of rib meristem and its derivatives and the relation of their activity to the pattern of growth in the pith can logically be considered as a continuous process rather than two distinct developmental processes. Although the specific pattern of pith rib meristem differs from species to species throughout a plant family and even within species, the patterns fall into two main types namely *Type A* (long shoot) and *Type B (short shoots).* In long shoots of Rosaceae, Rouffa and Gunckel, (1951) found that the pith-producing rib meristem and its derivatives retained the capacity to divide for a longer time compared to short shoots. Previously it had been found that in long shoots of Ginkgo, the pith-producing rib meristem retained their capacity to divide actively and the derivatives undergo uniform transverse divisions and uniform enlargement with very little intercellular space so that the linear order was not lost during maturation of pith cells, named as '*Type-A shoot's* (Gunckel and Wetmore, 1946). They also found that in short shoots of Ginkgo, the pith-producing rib meristem produced very short chains of cells with short duration of cell division and the derivatives with limited and unordered cell division and unequal cell enlargement. This resulted in broad cylinders of large cells with extensive intercellular spaces, named as '*Type-B shoots'*. Therefore, the amount of cell activity (cell division and cell enlargement) in the rib meristem and its derivatives corresponds to the amount of shoot growth. On the contrary, this type of correlation between the development of derivatives of pith rib meristem and growth habit was not correlated in peach seedlings, which were subjected to insufficient after-ripening. After-ripening is the term used to describe processes and changes, such as exposing seeds to specific environmental conditions for

a period of time, which depends upon species and the depth of seed dormancy that lead to non-dormant state. The peach seedlings which were subjected to short after-ripening were described as dwarf-like in character with short internode. These seedlings on the basis of external morphology, such as short internodes were comparable to short-shoot conditions i.e., to '*Type-B shoots'* and normal peach seedlings were comparable to long-shoot conditions i.e., to '*Type-A shoots'*, but internally, however, normal seedlings were found to have irregular groups of cells whereas short-internode seedlings were found to have regular files of cells. Therefore, the relationship between development of derivatives of pith rib meristem and growth habit was reversed (Holmsen, 1960).

In Chapter 2, 'RG' scions, when grafted onto dwarfing rootstock ('M.9'), were shorter than those grafted on vigorous ('RG') rootstocks. The length of 'RG' scion on 'M.9' rootstock was increased with GA treatment and became equal in length with 'RG' rootstock (control). There was a significant increase in the mean internode length of 'RG' scions on 'M.9' rootstock (refer 2.4.1.2 and Table 2.6) with GA treatment compared with 'RG' rootstock control. However the extent to which internode length was limited by cell division or cell expansion and how gibberellins affected those cellular activities were unknown. Therefore, the main objective of this Chapter was to investigate the role of GA in the elongation of internodes of apple scions grafted on to dwarfing ('M.9') and a vigorous rootstock ('RG') in terms of cellular activity, cell division and/or cell elongation of pith cells. Further objectives were to make histological observations on section cuttings of apple internodes with or without GA of the scions on dwarfing ('M.9') and vigorous rootstocks (RG) in order to analyse whether an increase in length of the internode was due to an increase in the number of cells or length of cells and, to analyse the dynamics of cell production and extension and how these were affected by exogenous gibberellin application during internode elongation in composite apple trees.

3.2 Materials and methods

3.2.1 Selection of material

Two rootstocks were selected for their contrasted vigour: 'M.9' dwarfing rootstock characterised by producing low vigour trees with shorter shoots and with a tendency to terminate active vegetative growth earlier, and 'Royal Gala' ('RG') used as a vigorous self-rooted rootstock control that produces trees with longer shoots because of later shoot termination. The 'RG' scion was grafted to each of these two rootstocks as described in Chapter 2. The plant material for this experiment was taken from the previous experiment (Chapter 2). The composite trees with 'RG' scions on 'M.9' and 'RG' rootstocks, with and without gibberellins, were taken for histological studies to assess the role of gibberellins in internode elongation. Mature internodes were harvested at growth cessation. The internodes to be harvested were decided based on the accurate numbers (position) of internode on the primary shoot that was formed when the rootstock and GA effects on the primary shoot length for scions on 'M.9' and 'RG' rootstocks were significant (12th of January, 2008) (refer Figure 2.6). For each treatment and for each tree, the nodes formed during that period were calculated and two consecutive internodes from each primary shoot of scion on both rootstocks, with and without gibberellins treatment, were selected, and internode length (mm) measured. The corresponding mean nodal positions harvested for 'scions on 'M.9' rootstock were 58^{th} and 59^{th}, on 'RG' rootstock were 67^{th} and 68^{th} and, with GA treatment for scions on both rootstocks, the mean nodal position harvested was 63^{rd} and 64^{th} from stem apex downwards. Each treatment was replicated four times so that altogether 32 internodes were harvested. The harvested internodes were immediately immersed in formalin: glacial acetic acid: alcohol (FAA) fixative solution composed of formaldehyde, acetic acid, ethanol and water at 2:1:10:7 by volume. After 24 hours, the internodes were transferred to 70% ethanol solution (Ripetti et al., 2008) and stored until tissue sections were made for microscopy studies.

3.2.2 Sample preparation

The length between the upper portion of one node at one end and the lower portion of the subsequent node at the other end was measured (Internode length) and, was divided into three equal portions. The middle portion was selected for the fresh longitudinal sections as it was found in previous studies on apple trees, and confirmed in this study (section 3.3.1), that there were no differences between bottom, middle and top portions of the internode (Ripetti et al., 2008); they also observed that the cell shape was similar along longitudinal sections and also across horizontal sections. Therefore, only longitudinal sections were taken for observations. A moderately thin (0.5mm), even, free-hand longitudinal section was made along the pith region of the middle portion internode (Figure 3.2). Six sample sections corresponding to each internode were taken for sample preparation. They were immersed in approximately 1 ml of bleaching agent (K hypochlorite, 21.5gm per litre) separately for 15-30 minutes and rinsed with water 2-3 times until the odour of the bleaching agent disappeared. They were then stained with a few drops of a solution of 1% safranin for 3-5 minutes, rinsed with water again, then rinsed with 50% alcohol (ethanol) once, 96% alcohol 2-3 times, to remove the excess stain, and finally rinsed once with 100% alcohol.

The corresponding sections were then placed on different slides with a 0.17 mm thick cover slip on top without air bubbles for observation under a confocal microscope (section 3.2.2). Images of the best histological section were taken along the longitudinal axis through the pith region. Care was taken to avoid the peripheral cell of the pith while taking the photographs as the peripheral cells are narrower and thick walled. The image area (1024x1024 pixcels; picture elements) and the objective magnification (40x) were fixed for all the images. all the visible cells of each photograph were measured to give the total number, the length and width of all cells (Figure 3.4 & 3.5), using Image J software, a Java-based image processing programme developed at the National Institutes of Health (Collins, 2007).

3.2.3 Confocal microscope

Confocal microscopy allows one to focus deep into tissue or cells and thus very thin sections are not required (Rezai, 2003). The term confocal refers to the condition where two lenses are arranged to focus on the same point, therefore, sharing the same foci (Hibbs, 2000). In this microscope, the sample, illumination and the detection of pinholes are all co-aligned against focal volumes of the optical system (Figure 3.3). A series of images at different positions can be produced through the thickness of the object, i.e., a series of X-Y images at different Z positions. As a result, in this technique, physical sectioning is no longer necessary, as it allows "optical sectioning" (Ferrando and Spiess, 2000).

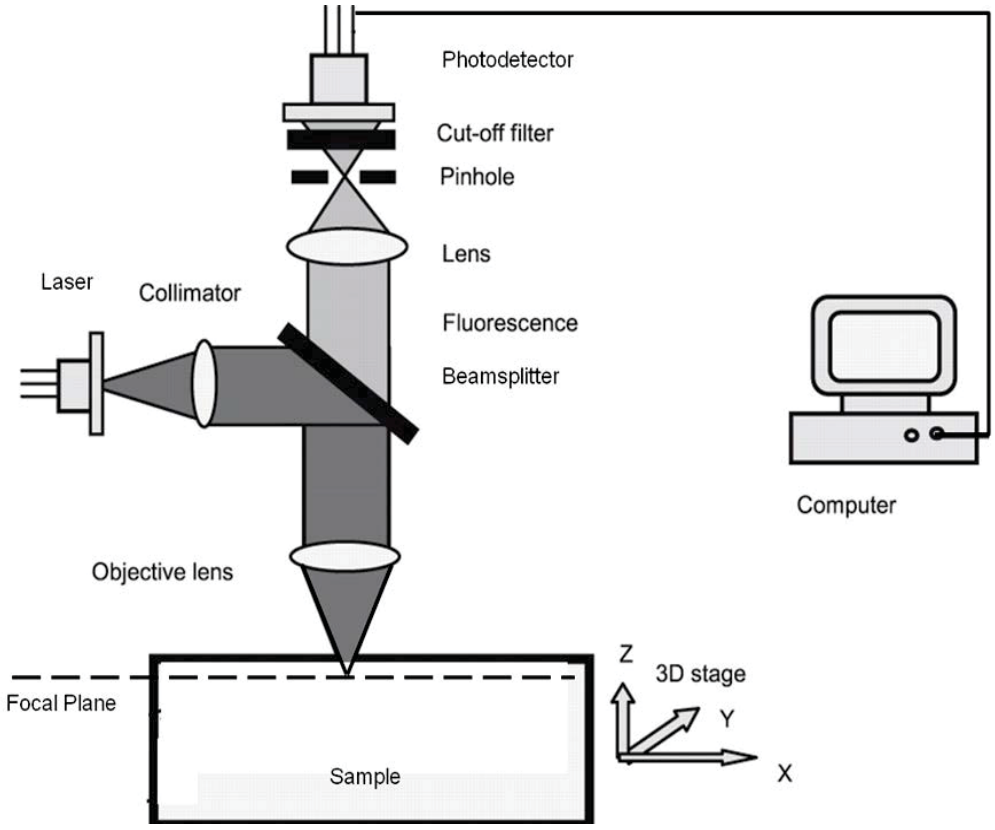

Figure 3.3. Schematic diagram of confocal microscope. The lens focuses the light beam at a spot within the specimen. The fluorescent light, because of its longer wavelength, comes to a focus at the plane of the detector pinhole. Only that fluorescence emission from the in-focus spot is able to pass through the pinhole detector to the computer screen (Modified after http://rpd.oxfordjournals.org/content/119/1-4/357/F2.large.jpg)

3.2.4 Confocal imaging and image analysis

The confocal laser scanning system (CLSM) used to obtain images through the depth of the tissue sections was a Leica TCS SP5 (6000B) (Leica Microsystems GmbH, Wetzlar, Germany). The Diode-pumped solid state (DPSS) laser provided the 405 nm wavelength excitation source for the epi-fluorescence detected at the emission range 569.9 nm – 627.8 nm. Sections were examined using 40x oil immersion objectives with cover slip applied to produce good quality images. Captured images were displayed on computer screen of the CLSM system. The images were digitized at a resolution of 1024x1024 pixels (picture elements). Confocal images from CLSM system were subjected to quantitative analysis using 'ImageJ' (National Institute of Health, USA) image analysis software. The 2-D image analysis was used to process the Z-series (stacked images) of confocal images taken by using CSLM system. The length and width of each cell (Figure 3.4) in each photograph were measured by drawing a boundary manually along cell walls using computer mouse and corresponding measurements (Figure 3.5) including total number of cells in each photograph were quantified by 'ImageJ' software.

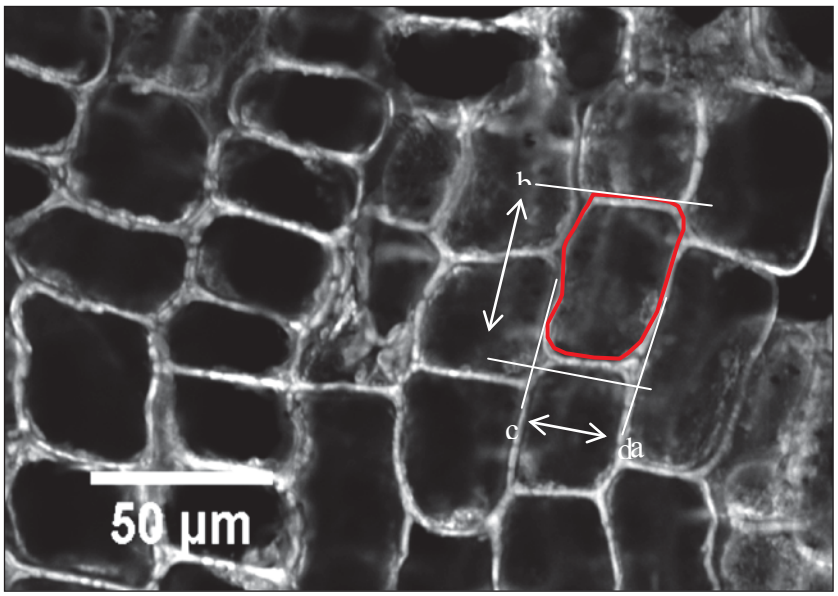

Figure 3.4. The boundary was drawn manually along the cell wall of each cell using the computer mouse and the corresponding measurements were given by 'ImageJ' software. For each cell, 'ab' denotes the length and 'cd' the width of the cells measured.

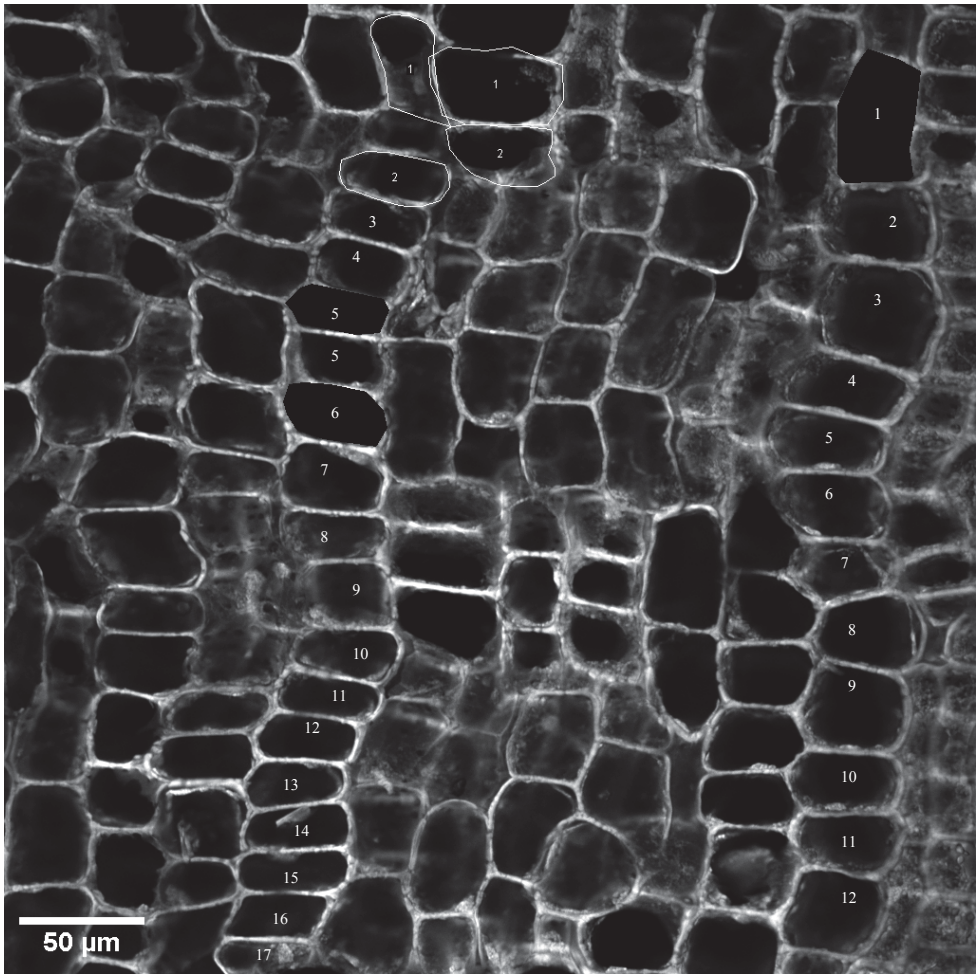

Figure 3.5. Image showing the cells that were counted: number of cell files in rows, columns and the total number of cells. The boundary was drawn manually along the cell wall of each cell using the computer mouse and the corresponding measurements were given by 'ImageJ' software.

3.2.5 Measurements and calculations

In this experiment, mean internode length was the average internode length of eight sub-sampled internodes collected from the primary shoot of composite apple trees from the previous experiment (Chapter 2) for microscopy studies. The length and width of each sub-sampled internode and the pith width were measured using digital callipers. Using confocal microscope, the focused areas of the sampled internodes of fixed dimensions

(section 3.2.4) 2-D images were captured. Using 'ImageJ', length, width and total number of cells of each image area (photograph) for each treatment were measured. The total number of cells in columns and rows for each photograph were counted. The total number of cells in a column along the length of the pith and the total number of cells in a row across the width of the pith were also calculated. The total number of cells in the pith along the full length of internode was calculated by multiplying cell number in a column in each photograph by the total number of image areas for the entire internode length.

3.2.6 Data analysis

The experiment consisted of two factors: rootstocks ('M.9' and 'RG') and GA treatment (with and without GA foliar sprays). Data were analysed using two-way ANOVA when normally distributed, otherwise non parametric ANOVA was performed when the data were not normally distributed, even after transformations. Firstly, the internode length, width and pith width were analysed and checked for main effects (rootstock and gibberellins) and rootstock × GA interactions. Secondly, the mean cell length, width and total number of cells were analysed as mentioned above. Correlation coefficients were calculated between mean total number of cells in a row and pith width, and, between mean total numbers of cells in a column along the longitudinal axis and internode length using regression. All statistical analyses used SAS version 9.2. The treatment effects are presented at $P \leq 0.05$ unless otherwise stated.

3.3 Results

3.3.1 Comparing mean cell number, length and width between samples at different positions within the pith of an internode

All three positions (bottom, middle and top) within one internode per treatment were observed. There was no position effect (bottom, middle and top) on the length, width and, cell number when data were analysed for all the treatments, except for 'RG' scion on 'M.9' rootstock , where the middle position of the pith had narrow cells compared with the other two positions (data not shown). Therefore, in subsequent measurements only the cells in the central portion of the internode were examined.

3.3.2 Effect of rootstocks and gibberellins treatment on internode length, cell expansion and cell division

The effect of rootstock and gibberellin treatment on internode length, cell length and cell number were compared with control ('RGRG without GA) over the middle portion of mature internodes, sub-sampled on one year old primary shoot.

3.3.2.1 Mean internode length

The rootstock × GA treatment interactions for the mean internode length of fully matured internodes taken for this study were significant (*P=0.007*). In addition, there was a significant GA main effect (*P=0.0001*). The sub-sampled internodes for scions on 'M.9' rootstock were shorter compared with those on 'RG' rootstocks without GA (Figure 3.6 and Table 3.1). GA treatment increased the internode length of scions on both rootstocks. The internodes of scions on 'M.9' and 'RG' rootstocks were statistically similar in length after GA treatment (Figure 3.8). The internodes for scions on 'M.9' were 38% shorter without GA compared with those on 'RG' rootstock without GA (Table 3.1). The internodes for scions on both rootstocks were similar in length with GA.

The internodes that were collected for this experiment were formed well before the time of termination. For 'RG' scions on 'M.9' rootstock without GA termination started one month earlier compared with that on 'RG' rootstock. In Chapter 2, when mean internode length was calculated there was no rootstock effect on internode length. However, the primary shoots were shorter due to early termination and slow plastochron rate for scions on 'M.9' rootstock. Towards the end of the growing season, internodes of all treatments started growing slowly and exhibited shorter internode length (Figure 3.6). The last formed ten internodes were plotted to show how internode length decreased towards the end of the growing season (June, 2008).

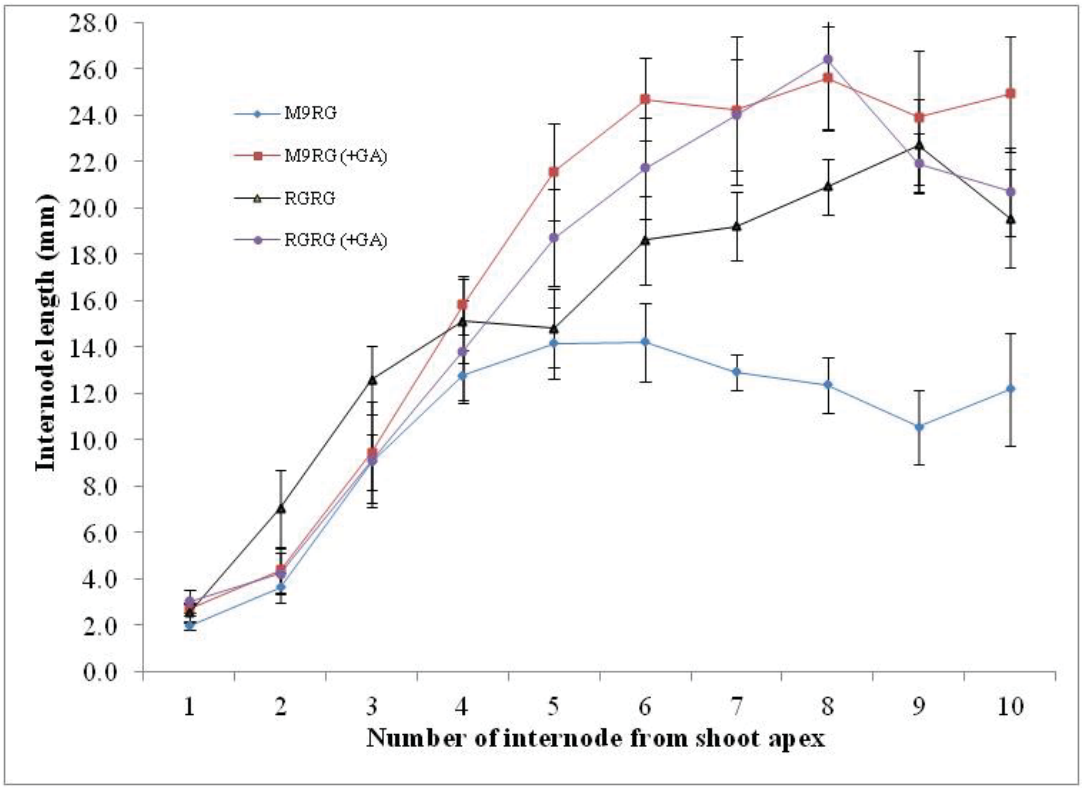

Figure 3.6. Internode lengths from shoot apex until the 10th node on the primary shoot of 'M9RG' and 'RGRG' without GA and with GA (+GA). 'M9RG' and 'RGRG' represent 'M.9' and 'RG' rootstock with 'RG' scion, respectively, without GA and, 'M9RG' +GA' and 'RGRG' +GA' represent GA_3+GA_{4+7} treatment applied from November(Nov) continued until the end of the growing season. Notice the gradual decrease in the internode elongation towards the end of the growing season (June, 2008).

3.3.2.2 Mean cell length and number

Regarding cell length, no difference was detected between rootstocks. However, a significant difference was detected (*P=0.003*) with GA treatment for 'RG' scions on both rootstocks (Figure 3.7 and Table 3.1). Therefore, GA increased the mean cell length for scions on both rootstocks. Regarding cell number, GA significantly increased for scion on 'M.9' but not for scion on 'RG' (see Section 3.3.2.3).

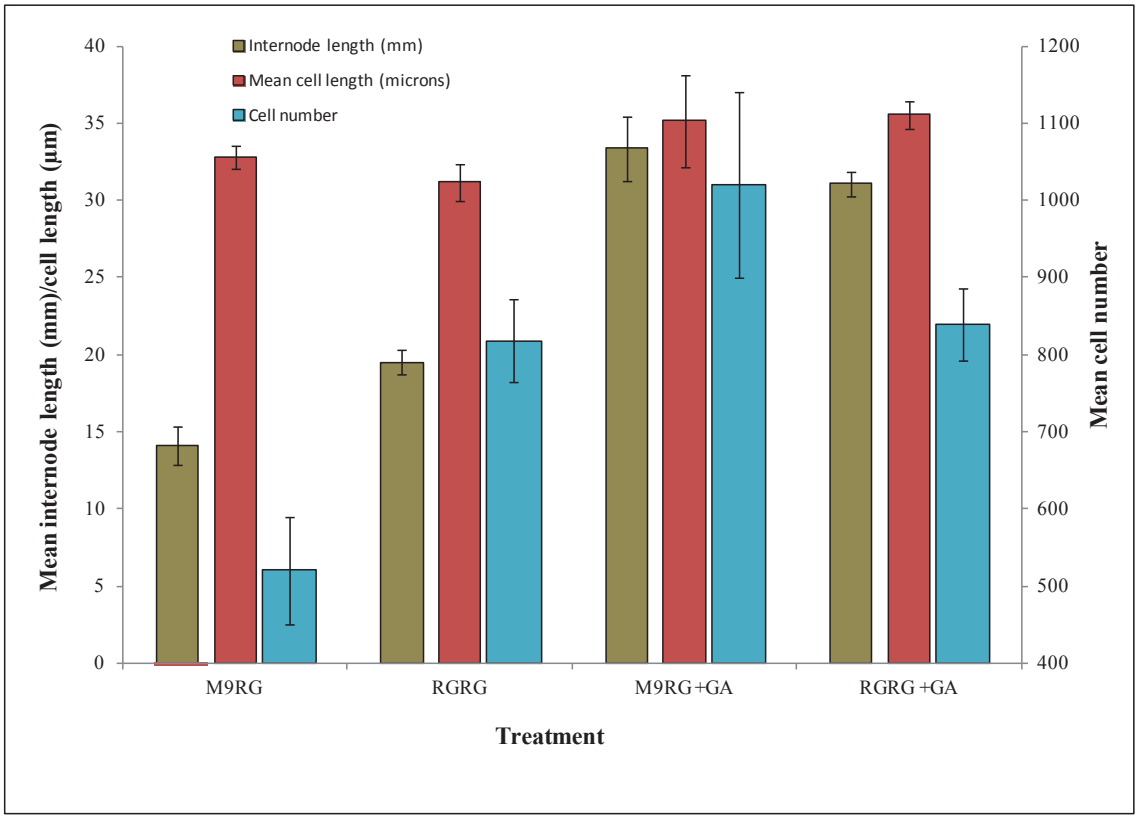

Figure 3.7. Effect of rootstock and gibberellin interaction on mean internode length and cell number and GA effect on mean cell length of sub-sampled internodes of 'RG' scions on 'M.9' and 'RG' rootstocks 'M9RG' and 'RGRG' represent 'M.9' and 'RG' rootstock with 'RG' scion, respectively, without GA and, 'M9RG' +GA' and 'RGRG' +GA' represent GA_3+GA_{4+7} treatment applied from November (Nov) continued until the end of the growing season.

Table 3.1. Mean internode length, cell length and the total number of cells along the length of the internode in pith tissues sampled along the longitudinal section of the internodes of scions on 'M.9' and 'RG' rootstocks without (-GA) or with (+GA) treatment. 'M9RG' and 'RGRG' represent 'M.9' and 'RG' rootstock with 'RG' scion, respectively, without GA and, M9RG +GA' and 'RGRG +GA' represent GA_3+GA_{4+7} treatment applied from November (Nov) continued until the end of the growing season The percentage increase in internode length, cell number and cell length were calculated in comparison with control 'RGRG' without GA. Mean values with standard errors

Treatment	Mean internode length (mm)	Mean total number of cells along internode length	Mean cell length (µm)	Percentage increase in		
				internode length (mm)	number of cells	Cell length (µm)
M9RG	14.1 ± 1.2 c	520 ±70 c	32.8 ± 0.7 b	-27.7	-36.4	5.1
RGRG	19.5 ± 0.8 b	818 ±53 ab	31.2± 1.2 b	-	-	-
M9RG +GA	33.4 ± 2.1 a	1020 ±121 a	35.0± 3.0 a	71.3	24.7	12.2
RGRG +GA	30.0 ± 0.8 a	839 ±47 ab	35.5 ± 0.9 a	53.8	2.6	13.8

Cell number along the internode length = mean no.of cells in a column in a photograpgh x number of photograpghs along the length of internode. Within a column means sharing the same letter are not significant at *P=0.05* using the multiple Tukey's test except for cell length, which used non-parametric ANOVA using multiple t-Test (LSD) at *P=0.05*.

When the frequency of distribution of cells with similar length was observed for all replicates for each treatment, most cells appeared to be around 25 µm to 40 µm for 'RG' scions on 'M.9' and 'RG' rootstock (Figure 3.8) with mean cell length 32.8 µm and 31.2 µm respectively. With GA treatment there was a greater spread of cells from 15 µm to 65 µm, with a greater preponderance of longer cells so that mean cell length increased by 13.8% (Table 3.1). The GA effect was greater for 'RG' scions on 'RG' rootstocks, in increasing mean cell length from 31.2 µm to 35.5 µm. Nevertheless, there was no significant difference between cell lengths of scions on both rootstocks with GA treatment (Table 3.1) and there were no significant interactions between rootstock and gibberellin treatment.

The figures 3.9A and 3.9B are representative images of 'M9RG' without GA and 'M9RG' with GA respectively and the figures 3.10A and 3.10B are representative

images of 'RGRG' without GA and 'RGRG' with GA respectively showing the arrangement of cells in a photograph captured using confocal microscopy.

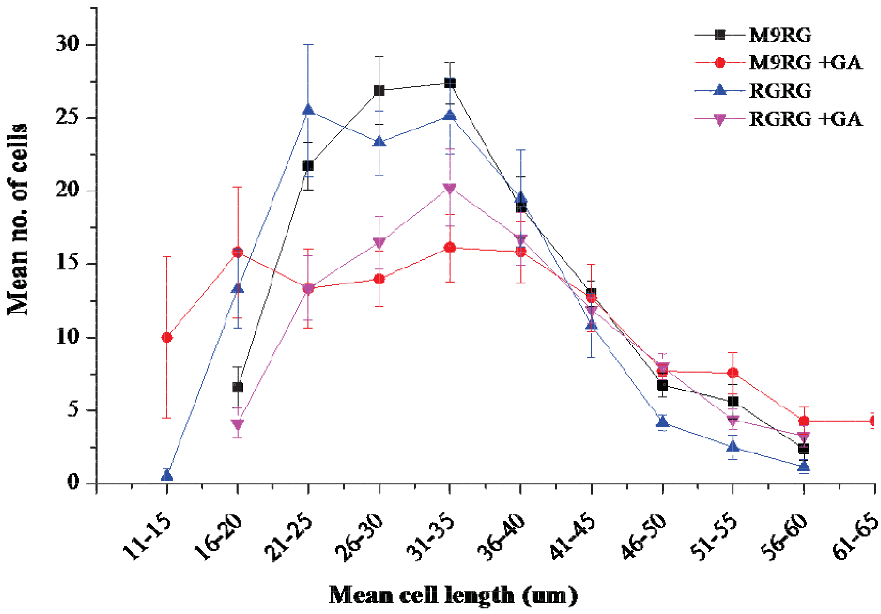

Figure 3.8. Rootstock and gibberellin treatment effect on range and distribution of individual cells with similar mean cell length (µm) of middle portion of the sub-sampled zone of the internode of 'RG' scions on 'M.9' and 'RG' rootstocks that was photographed 'M9RG' and 'RGRG' represent 'M.9' and 'RG' rootstock with 'RG' scion respectively, without GA and, M9RG +GA' and 'RGRG +GA' represent GA_3+GA_{4+7} treatment applied from November (Nov) continued until the end of the growing season (June, 2008).

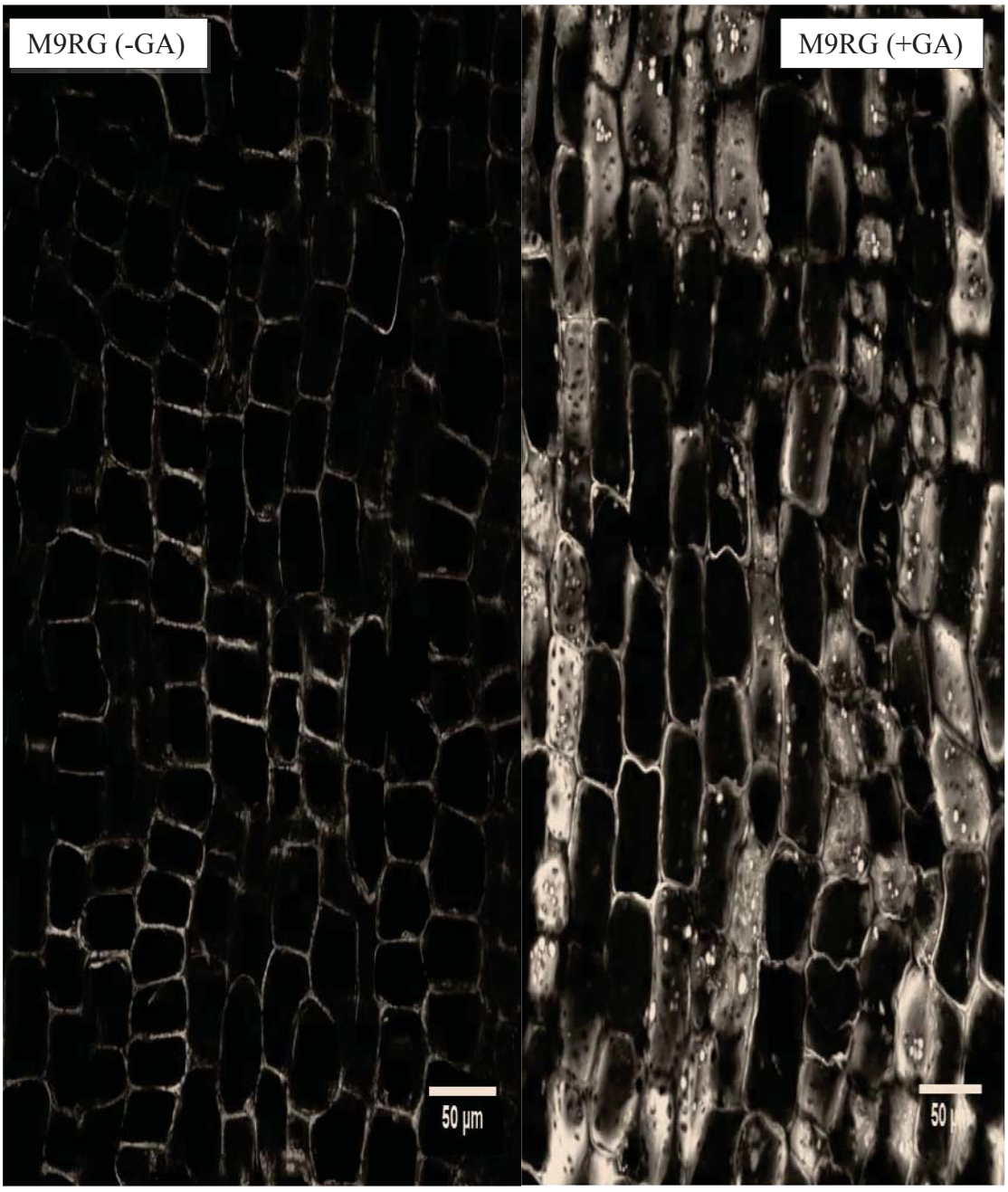

Figure 3.9. Representative images of longitudinal sections through middle portion of the pith region of internodes of 'RG' scions on 'M.9' without GA (A) and with GA (B) observed under a confocal microscope. 'M9RG' represent 'RG' scion with 'M.9' rootstock. The +GA represents the GA_3+GA_{4+7} treatment from November continued until the end of the growing season. Observe the longer cells of 'M9RG' with GA in B.

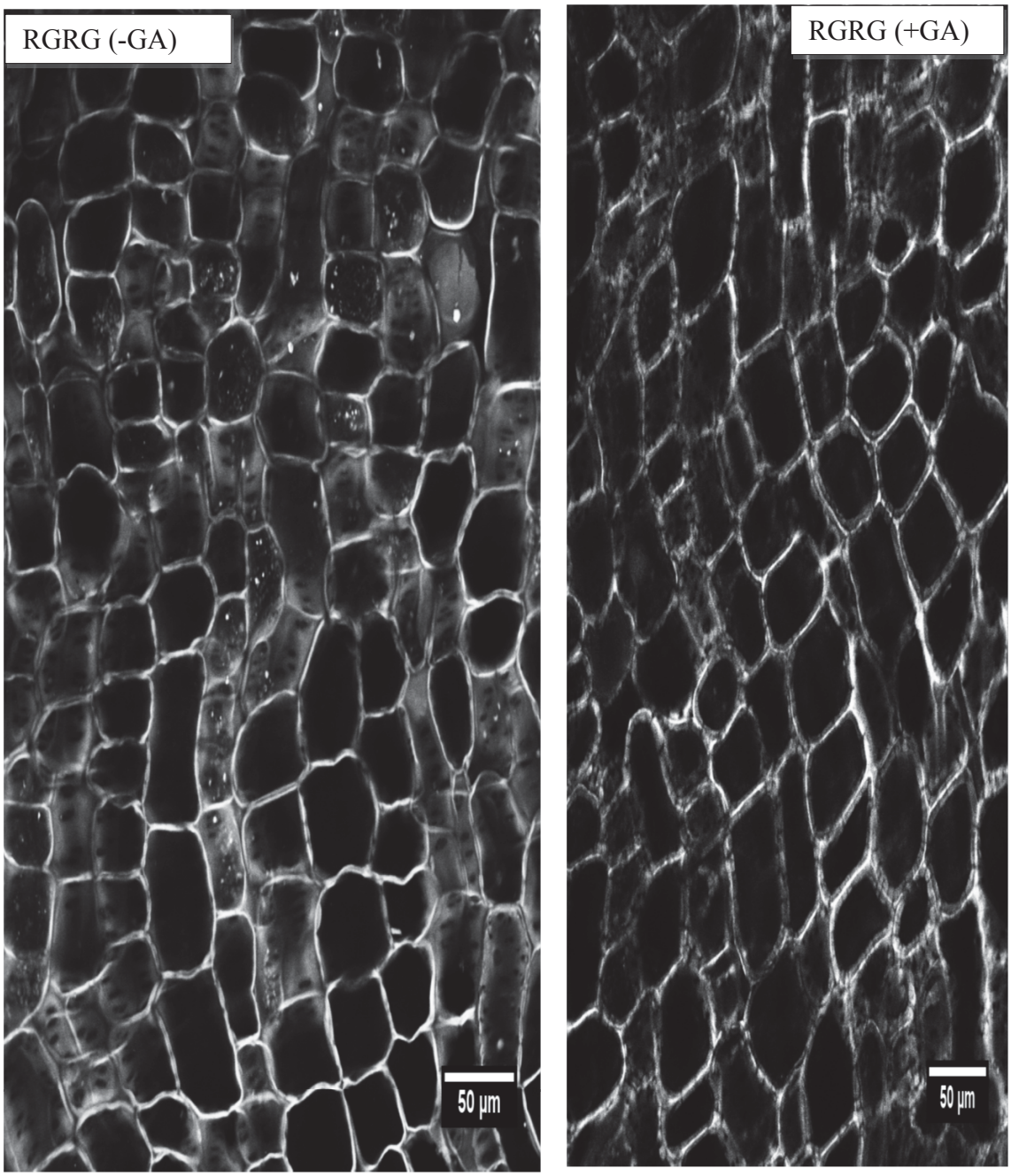

Figure 3.10. Images of longitudinal sections through middle portion of the pith region of internodes of 'RG' scions on 'RG' rootstocks without GA (A) and with GA (B) observed under confocal microscope 'RGRG' represent 'RG' scion on 'RG' rootstock. The 'RGRG' +GA represents 'RGRG' with GA_3+GA_{4+7} treatment from November continued until the end of the growing season.

3.3.2.3 Estimating total cell number for each internode: contribution of cell elongation and cell division for internode length increase

The rootstock × GA interactions were significant for cell number. The total number of cells along the internode length for scions on 'M.9' rootstock without GA were less (*P=0.008*) compared with those on 'RG' rootstock control (Table 3.2). With GA, the total number of cells for scions on the 'M.9' rootstock was not significantly higher compared with those on 'RG', both with and without GA. There were no significant differences between the cell numbers of internodes of scions on each rootstocks with GA treatment. Without GA, the shorter internodes of the scion on 'M.9' rootstock had fewer cells compared with the longer internodes of the scion on 'RG' rootstock although the cells were similar in length (Table 3.1).

The 'M.9' rootstock significantly decreased the length of internodes compared with 'RG' rootstock (Table 3.1). There was a 5.4 mm decrease (27.7%) in internode length for scions on 'M.9' rootstocks compared with those on 'RG' rootstock control. Despite highly significant differences in internode length between the rootstocks, mean cell length did not increase. In particular, the dwarfing 'M.9' rootstock had approximately equal mean cell length compared to the vigorous 'RG' rootstock (32.8 µm and 31.2µm, respectively), but presented shorter internodes (14.1mm) compared with those of vigorous rootstock (19.5mm). Therefore, the 27.7% decrease in internode length of scions due to 'M.9' rootstock was entirely due to decrease in cell number (Table 3.1). With GA treatment however, there was a significant increase in the length of internodes along with the increase in mean cell length and number (Table 3.2). There was a 71.3% increase in the internode length of 'RG' scion on 'M.9' rootstock with GA compared with 'RG' rootstock control (Table 3.2). The increase in mean cell length for scions on 'M.9' rootstock with GA treatment approached significance (*P=0.06)*, but its contribution to increased internode length was small (12.2%) compared with the large increase in mean cell number along the pith region with GA treatment (Table 3.1). However, for scions on 'RG' rootstock with GA although there was a slight increase of 20.6 cells (Table 3.2) in mean total cell number there was a significant increase (*P=0.01*) in the mean cell length (Table 3.2). Therefore, the 10.5 mm (53.8%) increase

in mean internode length of scions on 'RG' rootstocks with GA compared with 'RG' rootstock control resulted from an increase cell length (13.8%) primarily and cell number (2.6%) secondarily (Table 3.1).

There was a linear relationship between total number of cells in a column and internode length (R^2=0.60) for scions on 'M.9' and 'RG' rootstocks with and without GA, (Figure 3.11). As the data points are fewer and also due to limited range of internode lengths, the correlation is poor when checked for individual treatment separately. However, there was a positive trend for increased cell number as the internode length increased, but it can be seen that all the data points for 'RGRG' fall above the trend line.

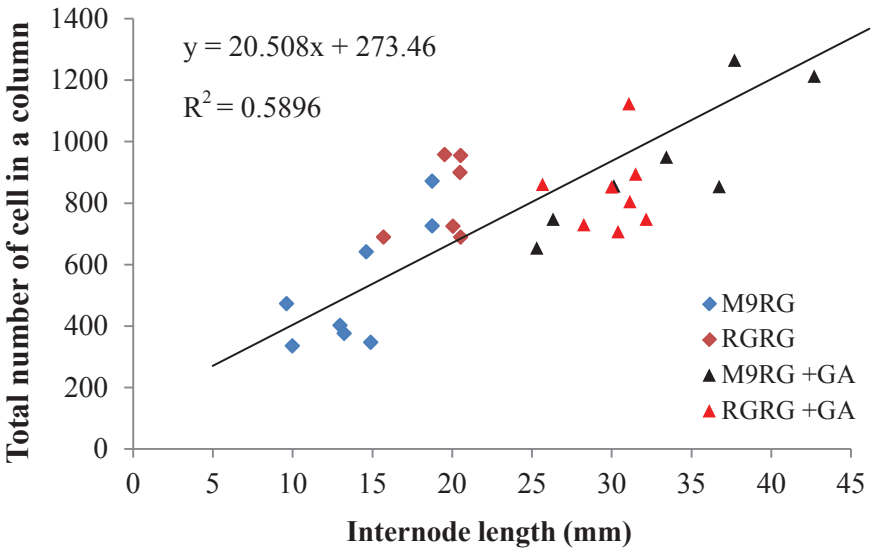

Figure 3.11. Relationship between internode length (mm) and number of cells in a column along the length of internode of 'RG' scions on 'M.9' and 'RG' rootstocks with and without GA treatment. 'M9RG' and 'RGRG' represent 'M.9' and 'RG' rootstock with 'RG' scion respectively without GA and, 'M9RG +GA' and 'RGRG +GA' represent GA_3+GA_{4+7} treatment applied from November (Nov) continued until the end of the growing season.

Table 3.2. Over-all rootstock, gibberellin effect and their interactions on internode length, cell length and number along the length of the internode The increase due to interaction and their significant difference (P-values) were given

	-GA	+GA	Increase due to interaction between rootstock × gibberellin	Significance due to GA effect
Internode length				
'M.9'	14.1 b	33.4 a	33.4-14.1 = 19.3 a	*0.0001*
'RG'	19.5 a	30.0 a	30.0-19.5 = 10.5 b	*0.0001*
P value	*0.007*	*0.08*	*0.04*	
Cell length				
'M.9'	32.8 b	35.0 a	35.0-32.8 = 2.2a	*0.06*
'RG'	31.2 b	35.5 a	35.5-31.2 = 4.3a	*0.01*
P value	*0.6*	*0.8*	0.4	
Cell number				
'M.9'	520.9 b (40cells/mm)	1029.0 a (31cells/mm)	1029.0-520.9 = 508 a	*0.0001*
'RG'	818.9 a (42cells/mm)	839.5 a (28cells/mm)	839.5-818.9 = 20.6 b	*0.8*
P value	*0.008*	*0.1*	*0.02*	

Means within columns sharing the same letter are not significant at *P*= 0.05 using multiple Tukey's test except for cell length, which used non-parametric ANOVA using multiple t-Test (LSD) and for increase due to rootstock × GA interaction used univariate ANOVA. For each parameter P values are given for differences between rootstocks. For the increase due to interaction between rootstock × gibberellin single factor ANOVA.

3.3.3 Effect of rootstocks and gibberellin treatment on different attributes of internode across the width

3.3.3.1 Mean width of internode, pith and cell

There was neither rootstock (*P=0.08)* nor GA treatment effect on internode width (Figure 3.12). However, the difference in internode width of scion on 'RG' rootstock with GA and the scion on 'M.9' rootstock without GA was significant at *P=0.02*. The

internode pith-width of 'RG' scion on 'M.9' rootstock was reduced compared with that on 'RG' rootstock (*P=0.007)*.

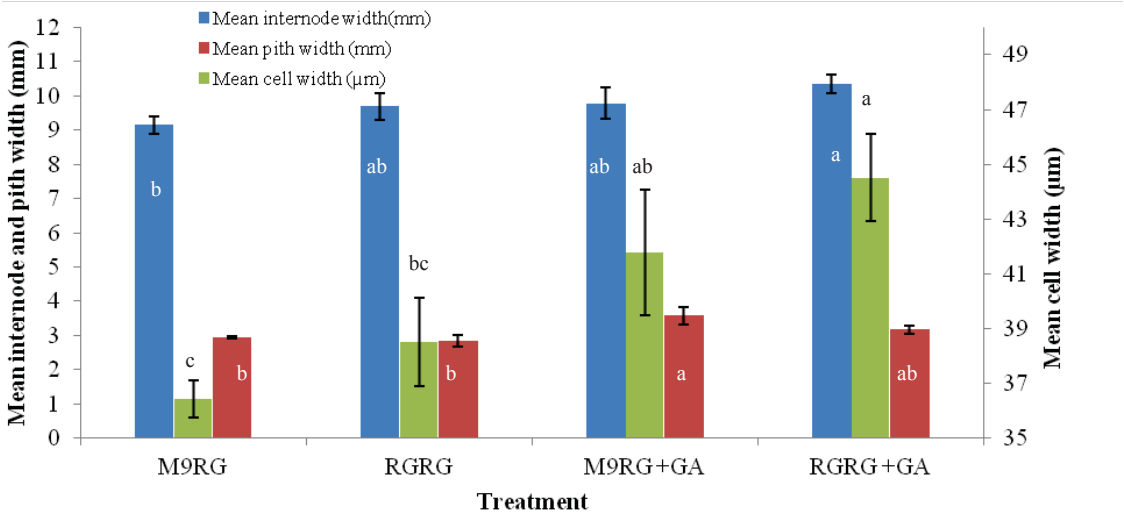

Figure 3.12. Effect of rootstock and gibberellin treatment on mean internode and pith width (mm) and mean cell width (µm) of 'RG' scions on 'M.9' and 'RG' rootstocks 'M9RG' and 'RGRG' represent 'M.9' and 'RG' rootstock with 'RG' scion respectively, without GA and, M9RG +GA' and 'RGRG +GA' represent GA_3+GA_{4+7} treatment applied from November (Nov) continued until the end of the growing season. Columns sharing the same letters for a single variable are not significantly different at P=0.05 using multiple t-Test (LSD) LSD. Bars represent the standard error.

Mean cell width for scions on 'RG' (41.8 µm) was higher (P=0.03).compared with those on 'M.9' rootstock (36.4 µm), (Figure 3.12). There was no significant effect of GA on cell width for scions on both rootstocks. However, with GA treatment for scions on 'RG' the cells were broader (44.5 µm) compared with those on 'M.9' rootstock with GA (36.4 µm).

When frequency distribution of cell width was obtained for all replicates for each treatment (Figure 3.13), most cells appeared to be around 30 µm to 45 µm for 'RG' scions on 'M.9' and 'RG' rootstock with mean cell width 36.4 µm to 41.8 µm respectively. Rootstock had an effect on the mean cell width of pith. Consequently, cells

for 'RGRG' were broader compared with those for 'M9RG'. With GA treatment mean cell-width for 'M9RG' increased slightly compared with those of untreated ones (Figure 3.13). Although the cells for 'RGRG' were wider than those of 'M9RG', there was little GA effect on the width of the cells of 'RGRG' (Figure 3.13)

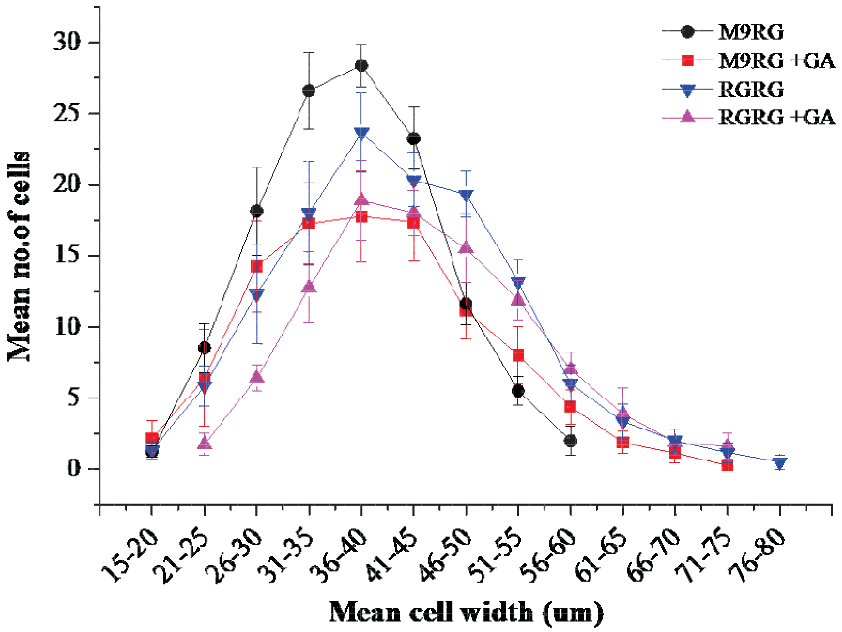

Figure 3.13 Rootstock and gibberellin treatment effect on range and distribution of individual measurements with similar mean cell width (µm) 'M9RG' and 'RGRG' represent 'M.9' and 'RG' rootstock with 'RG' scion respectively, without GA and, M9RG +GA' and 'RGRG' +GA' represent GA_3+GA_{4+7} treatment applied from November (Nov) continued until the end of the growing season. Bars represent the standard error.

3.3.3.2 Estimating the total number of cells across the pith and its correlation with internode width

The mean total cell number across the pith for scions on 'M.9' (95) and for those on 'RG' rootstocks (104) was not significantly different. With GA for scions on 'M.9' rootstocks the mean total number of cells across the pith decreased slightly, but approached significance (*P=0.06)* for the scions on 'RG' rootstocks compared with the

untreated ones. Thus, there was a decrease in the total number of cells across pith of 'RGRG' with GA to 77 from 104 of untreated 'RGRG'.

There was a positive correlation between the pith width and the total number of cells across the pith (Figure 3.14) of internodes of scions of 'M.9' rootstock (R^2=0.72) but no correlation for 'RG' rootstock (Figure 3.14). There was also a positive correlation between pith width and the total number of cells across the pith for GA treated internodes on 'M.9' rootstock (Figure 3.14) but no positive correlation with those on 'RG' rootstocks (Figure 3.14), with GA. Therefore, there was no correlation between the cell number across the pith and pith-width for scions on 'RG' rootstocks with and without GA.

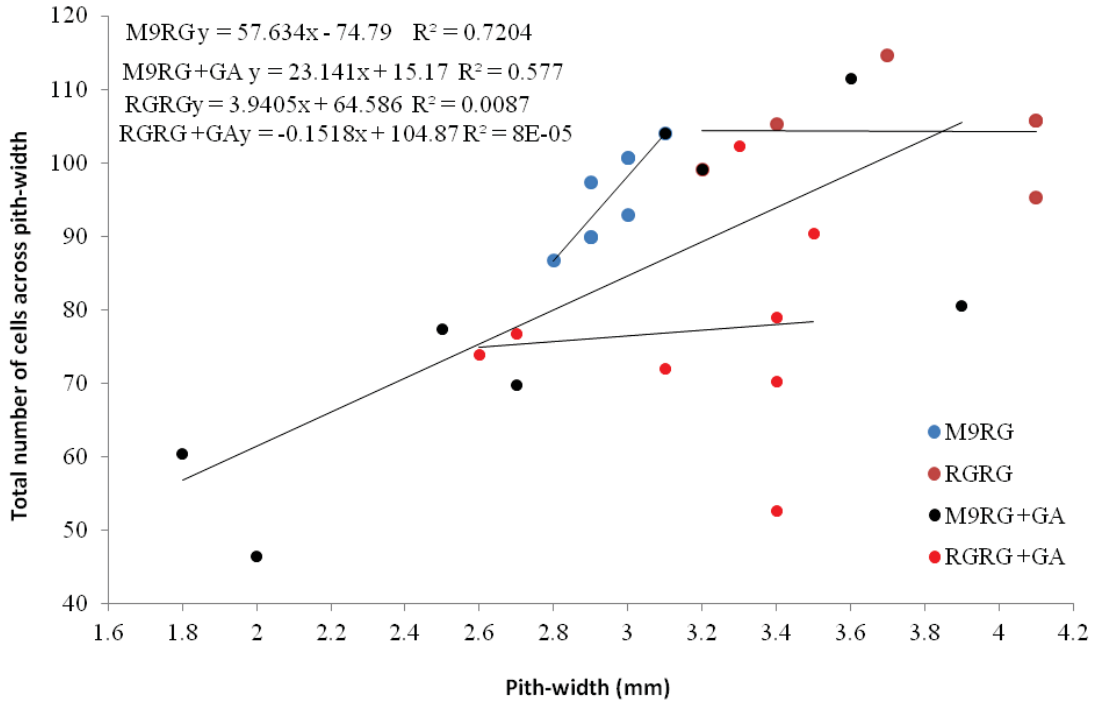

Figure 3.14. Relationship between pith width and total number of cells acroos the pith in mature internodes of 'RG' scions on 'M.9' and 'RG' rootstock with and without gibberellin.. 'M9RG' and 'RGRG' represent 'M.9' and 'RG' rootstock with 'RG' scion, respectively, without GA and, M9RG +GA' and 'RGRG +GA' represent GA_3+GA_{4+7}treatment applied from November (Nov) continued until the end of the growing season.

3.4 Discussion

The choice was made to focus histological examinations on stem pith tissue rather than on cortex and epidermis. This choice was based on the previous studies of cellular activities in different tissues carried out on *L. styraciflua* L. (Brown et al., 1995) and on apple trees (Ripetti et al., 2008), as the pith cells play a major role in increasing both cell number and cell length during internode elongation. It was also reported by Priestly (1929) that pith cell maturation factors such as vacuolization occur first, followed by maturation of the cortex and epidermis. The middle portion of the internode was selected to measure variables such as length, width and cell number as it was found in earlier studies that position had no significant effect (Ripetti et al., 2008). In this study also, it was observed that all three positions (bottom, middle and top) were similar (section 3.3.1). The peripheral narrower cells at the borders of the pith were ignored.

3.4.1 Rootstock effect on internode elongation in terms of cell division and/or cell elongation

In Chapter 2, it was observed that the scion on 'M.9' rootstock reduced the final mean primary shoot length without any significant difference in mean node number as well as mean internode length. It was also found in Chapter 2 that scions on dwarfing rootstocks were shorter because of early termination (refer Section 2.4.1.3) and slow rate of node production (refer Section 2.4.1.2.1). As a result, mean primary shoot was significantly shorter for scions on dwarfing rootstock compared with that on a vigorous rootstock. Therefore, there was no significant rootstock effect on the mean internode length in Chapter 2 as the internode length was calculated for the entire primary shoot and also because the last formed internodes on the shoot apical meristem do not elongate completely due to the act of termination influenced by the cold temperatures (Figure 3.6). Conversely, when individual fully matured internodes that were formed when the rootstock effect was significant were collected, there was a significant difference in their mean length (n=8). Consequently, the 'M.9' rootstock significantly decreased the internode length, when lengths of sub-sampled internodes were compared

with those on 'RG' rootstocks (Figure 3.8). Therefore, the internodes were longer on vigorous 'RG' rootstock (19.5 mm) compared with those on dwarfing rootstock (14.1 mm). The role of cellular activities, such as cell division and cell elongation, on the internode length was critically observed. When sample images were analysed, though there was no rootstock effect on the mean length of cells (Table 3.1), there was significant effect on the mean cell number. With approximately equal mean cell length values with dwarfing 'M.9' and vigorous 'RG' rootstocks (31.2 μm and 32.8 μm, respectively), the longer internodes of scions on 'RG' rootstocks (19.5 mm) can be attributed to more cells (Table 3.1) as the number of cells calculated for length of internode were more for scions on 'RG' rootstocks. Therefore, the decrease in the internode length of scion on 'M.9' dwarfing rootstock compared with 'RG' rootstock control was due to decreased cell number.

3.4.2 Gibberellin effect in the internode elongation in terms of cellular activity cell division and/or cell elongation

There was a 71.3% increase in mean internode length for scion on 'M.9' rootstocks with GA foliar sprays ('M9RG'+GA) compared with 'RG' rootstock control. For scions on 'RG' with GA foliar sprays ('RGRG'+GA) the increase was only 53.8% compared with 'RG' rootstock control. Hence, the effect of GA on internode elongation was more for scions on the 'M.9' rootstock compared with those on 'RG' rootstock (Table 3.2). Thus, there was a significant rootstock × gibberellin interaction as GA effect depended upon the type of rootstocks onto which the scion was grafted. With GA treatment, cells were significantly longer for internodes of scions on both the rootstocks ('M.9' and 'RG') compared with the untreated scions. There was a 2.2 μm increase on average in cell length for 'M9RG' when compared with untreated ones. With regards to cell number, there was an increase in mean cell number of 508 cells with GA treatment i.e., 96% increase in cell number for scions on 'M.9' rootstock with GA. Thus, when compared the effect of GA on cell length and cell number, it was very clear that the contribution of cell number to increased internode length with GA for scions on 'M.9' rootstocks was

significant compared to that for scions on 'RG' rootstock. Therefore, it may be concluded that the increase in mean internode length for scions on 'M.9' rootstocks with GA treatment was mainly due to the increase in the mean number of cells. Certainly, as there was mean increase of 2.2 μm cell length with GA for scions on 'M.9' rootstock there was also cell length contribution, which was much less, i.e. 6.7% .

It was reported by Brown and Sommer, (1992) that, in many woody species the differences in internode length was more due to cell number than to cell length. Although there was no significant increase in cell number for scions on 'RG' rootstocks with GA, there was a significant increase in mean cell length compared with untreated ones. Moreover, for scions on 'RG' rootstock with GA there was a significant increase in the internode length compared with untreated ones (Figure 3.7 and Table 3.1). Thus for scions on 'RG' rootstocks with GA a slight increase in mean cell number (20.6 cells) and a significant increase in cell length (4.3 μm) together contributed to the 10.5 mm increase in internode length. Therefore, the present histological investigations suggested cell division to be the primary reason for GA stimulation of internode length and cell elongation to be secondary. The decrease in internode length for scions on 'M.9' rootstock ('M9RG') compared with 'RG' rootstock ('RGRG') was due to decreased cell number (Table 3.1) and the increase in internode length of 'M9RG' compared with 'RGRG' with GA treatment was due to increased cell number. Thus if most of the rootstock and gibberellin effect is due to cell number, presumably one can conclude that most of the rootstock effect on increasing the internode length is due to differences in gibberellins.

The good correlation (R^2=0.58) between internode length and total cell along the length for 'M9RG' without GA ('M9RG') may be due to a better range of internode lengths (data not shown), whereas for scions on 'RG' rootstocks, the correlation was poor due to limited range of internode length. On the whole, for all internodes of all treatments, interestingly, there was a good correlation (R^2=0.60) between total numbers of cells along the length of internode (Figure 3.11). As the internode length increased the total number of cells increased.

From section 3.3.2, it was clear that internodes were shorter for scions on 'M.9' rootstocks compared with those on 'RG' rootstocks, and GA foliar spray increased internode length primarily by increasing the cell number and, to a lesser extent, cell length. This explains that scions on 'M.9' rootstocks were GA deficient. From section 3.1.2.2, it was suggested that for a significant GA response adequate amount of auxin is required. For apples, it was reported that the velocity of basipetal auxin transport was lower in the stem tissue of the dwarfing 'M.9' rootstock compared to the more vigorous MM.111 rootstock (Soumelidou et al., 1994a; Kamboj et al., 1999b). Furthermore, in this experiment the 'RG' scions on 'M.9' rootstocks were significantly longer when sprayed with GAs compared with the untreated scions. The fact that their effect was very significant may be due to sufficient amount of auxin in the 'RG' shoot, with which GA could interact. Although gibberellin treatment appeared to reverse dwarfing of 'RG' scion on 'M.9' rootstock compared with 'RG' rootstock control, the root system still remained smaller for 'M9RG' with GA compared with 'RG' rootstock control (refer Section 2.4.5.1) similar to previous studies (van Hooijdonk, 2009). The 'RG' scion on 'M.9' rootstock with gibberellins grown vigorously may be at the expense of root system and the scion effect on root system may not seem to be associated with carbon supply from shoot. Studies to determine the effects of scion and rootstock genotype on the biomass allocation within the young grafted grape vine, provided evidence for conferred root vigour by the scion did not relate to carbon supply from the shoot (Tandonnet et al., 2010).

3.4.3 Rootstock and gibberellins effect on the internode width

There was no rootstock or GA effect on internode width for scions on 'M.9' and 'RG' rootstocks. However, the broader internodes for scions on 'RG' rootstocks with GA could be attributed to increase in cortical tissue as there was no increase in pith width (Figure 3.12). For 'M.9' rootstock, there was a significant decrease in pith-width and cell-width without significantly affecting the total number of cells across the pith, compared with the scions on 'RG' rootstock. Although the cells were broader for scions

on 'RG' compared with the scions on 'M.9' rootstock, the pith could accommodate equal number of cells as there was 0.63 µm (21.6%) increase in pith width. Therefore, for 'RGRG' without GA, mean cell-width and total number of cells across the pith increased and also pith-width increased. Thus, for scions on 'RG', the cell number increased as the pith width increased. On the other hand, the cell-width for scions on 'RG' rootstocks with GA was 44.5 µm and thus increased from 41.8 µm without GA to 44.5 µm with GA and, as there was no increase in pith width, the number of cells across pith decreased.

Furthermore, Bland (1978) concluded that in apple trees long shoots have longer, but not more internodes than short shoots (spurs), and, the internode length depends upon the rate and duration of cell division rather than on the length of the pith cells. However, in previous studies and also in this study (see Section 2.4.2.4 and Figure 2.15), it was observed that the final length of an annual shoot is determined by the number of nodes and length of internodes (Seleznyova et al., 2003; van Hooijdonk, 2009). As the node number increased, the length of the annual shoot (sylleptic shoots) increased with increased internode length. Thus, in the Figure 3.10 the longer internodes of M9RG with GA resulted from more cells compared with the shorter internodes of M9RG without GA. There is plenty of information in the literature that supports these findings regarding increased internode length with increased cell number (Guttridge and Thompson, 1959; Stant, 1961; Arney and Mancinelli, 1966; Reid et al., 1983), and there is also a recent work on dwarf cucumber where it was reported short internodes were found to have fewer cells (Xin et al., 2012).

The longer shoots have longer internodes, which may be because of actively growing SAM with sufficient amount of GA and IAA to initiate node at the apical meristem and elongate node at the sub apical meristem. As a result of the activity of gibberellin with IAA interaction the sub apical meristem produced longer cells, which results in longer shoots.

3.5 Summary

The internodes, which were collected from sub-sampled zone on the primary shoot of 'RG' scions on 'M.9' dwarfing rootstock, were shorter compared with those on 'RG' rootstocks. The sub sampled zone of the primary shoot corresponds to that formed when rootstock effect was greater (i.e., January 12th) (Figure 3.8). With GA treatment the internodes were longer for scions on both rootstocks. However, there was a significant rootstock × gibberellin interaction, where the GA effect depended upon the type of rootstock the scion was grafted to. Therefore, the effect of gibberellins was greater for scions on 'M.9' rootstocks compared with those on 'RG' rootstocks in increasing the length of the internode. Histological studies using confocal microscopy showed that cells within sub-sampled internodes were similar in length for scions not treated with gibberellins on both rootstocks (Table 3.1), but there were more cells in the pith of scions on 'RG' rootstocks. Therefore, the longer internodes of scions on 'RG' rootstocks were due to the increased number of cells. The 'RG' scion on 'M.9' rootstock showed a great response to GA foliar sprays, suggesting they were deficient in GAs. The GA treatment significantly increased the length of internodes (Figure 3.8) of scions on both rootstocks (Table 3.1) and a significant increase in the number of cells for scions on 'M.9' rootstock. Therefore, the contribution of cell division was higher compared with cell elongation in the increased internode length of scions on both the rootstocks with GA. Thus, the role of GA in internode elongation was primarily in increasing the number of cells and secondarily in cell elongation as there was very little increase in the mean cell length. Therefore, for an apple internode, gibberellins acted on both cell division and cell elongation. The shorter internodes for scions on 'M.9' rootstock were collected from the sub sampled region of the primary shoot only, and were selected because they had formed at a critical time when dwarfing expression commence (Jan 12th). The internodes corresponding to this period with GA foliar sprays were significantly longer. Thus shorter internodes due to dwarfing rootstock may be due to GA deficiency. During the period which the dwarfing rootstock produced shorter internodes the GA treatment produced longer internodes. Thus one can conclude that the most of the rootstock effect was due to differences in gibberellins.

In Chapter 2, GA foliar sprays for scions on 'M.9' and 'RG' rootstocks increased node number, as well as node length, significantly compared with the untreated scions (Section 2.4.1.2.1. and Table 2.5). In such case, GA presumably acted both on apical and sub-apical meristems of apple shoots since:

(a) Leaf primordia initiation takes place in the peripheral zone of shoot apical meristem leading to greater node numbers (Table 2.5). Although GA appeared to increase the node number, it is actually IAA, which is essential for node initiation. Therefore when gibberellins were sprayed they must have increased the amount of IAA in the PZ of the SAM.

(b) Increased cell numbers in the internodes were probably due to gibberellin stimulation of the rib-meristem zone /sub-apical meristem of the SAM of apple shoot, although further research would be required to confirm this.

(c) Therefore, GA, by affecting apical meristem increased node number and by affecting sub-apical (rib-meristem) meristem, increased the length of internode. It was understood from the results of Chapter 3, that the increased length of internode was due to an increased number of cells in the internode, but what happened in the apical meristem, compared with sub-apical meristem with GA treatment could be clarified by comparing cell numbers of immature internodes to fully elongated internodes.

Chapter 4

Is the hormonal signalling in kiwifruit similar to apple?

4.1 Introduction

Primarily rootstocks were used as a method of propagation of selected scions that do not develop true-to-type when propagated from seeds (Webster, 2002). However, in temperate fruit trees they also are used as the principal method of controlling excessive vigour of the scion cultivar. For apple and pears, dwarfing rootstocks have been used for centuries, yet presently for kiwifruit, there are few or no reports of vigour controlling rootstocks in the literature. In New Zealand, the rootstock 'Kaimai' (TR-2) has been bred and selected for enhanced precocity of the scion variety, especially 'Hayward' (Warrington, 2000; Oliveira and Fraser, 2005). There is no information on the physiological mechanism by which an *Actinidia* rootstock might reduce scion vigour (Clearwater et al., 2004). Presently the rootstock 'Bounty 71' is under recommendation to be evaluated commercially as it showed potential during an evaluation trial from 2000 to 2007. This rootstock was found to have benefits relative to two known commercial rootstocks 'Hayward' and 'Kaimai'. The 'Bounty 71' rootstock caused 'Hort16A' to reduce vigour primarily by increasing self terminated short shoots thereby, producing a more open canopy and promoting number of flowers per winter bud. In addition, there was an increase in fruit size and fruit number without compromising dry matter. Thus, 'Bounty 71' was found to be more beneficial with higher yields of export quality fruit (Thorp et al., 2012). However, presently there are kiwifruit rootstock evaluation trials underway in Plant and Food Research, TePuke and Palmerston North. In Palmerston North, 13 inter-specific hybrid clones of *Actinidia,* obtained from Motueka have been grafted with *Actinidia deliciosa* 'Hayward' scion. These were used for evaluation trials for potential vigour controlling rootstock in kiwifruit. The rootstock parents used in order of vigour are: *A. chinensis* × *A. macrosperma, A.polygama; A. macrosperma x A. melandra, A. macrosperma*, *A. polygama* × *A.chinensis*. Vigour rating of these rootstocks was based according to the previous trials with 'Hort16A' scion grafted on each mentioned rootstock *(John Palmer, personal communication).* Rootstocks for kiwifruit would be of considerable value to the industry if they markedly

controlled excessive scion vigour and redirected vine carbon toward yield of high quality fruit.

The manipulation of plant architecture by controlling plant height and shape is an integral part of modern intensive agricultural and horticultural systems (Jackson, 2004). Vigour control can be achieved by using growth retardants that reduce stem elongation with minimal adverse effects on other aspects of plant growth. Plant growth regulators (PGR) are expensive but act very effectively in lower concentrations, hence vast quantities are not sprayed. Moreover, many PGRs are not particularly toxic in the environment. When compared to the use of herbicides, fungicides and insecticides, the use of PGR has been small (only 3.4% of the total usage of chemicals), and also the use of PGRs to reduce shoot growth conserve natural resources by exploiting them effectively. Inputs such as fertilizers, energy and labour could be converted to harvestable yield more efficiently under the influence of PGRs (Rademacher, 1993). Accurately timed foliar sprays of PBZ an anti-gibberellin were effective in managing reduction in tree growth and induce fruit production in young, non-bearing McIntosh apple trees on vigorous rootstocks (Estabrooks, 1993). Therefore, using PGRs to reduce shoot growth is not deleterious environmentally. However, public perception needs to be considered. In addition, architectural modifications imposed by exogenous hormones are the basis to understand regulation of vigour by endogenous hormones. Therefore, it is important to understand endogenous hormonal signalling mechanism in vegetative vigour of kiwifruit by supplying exogenous PGRs. This could also be a basis to identify genes responsible, and the function they perform, in vigorous growth of kiwifruit vines. In order to isolate such genes one should understand hormonal physiology behind vegetative vigour of kiwifruit vines. Apart from a few *in vitro* studies (see 1.5.4.1), there is little understanding how endogenous hormones: auxin, cytokinins and gibberellins act to influence vegetative growth and shoot architecture of kiwifruit vines.

The *in vitro* studies in kiwifruit revealed that axillary shoot formation was influenced by the type and concentration of cytokinin used (Arigita et al., 2005; Akbas et al., 2007) The further growth of axillary shoot was enhanced by the presence of GA_3 in the medium, by promoting cell division and elongation in the sub apical region of the newly formed shoots (Moncalean et al., 2003). Thus, kiwifruit requires cytokinins for axillary bud activity and gibberellins for elongation of secondary shoots, which is similar to

other trees. Growth retardants, paclobutrazol (PBZ) and succinic acid dimethly hydrazide (SADH) sprayed on 'Hayward' kiwifruit vines did not reduce shoot growth (Biasi et al., 1987), however, there was 44% decrease in shoot growth when Prohexadione-calcium (Ca-Pro) was used. Ca-Pro reduced the levels of highly active GA_1 and caused accumulation of its precursor GA_{20} (Evans et al., 1999) by blocking two oxoglutarate-dependant dioxygenases which catalyse the 3-beta hydroxylation of GA_{20} to GA_1 (Rademacher, 2000). Thus, vigorous kiwifruit vine growth can be attributed to high levels of GA_1, as Ca-Pro reduced the growth.

High vegetative vigour of grapevines was shown to be associated with high endogenous levels of gibberellins (Ziv et al., 1981) with double the amount of gibberellin in their buds compared with those on normally growing shoots. Although auxins were involved in developmental processes in grapevine (Weaver, 1956) they did not show any effect on vegetative growth. When both auxins and cytokinins applied to grapevines, there was a significant effect on fruit set, development and maturation, but no effect on vegetative growth. Reduction of vegetative growth rate during the floral induction and differentiation periods was achieved by application of Maleic hydrazide (MH) to the growing shoots (Lavee, 1986). Within five days after MH application plants exhibited a new slower rate of growth. The inhibitory effect of MH was reversible by IAA when applied during the first 5 days after applying MH i.e. before the new slow growth rate was established; however, GA_3 reduced the inhibitory effect of MH at any stage after treatment. Thus, the vigorous growth of grapevine is associated with high levels of gibberellins.

For apple, many previous studies focused on hormonal influence (Rogers and Beakbane, 1957; Lockard and Schneider, 1981; Kamboj et al., 1997) on the dwarfing of a scion by a rootstock. Recently it was observed in composite apple trees that there is a hormonal shoot-root-shoot signalling mechanism for the decreased scion growth on a dwarfing rootstock (van Hooijdonk, 2009). By the end of the first year of growth after grafting, mean total shoot length and node number of the scion grafted on 'M.9' dwarfing rootstock were significantly decreased compared with vigorous rootstocks 'MM.106, M.793 and 'Royal Gala'. Similarly, application of NPA to the rootstock stem of the vigorous rootstocks decreased the total shoot growth of the scion. Both treatments increased the proportion of primary and secondary shoot (if present) that terminated

early. For scions on a dwarfing rootstock or with stem-applied NPA treatment, the BAP re-instated the sylleptic shoot formation and, GA_{4+7} (GA_3+GA_{4+7} in Chapter 2) delayed the termination of both primary and sylleptic shoots thereby increased their final length and node number. Thus an endogenous signalling mechanism was proposed whereby the decreased IAA to the root system, decreased root-produced gibberellins and cytokinins transported to the scion (van Hooijdonk et al., 2010). The decreased supply of cytokinin and gibberellins from root modified the architecture of scion by reducing the SAS formation and, the duration of shoot extension growth.

As the size of many fruit trees was controlled by using size-controlling rootstocks, the lack of these rootstocks may be a limiting factor for kiwifruit vines. Therefore, the first objective of this chapter was to imitate the dwarfing effect of 'M.9' rootstock by applying NPA to kiwifruit rooted stem cuttings to restrict auxin supply to the root system. The second objective was to elucidate the effect of reduced IAA to root system on kiwifruit vine architecture.

Furthermore, peach tree vigour has been related to the volume of the soil available to the root system (Cockroft and Wallbrink, 1966). The physical restriction of peach and apple tree roots decreased plant growth and the plants had more flowers and higher yield efficiency compared with plants with non-restricted roots (Richards, 1977). Therefore, the third objective of this Chapter was to elucidate the effect of other vigour reducing practices like root restriction and possible involvement of root-produced hormones (cytokinins and gibberellins) on vine architecture.

4.1.1 Objectives

The over-all aim was to understand the shoot-root-shoot hormonal signalling on kiwifruit vine architecture. Specific objectives were to:

1) Elucidate the endogenous hormonal signalling from shoot i.e. decreased IAA supply to roots by applying NPA to the stem and its influence on the root-produced cytokinins and gibberellins to the shoot on kiwifruit vine architecture.

2) Elucidate the effect of other vigour reducing practices like root restriction and possible involvement of root-produced hormones (cytokinins and gibberellins) on kiwifruit vine architecture.

3) Elucidate similarities and/or differences in vigour reduction imposed on apple scion by dwarfing rootstock and, mimicking its effect by NPA application onto kiwifruit stem.

In order to achieve these objectives, an experiment (experiment one) was designed and conducted during 2007-2008. A further experiment (experiment two) was conducted during 2009-2010 to confirm the reproducibility of results in 2009-2010.

4.2 Materials and methods

4.2.1 Establishment of experimental plant material

4.2.1.1 *Propagation of self-rooted kiwifruit stem cuttings - background*

For kiwifruit stem cuttings, particular techniques such as bench heating or misting, temperature control are always required to get satisfactory rooting as they are characterized by very low intrinsic rooting ability (Biasi et al., 1990). Characteristics of plant material and the propagation conditions are important with kiwifruit similar to other plant species (Sim and Lawes, 1981). Indole-3-butyric acid (IBA) has been found to be critical for rooting of soft, semi hardwood and hardwood cuttings (Sim and Lawes, 1981; Biasi et al., 1990; Ercisli et al., 2003). The type of cutting and the time of collection also influenced rooting of kiwifruit cuttings (Ucler et al., 2004). Successful and consistent rooting of kiwifruit stem cuttings depended on the physiological status of the mother tree and the rooting environment as basal bench heating at 22-24ºC and lower air temperature 15-18ºC were essential for a high percentage of rooting (Stanica et al., 2002). The rooting percentage also depended on the rooting substrates as the highest rooting percentage was obtained when perlite on wood compost and perlite on wood flour were used (Stanica et al., 2002). A dipping time from 3-5 sec could be adequate when the cuttings were treated with plant hormones (Hartmann et al., 1975; Loach, 1987). Sim and Lawes, (1981) reported 74% stem cuttings of ‘Abbott’ kiwifruit

were rooted during January using 0.10% (1000 mg L^{-1}) IBA in 50% ethanol and 96% rooted using 5000 mg L^{-1} in the same month. As the formation of root system on stem cuttings of the kiwifruit can be markedly affected by a number of interacting factors (Sim and Lawes, 1981), a trial was conducted on 'Hayward' and 'Hort16A' using IBA 500 mg L^{-1} at Plant Growth Unit, Massey University, Palmerston North, New Zealand.

4.2.1.2 *Soft and semi hardwood cuttings*

Annual shoots of *Actinidia chinensis* 'Hort16A' (gold kiwifruit) and *Actinidia deliciosa* 'Hayward' (green kiwifruit) were collected during summer (third week of March, 2007) for soft and semi-hard wood cuttings. The shoots were cut into 3-node segments and the bases dipped in solution of 500 mg L^{-1} IBA in 25% ethanol for five seconds. An oblique cut was made and the bark was scraped below the bottom node before dipping the shoot base into the IBA solution. Out of the three nodes of the stem cuttings, one was positioned inside the propagation medium leaving two nodes remaining above the medium. The propagation medium used was Daltons™ base growing medium (C.A.N fines A grade 50%, Bark Fiber 30%, Pumice 7 mm 20%, Serpentine Super 1 kg/M^3; C.A.N stands for calcium ammonium nitrate a common additive to raw bark as a nitrogen source). Bench heating was used to maintain the medium at 20-22ºC, whilst the air temperature of the glasshouse was 15-18ºC. Each planting tray contained 60 individual rooting containers in which 60 stem cuttings were placed one in each rooting containers of the tray. Then they were placed under an overhead misting unit set (Figure 4.1) to mist the cuttings at regular intervals for 10 seconds at frequency of 10 minutes. The tops of the stem cuttings were sealed with pruning paste (BacSeal®Super, Bayer, New Zealand). After two weeks callus was formed and satisfactory roots were formed by 2nd week of June i.e., four weeks from callus formation (Figure 4.2). Eighty five percent of 'Hort16A' and 62% of 'Hayward' soft and semi hard wood cuttings rooted (Figure 4.2). The stem cuttings with healthy roots were transferred to space saver plastic standard sized pots with potting medium. The potting medium contained slow release long term fertilisers with trace elements, 150 g Dolomite [Osmocote 200 g, (8-9 months) and, Osmocote 100 g, (3-4 months)] per 100 L of media. During August, 2007

these cuttings were transferred to the cold room with 7ºC air temperature and remained there till October, 2007 to receive the required chilling units for proper bud burst.

Though the percentage of softwood and semi hardwood cuttings that rooted was high for both 'Hort16A' and 'Hayward' cultivars, unfortunately there was no uniform spring bud break after the dormancy period. The reason for the failure of bud break after root formation may be due to lack of carbohydrate reserves for subsequent bud break after root formation. The number of chilling hours received was more than 800CU, which should be sufficient to break dormancy (Lawes, 1984). One hour below 45°F (7°C) is equal to one chilling hour. Number of chilling hours was calculated by multiplying number of days with number of hours below 7^{o}C.

Figure 4.1. Misting unit used for soft and semi hardwood cuttings of kiwifruit 'Hort16A' and 'Hayward' for rooting with misting arranged at regular intervals for 10 seconds at frequency of 10 minutes.

Figure 4.2. Formation of roots for IBA treated (500 mg ml^{-1}) soft and semi-hard wood cuttings of kiwifruit 'Hort16A' and 'Hayward'. Root balls formed after 6-8 weeks of misting (June, 2007).

4.2.1.3 *Hardwood cuttings*

Annual shoots of *Actinidia chinensis* 'Hort16A' (gold kiwifruit) and *Actinidia deliciosa* 'Hayward' (green kiwifruit) were collected while dormant during winter i.e., 1st week of August, 2007. The stem cuttings taken were similar to that described in section 4.2.1.1. The concentrations of IBA used were 500 mg L^{-1} and 1000 mg L^{-1} in 25% ethanol. Dormant hardwood cuttings of kiwifruit 'Hayward' and 'Hort16A' were dipped in IBA solution for 5 seconds, planted in trays and directly kept in a cold room maintained in darkness at 7ºC air temperature and bench heating was used to maintain the medium at 20-22ºC (Figure 4.3).

Figure 4.3. Hardwood cuttings of kiwifruit 'Hort16A' and 'Hayward' arranged for rooting in the cold room maintained at an air temperature of 7ºC and bench heated to maintain the medium at 20-22ºC during winter (August, 2007) .

The percentage of hardwood stem cuttings rooted for the 'Hort16A' was 70% in 500 mg L^{-1} and 60% in 1000 mg L^{-1}. A few, long healthy roots were formed (Figure 4.4) and were not formed in clusters as for soft and semi hardwood stem cuttings (Figure 4.2).

After root formation, second week of October, 2007 (Figure 4.4) and spring bud break (25.10.07), cuttings were transplanted into space saver pots (Figure 4.5). Following transplanting, the potted plants were moved to the glass house. Each plant was

debudded to a single shoot. The kiwifruit 'Hayward' did not form roots on hardwood stem cuttings.

Figure 4.4. Formation of roots for IBA (500 mg ml^{-1} and 1000 mg ml^{-1}) treated hardwood cuttings of kiwifruit cultivars 'Hort16A' and 'Hayward'. Long and healthy roots were formed by October, 2007.

Therefore, only hard wood stem cutting of the 'Hort16A' were available as plant material for the experiment one during 2007-2008 experiment. Out of these rooted stem cuttings, the required number of plants with uniform growth were selected for application of treatments and were transferred to a tunnel house for the period of experiment.

Figure 4.5. Rooted hardwood stem cuttings of *Actinidia chinensis* 'Hort16A' (gold kiwifruit) transferred into space-saver pots after spring bud break.

4.2.1.4 *Experimental material for experiment two*

Two year old tissue cultured seedlings of kiwifruit 'Hort16A' grafted on rooted stem cuttings of gold kiwifruit wood were purchased during early summer of the year 2009 from Heather Dale nursery, Katikati, Western Bay of Plenty, New Zealand.

4.2.2 Synthesis of the auxin transport inhibitor 1-N-naphthylphthalamic acid (NPA)

NPA was synthesised in the laboratory at Massey University. 1-Naphthylamine (5 g) was dissolved in 500 mL of toluene, and phthalic anhydride (5 g) was dissolved in a further 500 mL of hot toluene (Currie, 1997). The two toluene solutions were mixed under a fume-hood and stored at 25°C for 24 hours to enable precipitation. Precipitated NPA was filtered through Whatmans No.1 filter paper and the precipitation was washed five times in clean toluene to remove any unreacted reagents. The synthesised NPA was tested by lettuce root bioassay. NPA appeared to be effective in inhibiting auxin

transport because it caused roots to lose their gravitropic growth and caused a significant reduction in the mean length of the radical from 10^{-6} to 10^{-4} M NPA (Figure 4.6).

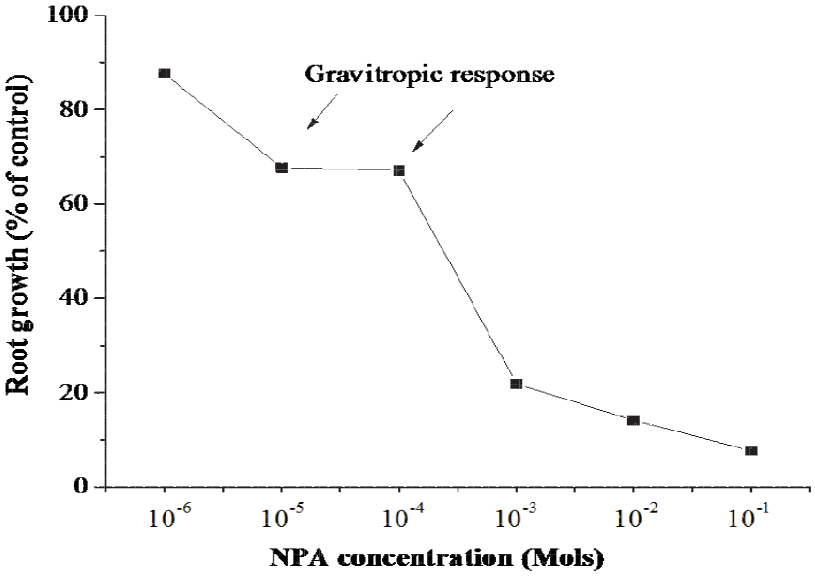

Figure 4.6. Effect of different concentrations of 1-N-naphthyphthalamic acid (NPA) on the mean length of lettuce roots expressed as a percentage of mean root length for control seedlings. The two arrows indicate the concentration of NPA at which roots lost their geotropism.

4.2.2.1 *Determination of suitable physiological concentration of NPA to restrict auxin transport*

Self-rooted hard wood stem cuttings of *A. chinensis* 'Hort16A' as described in section 5.2.1.1.2 were taken to determine the physiological concentration of NPA to be applied to plants for the period of the experiment. A three centimeter long region of epidermis below the first node of the primary shoot around the stem was scraped with a scalpel and NPA dissolved in lanolin as ammonium salt at concentrations of 1, 10, 25 mg/ml^{-1} per plant was applied. The portion of the stem where NPA applied was immediately wrapped with tinfoil to keep the lanolin in place and to maintain the activity of NPA for as long as possible.

On the third day after the application of NPA, leaves showed epinasty that increased in severity with concentration. Epinasty disappeared slowly after 10 to 14 days. Axillary

bud activation was observed two weeks after NPA application below the portion where NPA was applied. Among the three concentrations the 10 mg/ml^{-1} NPA reduced the length of the primary shoot much more compared to other two concentrations 1 and 25 mg/ml^{-1} NPA (Figure 4.7). Therefore, 10 mg NPA concentration was used in this experiment.

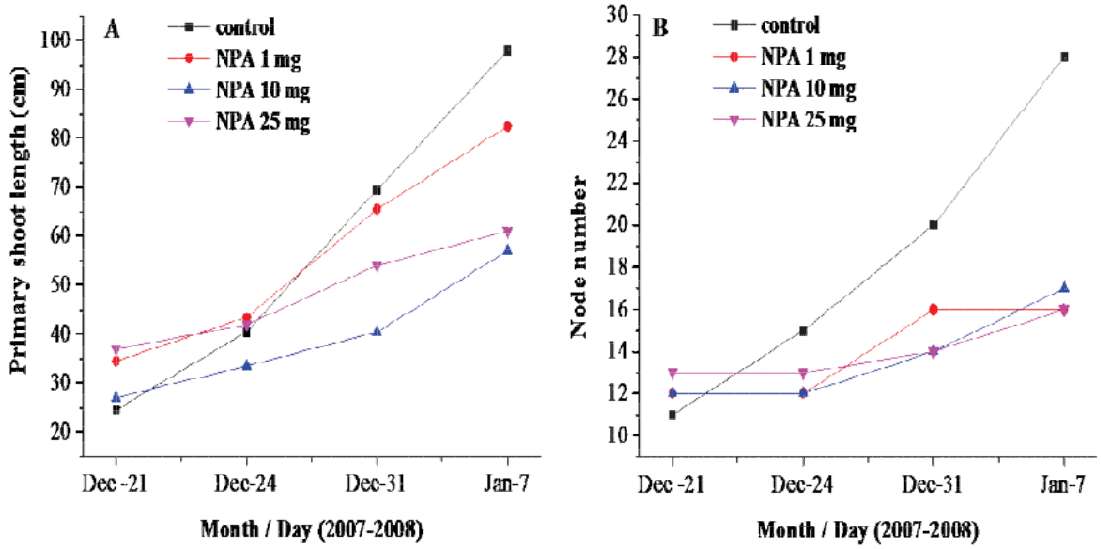

Figure 4.7. Effect of single application of NPA at 0 (control), 1, 10 and 25 mg/ml^{-1} per plant applied to the stem portion below the first node of the primary shoots of *A. chinensis* 'Hort16A' on primary shoot length (A) and node number (B). NPA in lanolin paste was applied on 21st December, 2007.

4.2.3 Treatments

4.2.3.1 *Treatments for experiment one*

This experiment had eight treatments each replicated eight times and arranged in a randomised complete block design (RCBD). The following were the treatments applied:

Untreated control ……	(Control)
N-1-naphthylphthalamic acid applied to the stem below the first node ……	(NPA)
NPA with Indole-3-acetic acid below……..	(NPA+IAA)
Root restriction ……	(RR)
Root restriction with NPA ……	(RR + NPA)
Stem girdling ……	(G)

Stem girdling with root restriction ……..... (G + RR)
Stem girdling with NPA …… (G + NPA)
(See following section 4.2.3.3 for details)

4.2.3.2 *Treatments for experiment two*

This experiment had four treatments each replicated eight times and arranged in a randomised complete block design (RCBD). The following were the treatments applied:

Untreated control ……. (Lanolin/Lanolin)
N-1-naphthylphthalamic acid …….. (NPA/Lanolin)
Indole-3-acetic (IAA)…… (Lanolin/IAA)
NPA with Indole-3-acetic acid below…. . (NPA/IAA)

4.2.3.3 *Application of treatments*

4.2.3.3.1 Control, NPA, NPA+IAA and IAA

For experiment one, a 3 cm long portion except for NPA+IAA treatment (two 3 cm long portions) and, for experiment two, two 3 cm long portions with a gap of one cm in between (Figure 4.8) were scraped around the stem below the first node of the primary shoot with a scalpel to remove the epidermis. For control, only lanolin paste was applied for both experiments to the scraped portions. For NPA treatment: 10 mg ml^{-1} NPA dissolved in lanolin as ammonium salt was applied in the 3 cm scraped portion for experiment one and, for experiment two only lanolin paste in the upper and NPA in the lower portions were applied. For NPA+IAA treatment: NPA in the upper and 5 mg ml^{-1} IAA in lanolin paste to the lower portion were applied for both experiments. For IAA treatment: 5 mg ml^{-1} IAA in the upper portion and only lanolin paste in the lower portion were applied only for experiment two. IAA application was repeated every week and NPA fortnightly over the growing seasons. The lanolin application for all the treatments was repeated every week. The portions of the stem where lanolin, NPA and IAA applied were immediately wrapped with tinfoil to keep the lanolin in place and to maintain the activity of NPA and IAA for as long as possible**.**

4.2.3.3.2 Girdling ± NPA and ± Root restriction

For the girdling treatment, a 3 cm strip of bark around the circumference (girdle) of the stem was removed with a scalpel and only lanolin was applied to girdled region. For girdling + NPA treatment, 10 mg ml^{-1} NPA in lanolin was applied to the girdle. For root restriction treatment, plants were grown in 1 L pots compared to 10 L for the non-restriction plants for the duration of experiment.

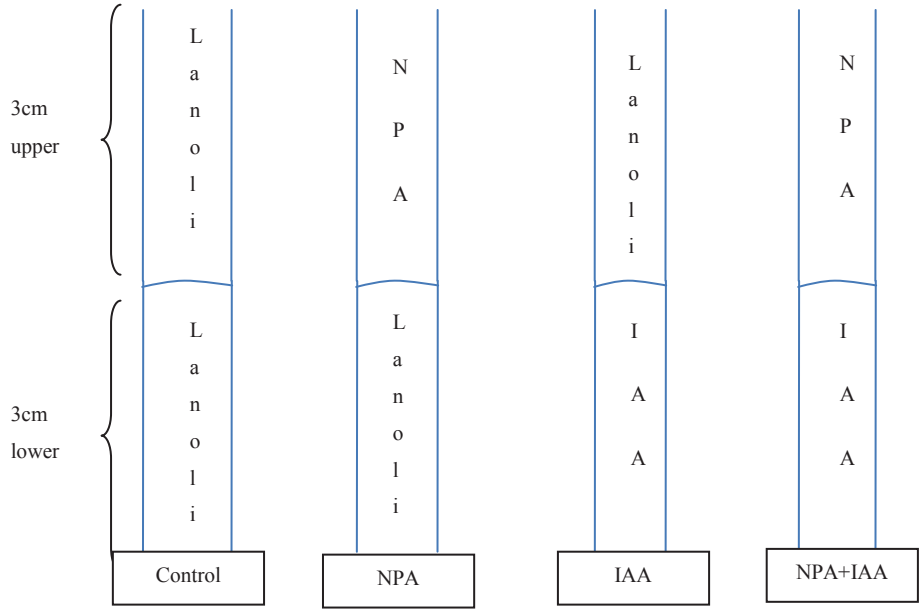

Figure 4.8. Diagrammatic representation of stem portion with two three cm long regions separated by one cm gap on the primary shoot below the first internode where only lanolin applied on both regions for untreated control vines;NPA and lanolin to the upper and lower regions respectively for NPA treatment; lanolin and IAA to the upper and lower regions respectively for IAA treatment; NPA and IAA to the upper and lower regions respectively for NPA+IAA treatment during experiment two from October to March (2009-2010).

4.2.3.4 *Growing medium and irrigation*

For both experiments the growing medium used was a long-term potting medium that contained 300 g per 100 L of 14-month slow release fertiliser (N: 15, P: 4, K: 17, Mg: 1.8) (Osmocote, Scotts, USA). An automatic irrigation controller (Hunter, Smart Valve Controller, USA) was arranged and irrigation was scheduled four times a day for 15

min. The root-restricted plants were overhead watered both morning and evening. Liquid fertiliser (Peters Professional, Scotts, USA: 20% N, 8.7% P, 16.5% K) was applied by hand for the root restriction treatment once every week from the second week of February, 2008 to ensure that root restriction was not resulting in nutrient deficiency.

4.2.3.5 *Measurements of shoot growth*

Shoot growth of all vines was measured throughout the growing season. The length and node number of the primary shoot was measured weekly from December, 2007 for experiment-one. Primary shoot length was measured from the first node at the base of the primary shoot to the youngest expanded leaf. The number, length and node number of sylleptic axillary shoot (SAS) were also measured. The position of SAS on the primary shoot i.e., the number of node on the primary shoot from which SAS was produced was also recorded. At the end of the first growing season (March, 2008), plants growing in 10 L pots were transferred into 40 L pots and those that were growing in 1 L into 10 L pots. After all plants terminated (March 10th, 2008) they were transferred to a standing out area. Root systems of four untreated control and for four NPA treated plants were washed carefully free of potting mix. The thin white roots, the feeder roots were classified as very soft and soft; the diameter of these roots was ≤ 1 mm. Brown coloured structural roots were classified as medium roots (2-3 mm) and hard roots (≥ 5 mm). Their fresh weights were taken and then oven dried at 80°C to a constant mass and their dry weights recorded on a four-decimal place balance (METTLER AE200, Switzerland) within 30 sec of removal from the oven. During summer of the third growing season (January, 2009), annual shoots of all treatments were pruned, wrapped in plastic bags and fresh weights were taken immediately.

For experiment two during 2009-2010 growing period length, node number of primary and number and length of SAS were taken in a way similar to experiment-one. For experiment two tertiary shoots were also measured. At the end of the growing season (May, 2009), individual leaf length and width (cm) of leaves on the primary shoot were measured using calipers and the leaf area was calculated by multiplying leaf length by leaf width.

4.3 Results

Two days after NPA application, leaves showed epinasty (Figure 4.9) similar to that observed for apple trees (van Hooijdonk et al., 2010). After approximately 14 days, axillary bud activation below the site of NPA application (Figure 4.10) was observed and these axillary buds were removed to prevent IAA signalling to the root system.

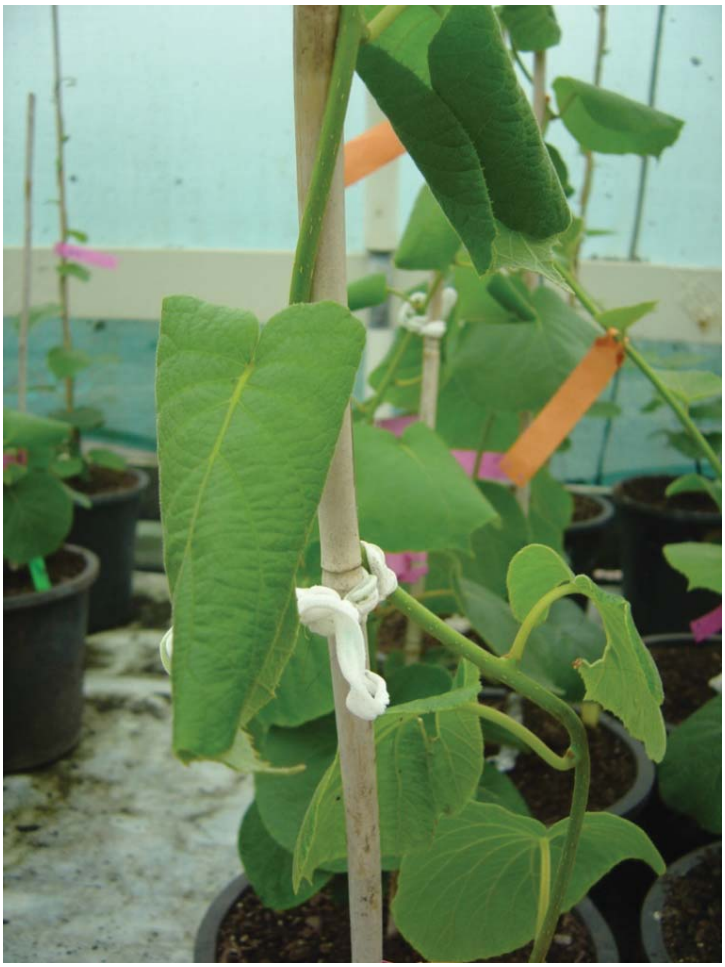

Figure 4.9. Kiwifruit vines of *A. chinensis* 'Hort16A' exhibiting epinasty three days after application of NPA to the potted vines in the tunnel house.

The area of stem where NPA was applied showed an increase in diameter. Unfortunately, all the plants with girdle, girdle + NPA and girdle + RR, died within a week after making girdles (data not shown).

Figure 4.10. The yellow circle shows the activation of axillary buds below NPA application for kiwifruit vines 'Hort16A' where lanolin applied on the upper and NPA on the lower portions covered with aluminum foil.

4.3.1 Experiment one

4.3.1.1 *Treatment effect on primary shoot length*

The primary shoot of NPA treated plants did not terminate any earlier than the controls (Figure 4.11) even after repeated fortnightly applications. However, NPA reduced the mean length of the primary shoot compared with untreated control vines by 21st January 2008 (i.e., after two weeks of NPA application) and continued until the end of the growing season (10th March, 2008). The final mean length of the primary shoot with NPA treatment was shorter at *P=0.03* compared with untreated control vines. Although there was an initial reduction in the growth of the primary shoot for the NPA+IAA treatment, there was a gradual increase in the cumulative mean length of the primary shoot toward the end of the growing season; shoots treated with NPA+IAA were significantly shorter (*P=0.001*) compared with control plants on 4th February, 2008 and by the end of the growing season (10th March, 2008) they were similar (*P=0.2*) with untreated control shoots. Hence, IAA below NPA made the primary shoots longer

towards the end of the growing season (Figure 5.11) Although, the length of primary shoots for NPA+IAA treatment was increased compared with NPA treatment, the difference approached significance only at *P=0.06*.

With root restriction i.e., plants grown in 1 L pots with or without NPA reduced final mean length of primary shoot significantly (P≥0.0001) compared with the untreated control plants. The reduced extension growth caused by the NPA+RR treatment was evident as the initial length of primary shoot (from 17/12/07 to 31/12/07) before application of treatments was higher than the other treatments (Figure 5.11), but then showed a marked decrease in growth with NPA application. The primary shoots of NPA+RR were shorter than RR vines at *P=0.07* (Figure 4.11). The effect of NPA+RR appeared to be additive since the reduction in the length of the primary shoot due to NPA or RR was 16% or 31%, respectively, when compared with the control, while the combined effect of NPA+RR was 45%.

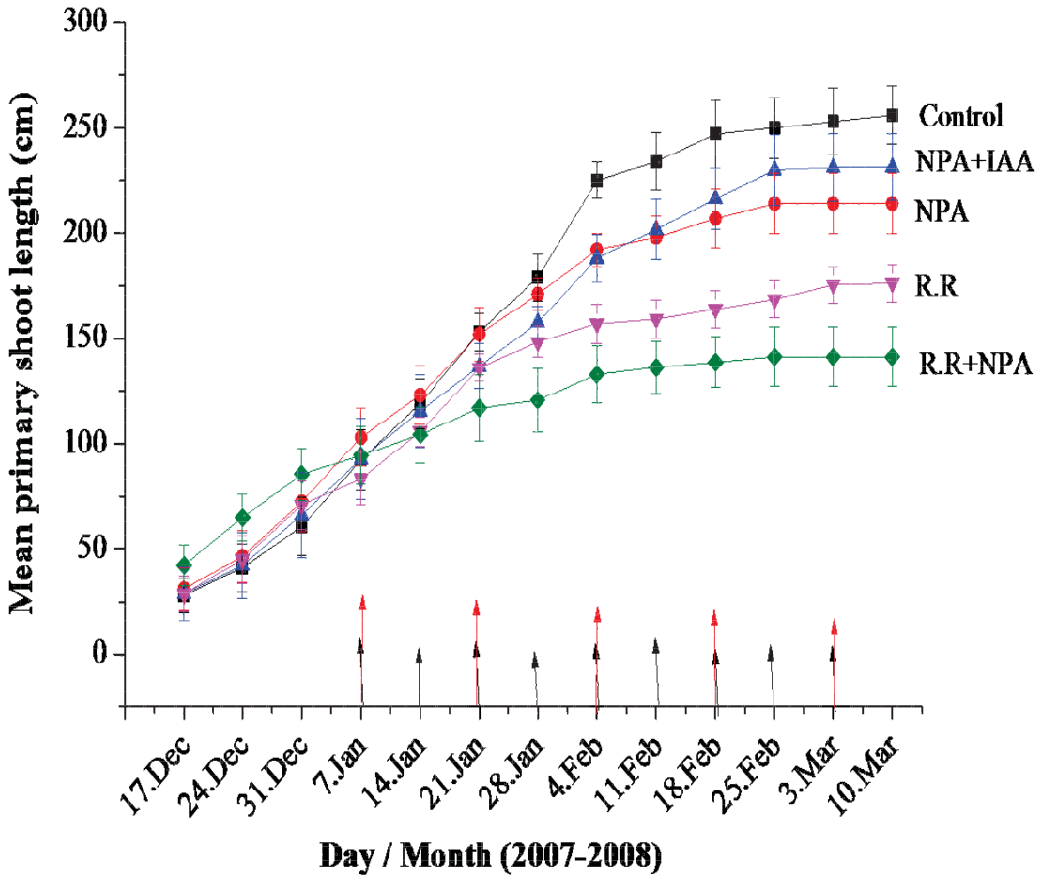

Figure 4.11. Mean primary shoot length (cm) of kiwifruit vines A.chinensis 'Hort16A' for untreated control vines, auxin inhibitor naphthylphthalamic acid (NPA) applied below first node of the stem, indole-3-acetic acid applied below NPA (NPA+IAA), root restriction (RR: plants grown in 1 L pots), root restriction with NPA (RR+NPA). The red arrows indicate the dates of NPA application and the black arrows indicate the dates of IAA application to the stem. Bars represent standard error.

4.3.1.2 *Treatment effect on the mean node number and internode length of the primary shoot*

The significantly shorter primary shoots of NPA treated vines compared to control appeared to be due to a combination of node number and internode length as neither were significantly different alone (Table 4.1). RR and RR+NPA treatments resulted in shorter primary shoots compared to controls due to reduced mean node number at *P=0.04* and *0.001* respectively and decreased mean internode length at *P=0.05* and *0.008* respectively (Table 5.1).

There was a strong positive correlation ($R^2 \geq 0.70$) between node number and length of primary shoots for all treatments except for NPA+IAA (R^2=0.43) (Figure 4.12). The length of primary shoot increased as node number increased. For NPA+IAA treatment with similar number node compared with NPA, the primary shoots were longer; hence the mean internode length was greater (Table 5.1).

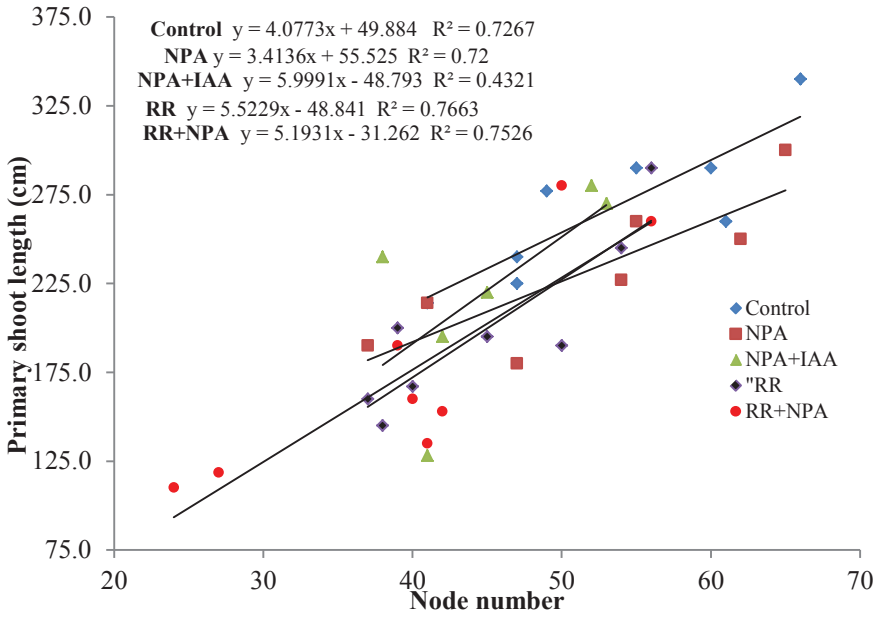

Figure 4.12. Treatment effect on the relationship between primary shoot length and node number for kiwifruit vines *A.chinensis* 'Hort16A' for untreated control vines, auxin inhibitor Naphthylphthalamic acid (NPA) applied below the first node on the primary shoot, indole-3-acetic acid applied below NPA (NPA+IAA) root restriction (RR: plants grown in 1 L pots), root restriction with NPA (RR+NPA).

Table 4.1. Treatment effect on the mean length, node number, internode length of the primary shoot for kiwifruit vines *A.chinensis* cultivar 'Hort16A' at the end of the growing season March, 2008. The values are mean ± standard error.

	Primary shoot		
Treatment	**Length (cm)**	**Node number**	**Mean internode length (cm)**
Control	256 ± 13.8 a	50 ± 2.7 a	5.13 ± 0.1 ab
NPA+IAA	231 ± 15.8 ab	45 ± 3.1 a	5.32 ± 0.3 a
NPA	214 ± 14.1 bc	47 ± 3.5 a	4.69 ± 0.2 abc
RR	176 ± 9.0 dc	41 ± 1.2 ab	4.47 ± 0.2 bc
RR+NPA	141 ± 14.2 d	35 ± 3.7 b	4.16 ± 0.2 c

Means sharing the same letters in each column are not significantly different at *P=0.05* using the Duncan's Multiple Range Test.

4.3.1.3 *Treatment effect on the formation of sylleptic axillary shoots (SAS)*

NPA treatment had no significant effect (*P=0.4*) on the mean number of SAS compared with untreated control vines, whereas NPA+IAA significantly (*P=0.01*) decreased their number. For vines treated with RR, there were no secondary shoots (Figure 4.13). With RR+NPA, the mean number of secondary shoots formed was significantly reduced compared to control and NPA at *P=0.03, 0.01* respectively. The activity of axillary buds in RR+NPA was much less compared to control and NPA treated vines (Figure 4.13).

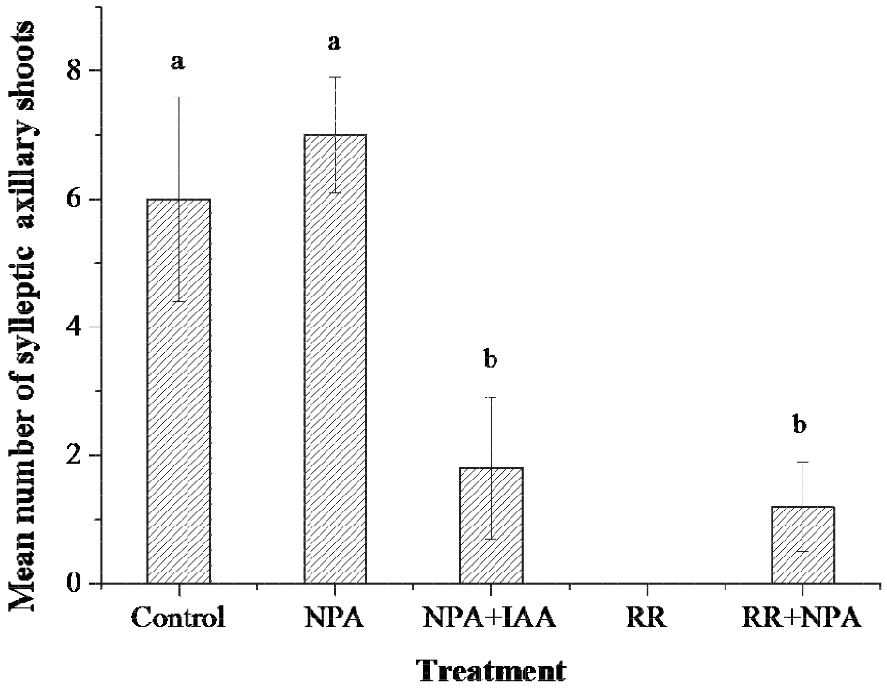

Figure 4.13. Mean number of SAS formed for kiwifruit vines *A.chinensis* 'Hort16A' by the end of the growing season (10th March, 2008) for untreated control vines, auxin inhibitor naphthylphthalamic acid (NPA) applied below first node of the stem, indole-3-acetic acid applied below NPA (NPA+IAA), root restriction (RR: plants grown in 1 L pots), root restriction with NPA (RR+NPA). Bars represent standard error. Columns sharing the same letter are not significantly different using Duncan's Multiple Range Test at *P=0.05*.

4.3.1.4 *Treatment effect on the mean total secondary shoot length*

The RR+NPA treatments significantly reduced the mean total SAS length (*P=0.01)* compared with control. As the number and length of SAS formed for NPA+IAA were less (Figure 4.13 and 4.14) the mean total length was less compared to NPA treatment (*P=0.02*). Untreated control vines were not different from NPA treated ones in their mean total length of SAS (Figure 4.14).

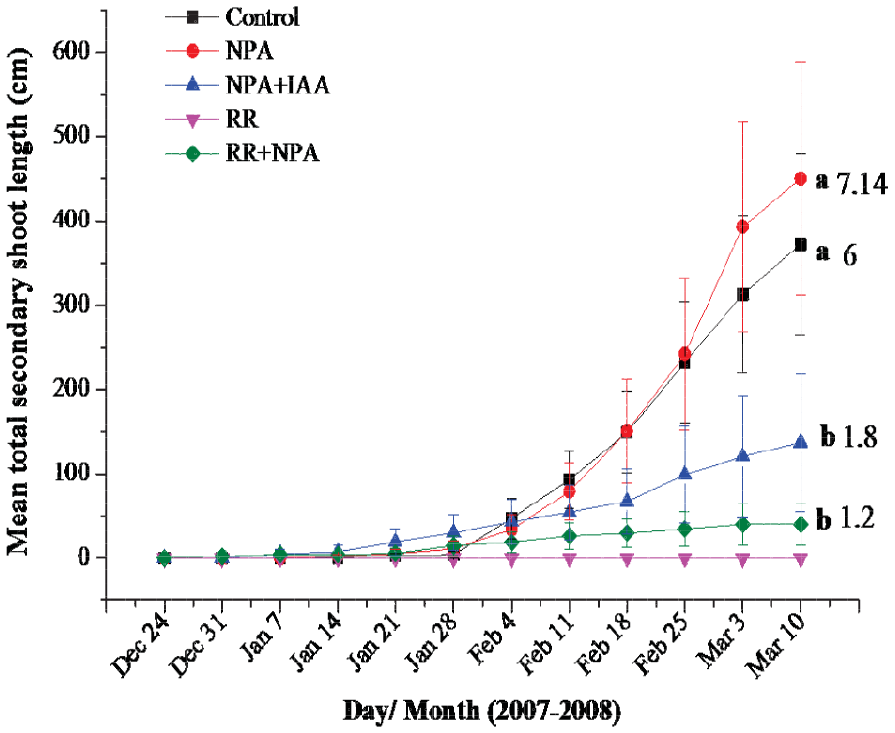

Figure 4.14. Treatment effect on the mean total SAS length formed on kiwifruit vines *A.chinensis* 'Hort16A' for untreated control vines, auxin inhibitor naphthylphthalamic acid (NPA) applied below first node of the stem, indole-3-acetic acid applied below NPA (NPA+IAA), root restriction (RR: plants grown in 1 L pots), root restriction with NPA (RR+NPA). Bars represent standard error. Means sharing the same letter are not significantly different using Duncan's Multiple Range Test at *P=0.05*. Mean number of SAS formed in response to each treatment are shown on the right.

4.3.1.5 *Treatment effect on the total shoot growth per vine*

The mean total shoot length (primary and sylleptic shoot together) per vine for the NPA and untreated control was similar (Figure 4.15). Compared to control vines, the mean total shoot length per vine was reduced (*P=0.06*) when IAA was applied below the site of NPA application. Due to less number of SAS for NPA + IAA, the reduction in the total shoot growth was significant at *P=0.04* compared with those with only NPA. The RR+NPA and RR treatments were not different to each other, but these treatments significantly decreased (*P=0.001*) total shoot length per scion compared with the control (Figure 4.15) by reducing mean primary shoot length (Figure 4.11), number (Figure 4.13) and length of sylleptic shoots (Figure 4.14).

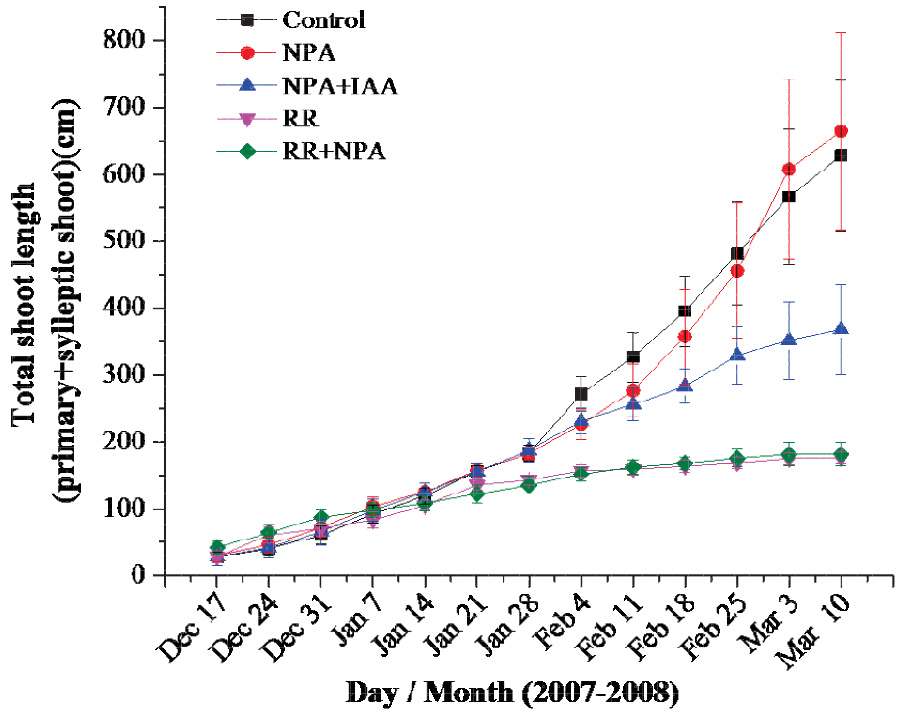

Figure 4.15. The effect of treatments on the total shoot length (primary + sylleptic shoots) of kiwifruit vines of *A. chinensis* 'Hort16A' for untreated control vines; the auxin transport inhibitor naphthylphthalamic acid (NPA) applied below first node of the stem; indole-3-acetic acid applied below NPA (NPA+IAA); root restriction (RR: plants grown in 1 L pots); root restriction with NPA (RR+NPA) treatment of the stem. Bars represent standard error.

4.3.1.6 *NPA effect on the dry weight of the root system*

NPA appeared to decrease the dry weight of all root categories slightly although the differences were not significant (Figure 4.16). Thus NPA had no effect on the mean dry weight of the root system.

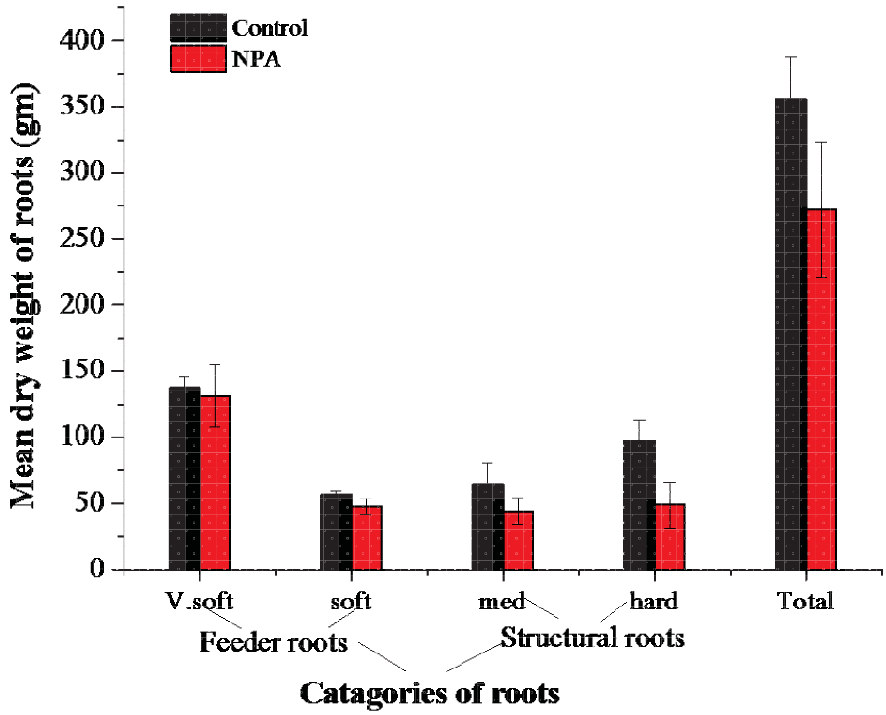

Figure 4.16. Mean dry weight of very soft, soft (feeder roots); medium (med) and hard roots (structural roots) and of the total root system for untreated control vines and NPA (auxin transport inhibitor naphthylphthalamic acid) applied below first node of the stem of *A.chinensis* 'Hort16A'. Bars represent standard error.

4.3.1.7 *Treatment effect on pruning weight*

During summer (January, 2009), when all the annual shoots of all treatments were pruned, the fresh weight of shoots of vines for RR and RR+NPA treatments were similar and significantly less compared with that of vines treated with NPA (*P=0.03* and *0.06* respectively). There was no significant difference between the vines treated with NPA and untreated control vines in their pruning fresh weight. However, the vines treated with NPA+IAA exhibited vigorous growth and their pruning fresh weight approached significance at *P=0.06* compared with NPA treated vines (4.17).

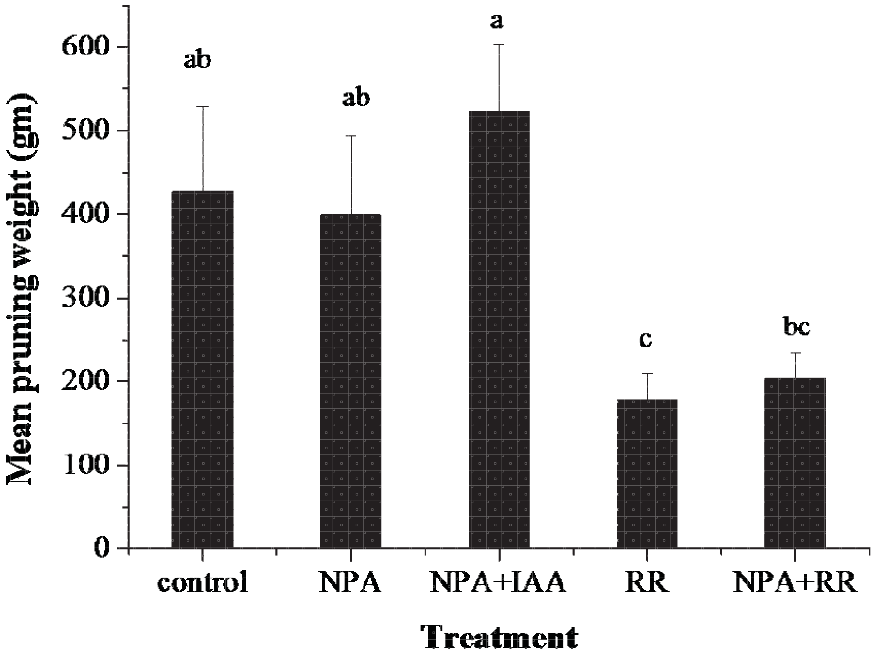

Figure 4.17. Summer pruning fresh weights of kiwifruit vines 'Hort16A' in response to vigour modifying treatments; the auxin transport inhibitor naphthylphthalamic acid applied below the first node of the stem (NPA),; indole-3-acetic acid applied to the stem below NPA (NPA+IAA); root restriction (RR: plants grown in 1 L pots); root restriction with NPA (RR+NPA) and untreated control. Bars represent standard error. Means sharing same letters are not significant at *P=0.05* using Duncan's Multiple Range Test.

4.3.2 Experiment two

4.3.2.1 *Treatment effect on primary shoot length*

The primary shoot of NPA treated plants did not terminate any earlier than the controls (Figure 4.18) even after repeated fortnightly applications. However, NPA significantly reduced the mean length of the primary shoot compared with untreated control vines at *P=0.01*. Vines treated with NPA+IAA and only IAA had primary shoots that were similar in length and not significantly different from untreated vines (*P=0.28*).

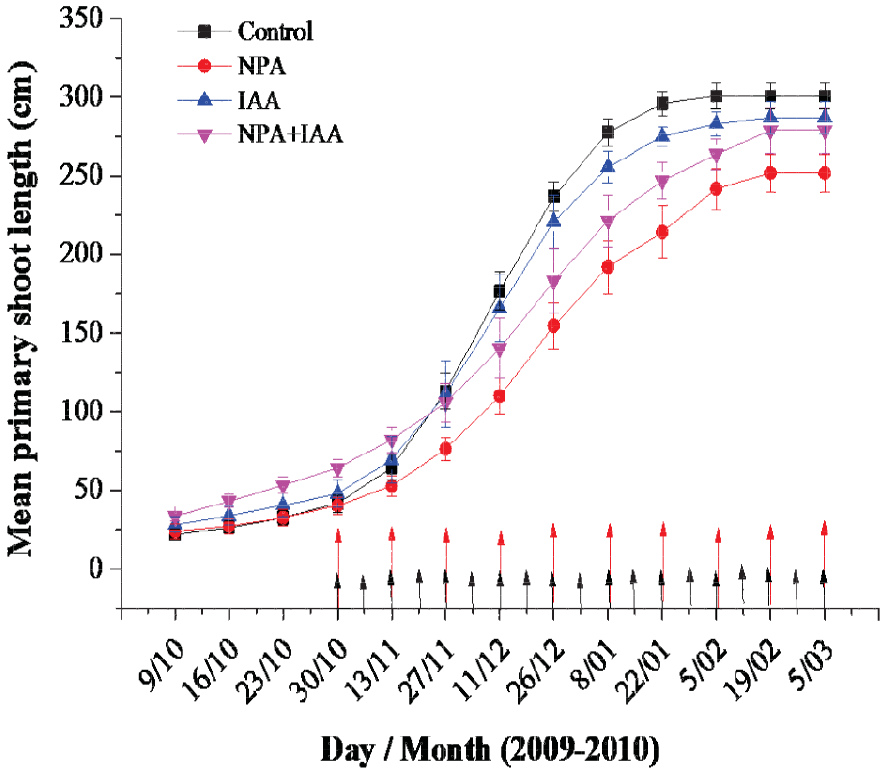

Figure 4.18. Mean primary shoot length (cm) of kiwifruit vines *A.chinensis* 'Hort16A' for untreated control vines; auxin inhibitor naphthylphthalamic acid (NPA); indole-3-acetic acid (IAA), indole-3-acetic acid applied below NPA (NPA+IAA) applied below the first node on the stem. The red arrows indicate the date of NPA and black arrows IAA application to the vine stem. Bars represent standard error.

4.3.2.2 *Treatment effect on the mean node number and internode length of the primary shoot*

There was no treatment effect on the mean node number on the primary shoot (Table 4.2). NPA treatment significantly (*P=0.01*) reduced mean primary shoot length and with similar node number compared with control the mean internode length was reduced significantly (*P=0.0003*) compared with control. There were no differences in primary shoot length, node number and internode length among untreated control, IAA and NPA+IAA treatments (Table 4.2).

There was a strong positive correlation between node number and length of the primary shoots of all treatments (Figure 4.19). As the node number increased the primary shoot length increased, but NPA treated vines with similar number of node had significantly

shorter primary shoots compared with control vines thus the mean internode length was less compared with untreated control vines. For untreated control and IAA treated vines, the primary shoots were longer with similar node number compared with NPA treated vines (Figure 4.19).

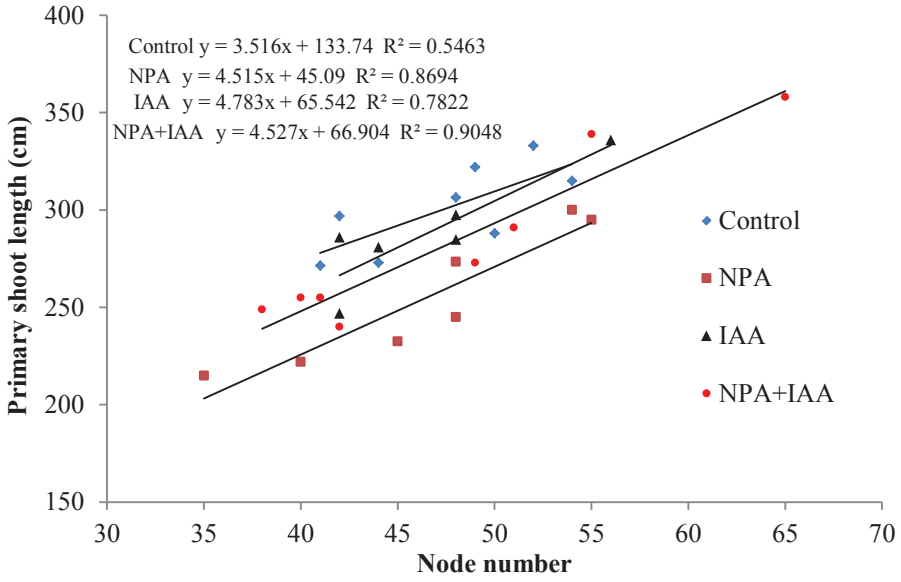

Figure 4.19. Treatment effect on the relationship between primary shoot length and node number for kiwifruit vines *A.chinensis* 'Hort16A' for untreated control vines, auxin inhibitor naphthylphthalamic acid (NPA) and indole-3-acetic acid (IAA) applied below the first node on the primary shoot, indole-3-acetic acid applied below NPA (NPA+IAA).

4.3.2.3 *Treatment effect on mean leaf area*

At the end of the growing season (May, 2009), individual leaf length and width (cm) of leaves on the primary shoot were measured using calipers. The mean leaf area for NPA treated vines was significantly less compared with untreated control and IAA treated vines (Figure 4.20). The vines with NPA+IAA treatment the leaf area was less than control and IAA but not significantly different. Therefore, for NPA leaf area was significantly less (*P=0.005*) compared with untreated control, IAA and NPA+IAA (Figure 4.21).

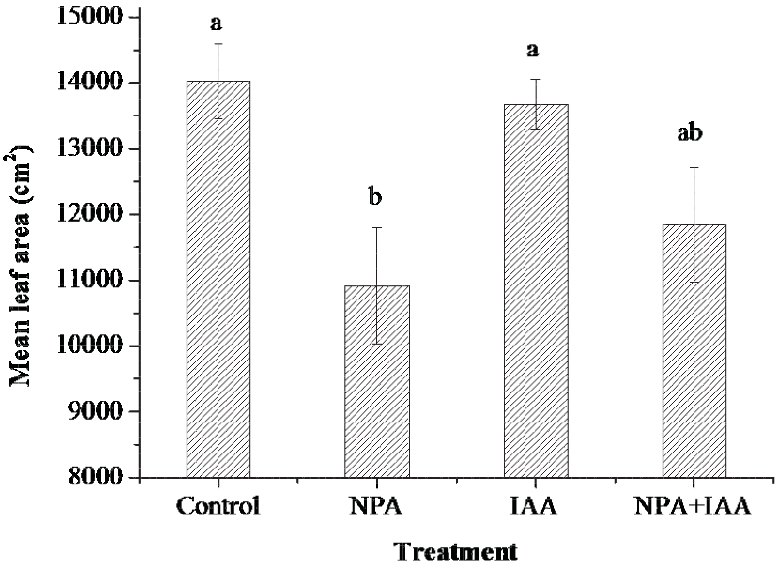

Figure 4.20 Treatment effect on mean leaf area (cm^2) of kiwifruit vines *A.chinensis* 'Hort16A'at the end of the growing season (April, 2010) for untreated control vines, auxin inhibitor naphthylphthalamic acid (NPA) and indole-3-acetic acid (IAA) applied below the first node on the primary shoot, indole-3-acetic acid applied below NPA (NPA+IAA) Columns sharing the same letters are not significantly different at *P=0.05* using the Duncan's Multiple Range Test. Bars represent the standard error.

Table 4.2. Treatment effect on length, node number, internode length of primary shoot; number and length of sylleptic (secondary and tertiary shoots) and total shoot length at the end of the growing season (April, 2010) of kiwifruit vines 'Hort16A' for untreated control vines, auxin inhibitor naphthylphthalamic acid (NPA), indole-3-acetic acid (IAA), indole-3-acetic acid applied below NPA (NPA+IAA) on the stem.

Treatments	Primary shoot **A**			Sylleptic secondary shoot **B**		Sylleptic tertiary shoots **C**		Total shoot length per vine **A+B+C (cm)**
	Length (cm)	Node number	Mean internode length (cm)	Mean total number	Mean total length (cm)	Mean number	Mean total length (cm)	
Control	300.8 a	47.6 a	6.4 a	6.2 a	538.3 a	0.13	13.0	852 a
NPA	254.7 b	46.0 a	5.5 b	7.0 a	620.7 a	1.80	53.4	957 a
IAA	288.8 ab	47.0 a	6.2 a	5.0 a	588.8 a	0.00	0.0	875 a
NPA+IAA	282.5 ab	48.0 a	6.0 a	7.1 a	555.7 a	0.25	4.1	839 a

Means sharing the same letter in each column are not significantly different at *P=0.05* using Duncan's Multiple Range Test.

4.3.2.4 *Treatment effect on growth attributes of sylleptic (secondary and tertiary) shoot*

There was no significant treatment effect on mean total number of SAS produced (Table 4.2). The mean number of SAS for NPA treatment and for control vines was similar. The IAA treatment showed the least number of SAS compared with control, NPA and NPA+IAA treatments (Table 4.2). For all these treatments, more sylleptic shoots were formed after bending of the primary shoot. The primary shoot after reaching certain height was bent on horizontal wires to avoid tangling of shoots with other shoots. The bending was done during the second week of January, 2010. Therefore, the number of secondary shoots formed after the 2nd week of January, 2010 was almost similar in all treatments and as a result of this, for the total number there was no significant treatment effect on the number of SAS formed (Table 4.2). When the number SAS formed before bending were counted the treatment effect was closer to significance (*P=0.06*). However, the IAA treatment reduced the number of SAS compared with NPA and NPA+IAA (*P=0.01*) (Table 4.3), in experiment-two, the NPA+IAA increased the SAS formation, where as it decreased in experiment one (Figure 4.3).

Table 4.3 Treatment effect in comparison with experiment one on number of SAS formed for untreated control, auxin inhibitor naphthylphthalamic acid (NPA) and indole-3-acetic acid (IAA) applied below the first node of the primary shoot, indole-3-acetic acid applied below NPA (NPA+IAA) on the stem. Number of SAS for the entire growing season 2007-2008 for experiment one and 2009-2010 for experiment two was recorded. For experiment two bending was done before 2nd week of January, 2010.

Treatment	Number of sylleptic shoots		
	Experiment-one	Experiment-two	
	Full growing season	Full growing season	Before bending *
Control	6.00 a	6.2 a	2.86 ab
NPA	7.14 a	7.0 a	3.86 a
IAA	-	5.0 a	0.83 b
NPA+IAA	1.18 b	7.1 a	3.88 a

Means sharing the same letter in each column are not significantly different at *P=0.05* using Duncan's Multiple Range Test. * indicates the date of bending, i.e., before 2nd week of January, 2010.

The mean total length of SAS was similar for all treatment. Regarding tertiary shoot, out of eight vines for untreated control one produced two tertiary shoots and for NPA two produced 11, as a result the number of tertiary shoots produced for NPA was more. There were no significant differences for mean total growth (primary plus secondary plus tertiary shoots) per vine (Table 4.2).

4.4 Discussion

The appearance of epinasty three days after NPA application was assumed to be due to auxin transport inhibition. This downward bending of leaves (Figure 4.9) may be due to ethylene production (Leather et al., 1972; Bradford and Dilley, 1978; Harbage and Stimart, 1996) as a result of auxin accumulation (Van Onckelen et al., 2003). Activation of axillary buds below the region where NPA (Figure 4.10) was applied also indicates that auxin transport was impaired, which removed apical dominance (Bangerth, 1992; Bangerth, 1994). The death of all the girdled plants may be due to the removal of functional phloem with the bark as a result of which availability of carbohydrates to the newly formed root system of the cuttings would be reduced and little storage root carbohydrate would be available during the spring. Death of girdled vines was also possibly due to the damage of functional xylem as death was so fast (within one week after girdling). Swelling at the site of NPA application was probably a response to auxin accumulation as IAA is involved in the control of cambial activity (Little and Savidge, 1987) and also ethylene produced due to accumulation of auxin (Love et al., 2009).

4.4.1 Treatment effect on the growth of the primary shoot

For apples, NPA reduced mean primary shoot length, node number and number of SAS on the primary shoot of the scion irrespective of the rootstock onto which it was grafted (van Hooijdonk et al., 2010). For kiwifruit during both experiments, NPA decreased only the length of the primary shoots (Figure 4.11) compared with the untreated control vines (Figure 4.9 and 4.18) but there was no effect on secondary shoot number or total mean shoot length (Figure 4.15 and Table 4.2). Therefore, for kiwifruit NPA did not decrease the growth in any attribute except for primary shoot length. The reason for

applying IAA below NPA in kiwifruit vines was to try and reverse the reduction caused by NPA. The decrease in the length of the primary shoot with NPA was partially reversed with IAA below NPA and the primary shoots were longer approaching significance at *P=0.06* (Figure 4.11 and Table 4.2) compared with NPA. Therefore, the reduced primary shoot length due to NPA application was partially reversed by the additional IAA application towards the end of the growing season. The IAA applied below NPA would have been transported basipetally where it may have increased root derived gibberellins to keep the shoot apical meristem (SAM) and sub apical meristem active for longer period compared with NPA treatment. This may be the reason for the longer primary shoots with elongated internodes (Table 4.1) for the NPA+IAA treatment (*P=0.06*) compared to the NPA-treated vines. Although mean node number and internode length were not significantly different for untreated control, NPA and NPA+IAA treated plants, the primary shoot for NPA treated plant was significantly shorter, which was contributed by both decreased node number and internode length (Table 4.1 and 4.2). However, the increased mean length of the primary shoot for NPA+IAA was due to increased mean internode length, which made it longer compared with NPA treated plants. For control and NPA for both experiments and, NPA+IAA and IAA for experiment two, there was an increase in the primary shoot length as the node number increased except for NPA+IAA of experiment one (Figure 4.12 and 4.19). For NPA+IAA treatments, for the same number of nodes they have longer primary shoots (Figure 4.12). There was no difference between controls, NPA+IAA and IAA treated vines in the length of the primary shoot (Figure 4.18) for experiment two. The reason for the similar vigour for all the treatments except for NPA treatment may be the natural vigour of the experimental plant material selected for experiment two i.e., tissue cultured ones as it was known that tissue cultured plants were more vigorous compared with rooted stem cuttings of kiwifruit (Loreti et al., 1991).

In case of RR+NPA, the primary shoots were significantly shorter (*P=0.0001*) because of shorter internodes (*P=0.008*) and fewer nodes (*P=0.001*) compared with control vines (Table 5.1). Thus, activity of gibberellins may have been reduced by the NPA treatment compared to control vines since gibberellins stimulate the apical meristem to produce more nodes, and their effect on sub-apical meristem increases internode length (Sachs et al., 1959; Ali and Fletcher, 1970; Potter and Fry, 1993). In Chapter 2 of this

thesis, the gibberellin foliar sprays for 'RG' scions on 'M.9' dwarfing rootstock increased number of node and internode length (see Chapter 2; Section 2.3.2.1) significantly compared with untreated 'RG' scions on the same rootstock. Therefore, the RR+NPA treatment, due to the deficiency in root-produced and gibberellins there was a significant decrease in node production and extension of node. For RR vines, the primary shoots were shorter and although there was 75% correlation between primary shoot length and node number, the mean internodes were significantly shorter (*P=0.05*) compared to control vines, which may be a gibberellin effect. As there was no significant difference between RR and RR+NPA, the decrease in the length of the primary shoot due to RR+NPA may be only due to root restriction.

4.4.2 Treatment effect on the growth of sylleptic axillary shoots

Any increase in SAS formation compared with control, when kiwifruit vines were treated with NPA (Figure 5.13 and Table 5.2) supports Bangerth's hypothesis that restriction of auxin supply to root system increases cytokinin production at least temporarily, which results in release of axillary buds, thus forming secondary shoots (Bangerth, 1992). This was also supported by findings of Currie that cytokinins, especially trans-zeatin riboside (ZR), were elevated in root xylem exudates when NPA was applied to the stem of rooted kiwifruit cuttings (Currie, 1997). The increase in cytokinin appears to be a short term transient increase as long term cytokinins from the roots may decrease when NAA was applied to the shoot (Currie, 1997). When NPA was applied to 'MM.106' and cytokinins measured 96 hrs after NPA treatment, there was a significant reduction in some cytokinin forms compared with control trees (van Hooijdonk, 2012). The concentration of ZR in root exudates of one year old rooted kiwifruit cuttings which had been decapitated increased in the first 24 hrs but decreased after 72 hrs. Similarly the decapitated cuttings with NPA applied to the base of the cuttings 48 hrs before decapitation, increased ZR levels immediately (0 hrs) but decreased cytokinin levels 48 hrs after decapitation.

Thus with decapitation there is short term increase and long term decrease in the levels of cytokinin. Nevertheless, Bangerth et al., (2000) reported decapitation of bean plants

increased cytokinin concentration in the xylem sap initially, which were then decreased by applying NAA to the cut stem. And also for kiwifruit Currie, (1997) reported that decapitation followed by NAA application to the cut surface decreased cytokinin levels compared with controls. Similarly, in this experiment IAA below NPA reduced SAS formation. The mean number of SAS formed with NPA treatment in experiment one of this chapter was 7.14 and with IAA below NPA it was only 1.8 (Figure 4.14). Thus there was a decrease in the mean number of SAS formed with IAA supply to the root system. The recent study conducted by Hayward et al., (2009) demonstrated that auxin regulation acts as a feedback mechanism for strigolactones synthesis. Strigolactones are novel hormones that inhibit branching (Ferguson and Beveridge, 2009; Beveridge and Kyozuka, 2010). Hayward et al., (2009) mentioned that increased auxin content with low strigolactones conditions, acts as a downstream communicator to increase strigolactones biosynthesis, thus suggesting that auxin regulated strigolactones biosynthesis may form a component of auxin mediated branching inhibition (Hayward et al., 2009). Therefore, the reduced secondary shoot formation in NPA+IAA treatment during experiment one may be attributable to auxin-mediated strigolactones biosynthesis inhibiting secondary shoot formation. Restricted auxin supply by 'M.9' rootstock restricted SAS formation in apple (Table 2.9); with kiwifruit restriction of auxin supply to the root system by NPA did not restrict, but applying IAA below NPA did. Thus, increased levels of IAA in the root system inhibited SAS formation, which may be by producing SLs.

Recently it was discovered that carotenoid cleavage dioxygenases genes (*CCD*), which are involved in the synthesis of strigolactones, play an integral role in the control of branching in *Arabidopsis*, pea, petunia and rice (Ledger et al., 2010). In tomato, the reduction in *CCD7* expression increased branching (Vogel et al., 2010) and tomato mutants, which are deficient in strigolactones (SLs) have reduced *CCD7* expression and increased branching (Koltai et al., 2010). Similarly, in rice and pea (Gomez-Roldan et al., 2008; Umehara et al., 2008), branching mutants lacking these genes reduced SLs concentration (more branching) and applications of synthetic SLs restored the wild-type branching phenotype to the mutant i.e., inhibition of branching. Thus CCD genes have been demonstrated to play an integral role in the control of branching development (Ledger et al., 2010).

The branching development controlled by the CCD pathway appeared to be conserved in woody perennials (Ledger et al., 2010). The CCD genes (*AcCCD7* and *AcCCD8)* were detected in root tissue of *A. chinensis* in high levels and were not detected in leaf, stem and vegetative buds. As *CCD7* and *CCD8* were not detected in vegetative bud and stem of A. chinensis, may be strigolactones levels are low so that so that kiwifruit plants have vigorously branching phenotype. However, Brewer (2009) and Hayward (2009) reported that high levels of auxin promoted SLs synthesis. It was also known, that the high auxin levels inhibited branching due to apical dominance. When auxin levels are high, SLs levels may be high and promote branching inhibition. Thus, reduced branching for NPA+IAA (experiment one) and IAA (experiment two) suggest the branching inhibition may be due to SLs synthesis mediated by high levels of IAA (Hayward et al., 2009).

The complete absence of SAS for RR may also indicate that insufficient cytokinins were produced to promote their formation. In the case of RR+NPA, although cytokinin production from the roots may have been reduced because of root restriction, restricting auxin transport using NPA might have stimulated cytokinins production, hence the release of axillary buds and the formation of sylleptic shoots. Since these secondary shoots did not terminate immediately, but grew into long shoots, it could be assumed that gibberellins production from the roots was enough to stimulate both the apical and sub-apical meristem to produce slightly longer shoots. However, there was 80% fewer sylleptic shoots formed with this treatment compared with control vines and their total length was also less compared to the control (Figure 4.13 and 4.14). Thus, the results obtained with the RR+NPA treatment may suggest that auxin restriction along with root restriction reduced cytokinins and gibberellins production from roots to shoot and, as a result, the number and length of the sylleptic shoots were reduced compared to control and NPA treated vines (Figure 4.13 and 4.14) to promote node formation to promote node formation to promote node formation.

Cytokinin has been widely reported to promote axillary bud activation and to antagonize auxin inhibition on branching (Cline, 1991). However, for further growth a supply of gibberellins is probably required (Prochazka and Jacobs, 1984; van Hooijdonk et al., 2010). Gibberellins may stimulate shoot growth via their effects on the primary apical meristem, which tends to affect node number and/or effects on the sub-apical

meristem, which affects mainly cell number and length of the internode (Ali and Fletcher, 1970).

The length and number of sylleptic shoots of the scion on a dwarfing rootstock and of the scion on a vigorous rootstock with NPA were reduced (Figure 1.3) (van Hooijdonk et al., 2010), whereas for kiwifruit there was no difference in the total shoot length of vines with NPA compared with untreated control vines. Thus, there is a striking difference between apple and kiwifruit; in the case of apples, the scion on a dwarfing rootstock ('M.9') due to reduced supply of auxin to the root system reduced total shoot length compared with the scion on a vigorous rootstock ('RG'), but, for kiwifruit vines when treated with NPA there is no difference in the total length of shoot per vine compared with untreated control vines.

The ability of NPA to decrease scion vigour significantly for the highly vigorous 'Royal Gala' rootstock (Section 1.3.4.1 and Figure 1.2) and, the general similarities in the architectural changes imposed on the scion by a dwarfing rootstock 'M.9' and NPA (compare Figures 1.1 and 1.2), makes the hypothesis stronger that decreased basipetal transport of IAA from shoot-to-root is an essential physiological process regulating scion vigour of composite apple trees on dwarfing rootstocks (Lockard and Schneider, 1981). This decreased IAA from scion-to-root (Soumelidou et al., 1994a), consequently reduced biosynthesis and supply of root produced cytokinins (Kamboj et al., 1999a) and gibberellins (van Hooijdonk et al., 2011) to the scion. On the other hand, for kiwifruit vines, NPA application although significantly reduced the length of the primary shoot, there was no difference in the outgrowth of SAS compared with untreated control vines. Thus the hormonal signalling mechanism for apple trees does not hold good for kiwifruit vine growth. Further study is required to quantify hormone levels in xylem sap of kiwifruit vines for untreated and NPA treated ones to differentiate hormonal signalling between trees and vines.

The number of SAS formed for control, IAA and NPA+IAA were similar to NPA treatment for experiment two (Table 4.2), which may be due to bending of the primary shoot at the top support wire. When the primary shoot was bent, basipetal polar auxin transport would be disturbed and as a result apical dominance removed, activating axillary bud to form SAS (Cline, 1991). In these treatments more SAS appeared on

primary shoot after bending i.e., after 2nd week of January, 2010. The primary shoot was bent on horizontal wires to avoid tangling with other shoots. Therefore, the number of SAS formed after bending the primary shoot was almost similar in all treatments and thus no significant treatment effect (Table 4.2). However, when the number of SAS were counted before bending i.e., 2nd week of January, 2010, the NPA and NPA+IAA significantly increased (*P=0.01*) them compared with IAA (Table 4.3). However, the results for experiment two were different from that of experiment one in that IAA did not reverse the effect of NPA (Table 4.3). The reason for this is unknown but may be related to the greater vigour of the tissue cultured plants used in experiment two.

Application of a girdle to a kiwifruit cutting and decapitation resulted in elevation of cytokinin levels in root xylem exudates. Similarly application of NPA to a kiwifruit cutting resulted in a change in cytokinin levels similar to girdling, however, there was a decreasing trend in cytokinin levels (Currie, 1997). Conversely, Currie in his study mentioned that in general, re-growth of new vegetative growth was well correlated to the levels of cytokinins in xylem sap as the percentage bud burst and the mean final length of re-growth increased. Therefore, girdling in kiwifruit increased vegetative growth, which is contrary to a number of species such as peach (Dann et al., 1984), and apple (Schechter et al., 1994) where there was a reduced vegetative growth as a result of girdling. Cytokinin levels were decreased in xylem exudates in apple trees as a result of trunk girdling associated with reduced vegetative growth (Skogerbo, 1992). Similarly in peach cytokinin and gibberellin levels decreased in xylem exudates, which was also associated with decreased growth rate of shoots on girdled branches (Cutting and Lyne, 1993). Although gibberellins were not measured in cane girdled kiwifruit, as the vegetative growth was not influenced, it was suggested that gibberellins might not have been affected by girdling (Currie, 1997). Therefore, girdling did not decrease vegetative growth in kiwifruit whereas in apple in fact it did. From this information and also from these experiments in this Chapter, the auxin restriction to roots by NPA did not reduce vegetative growth. However, there is discrepancy between apple and kiwifruit. From the literature it can be assumed that the increased IAA levels to the root system increased cytokinin and gibberellin levels and increased shoot growth for apple scions on vigorous rootstocks (van Hooijdonk et al., 2009)) and for kiwifruit increased IAA levels

to the root system decreased cytokinin and gibberellin levels and reduced shoot growth for vines treated with NPA+IAA (experiment one) and IAA (experiment two).

4.4.3 Treatment effect on the growth of root system

There was no difference in the mean total dry weight of kiwifruit vines with naphthylphthalamic acid (Figure 4.16). The dry weight of roots of composite tree with 'RG' scion on 'M.9' rootstock was less compared to 'RG' scion on the 'RG' rootstock. Interestingly the root dry weight of the vigorous rootstock 'RG' decreased because of 'M.9' scion (Figure 2.24). Thus when basipetal IAA transport was decreased through the stem of 'M.9' rootstock it influenced the growth of roots thereby decreasing the growth of the roots and thus reducing the dry weights (van Hooijdonk et al., 2010). However, for kiwifruit the decreased IAA supply to root did not reduce root growth. If kiwifruit vines are compared to 'RG' scion on 'RG', both can be categorised as vigorous. It was also observed that there was a reduction in the total root dry mass when 'RG' scion on 'RG' rootstock was treated with NPA (van Hooijdonk et al., 2010). In a similar way, reductions in the root dry weights were observed for the 'RG' on the 'M.9' rootstock (Figure 2.16). Such reduction in root dry weight was not observed for kiwifruit. In addition, the available data for kiwifruit root do not allow making specific conclusions, which may be due to less replicates.

4.5 Summary

In order to stimulate the dwarfing rootstock effect of 'M.9' to decrease the basipetal transport of auxin, kiwifruit vines were treated with NPA in both experiments of this Chapter. In both experiments, the length of the primary shoot was reduced with NPA treatment, which was similar to apple trees. In addition, IAA applied below NPA reversed the effect of NPA. The effect of IAA below NPA in both experiments increased the length of the primary shoot, which approached significance at $P=0.06$ compared with NPA treated vines. However, for kiwifruit there was no difference in the number of SAS and total shoot length with NPA treatment and control. Unlike apple trees, with NPA, for kiwifruit vines, the number of SAS was similar to untreated control

vines. Thus kiwifruit does not behave anything like apple when it comes to NPA except for primary shoot length. This decrease in primary shoot length may also be due to loss of apical dominance as the secondary shoots were produced on the primary shoot.

For apple trees, decreased auxin levels to root system by applying NPA on the rootstock stem decreased root growth and root-produced cytokinin and gibberellin (van Hooijdonk, 2009). Consequently, the scion growth was reduced, which was similar to the effect of 'M.9' rootstock. Therefore, for apple trees, reduced levels of IAA to root system reduced shoot growth as well as root growth. On the other hand, for kiwifruit reduced levels of IAA to the root system by applying NPA to the stem, did not affect either root or shoot growth. As a result there was no difference in the vegetative growth of vines compared with untreated control vines. Therefore, for kiwifruit vines, NPA did not give any dwarfing effect of 'M.9' rootstock as it did for an apple scion grafted on a vigorous rootstock. However, increased IAA levels decreased growth (NPA+IAA-experiment one; IAA-experiment two). Further studies are required to measure the levels of IAA reaching root system and its influence on the levels of root-produced hormones such as cytokinin, gibberellins in relation to the architectural changes in kiwifruit vines.

Moreover, architecturally there are many differences between apple trees and kiwifruit vines. Canopy structure is very different in apple compared with kiwifruit. Kiwifruit has a long period of vegetative growth compared with apples, which has a clear separation of the peaks of vegetative and fruit growth (Palmer, 2007). Apple fruits are exposed to sunlight for longer periods during the day compared with kiwifruit. At the time of termination of shoot extension growth, all the growing points in apple tree form a terminal bud which goes into dormancy, but in kiwifruit vines the apical tissue aborts and stops growing at the end of the growth season. Thus, further growth in the next growing season does not depend upon auxin flow from terminal bud but depends on the activity of axillary buds. Therefore, under most circumstances, almost all axillary buds burst during spring. The main problem that kiwifruit growers are facing presently is the lack of vigour controlling rootstocks to control vegetative growth and to increase 'harvest index'. High allocation of carbohydrates to the root system and the long period of vegetative growth in kiwifruit limit the possibility of increasing the harvest index (Palmer, 2007). Suitable rootstocks to reduce the vigour may increase both the harvest

index and dry matter content. Hence, there is a need to understand the internal signals of growth controlled by endogenous hormones in order to understand the physiological mechanism of vegetative growth, which in turn may help in breeding suitable rootstocks for kiwifruit.

Therefore, as architectural modifications imposed by exogenous hormones could be the basis for understanding the influence of endogenous hormones, another experiment (Chapter 6) using exogenous auxins, gibberellins, cytokinins and growth retardants was designed to elucidate the architectural differences and to understand the control that endogenous hormones have on the vigorous growth of kiwifruit vines. This understanding can then be extended to quantifying endogenous hormones that may regulate vigour and architectural responses of kiwifruit vines.

Chapter 5 Effect of foliar sprays of auxin, gibberellins, cytokinins, auxin transport inhibitor (NPA) and anti-gibberellin (PBZ) on the vegetative growth of 'Hort16A' and 'Hayward'

5.1 Introduction

In Chapter 4, for kiwifruit, restriction of IAA to the root system had no effect on its architecture. Although, NPA presumably restricted auxin supply to root system (see Chapter 4 Section 4.3; Figure 4.10), there was no effect on sylleptic axillary shoot (SAS) formation (Figure 4.13) and presumably no effect on cytokinin. There was also no effect on the dry weight of the total root system. However, for apple trees, NPA applied to the stem of vigorous rootstocks significantly reduced primary shoot growth, SAS formation, caused greater proportion of primary and SAS to terminate growth early and also reduced total root system dry weight (van Hooijdonk, 2009). Analysis of endogenous hormones for these apple trees provided some additional support for the hypothesis that reduced supply of IAA to the roots system reduced root growth and the amount of root produced cytokinin, reducing SAS formation and gibberellin promoting termination of growth early. There is no such published information available on endogenous hormonal influence on kiwifruit vine growth and little is known about the physiology behind the vigorous growth of kiwifruit vines.

Reducing vegetative vigour for kiwifruit is very important to increase harvest index and fruit dry matter content and also to reduce pruning cost as there are no vigour controlling rootstocks for kiwifruit. Moreover, reducing vegetative vigour is very much desired for kiwifruit as it may reduce the incidence of PSA as it can live or survive as an epiphytic bacterium on leaves (Vanneste et al., 2011). There are a few studies involved in the use of chemical to reduce vegetative growth for kiwifruit vines such as application of 1000 mg L^{-1} NAA or an auxin transport inhibitor, CPPP (5-(2-carboxyphenyl)-3-phenylpyrazole) in lanolin paste to the cut ends of non-terminating shoots at two leaves distal to the last fruit at first pruning in late spring inhibited sylleptic axillary bud development (Henzell et al., 1986). Foliar sprays of 2000 mg L^{-1} 2-chlorethylphosphonic acid (Ethrel) on two-year-old male clone M3 and female

cultivar 'Lande' of *Actinidia kolomikta* were used as an alternative method to reduce summer pruning and to increase productivity. It shortened vegetative growth period and significantly reduced length of proleptic shoots and number of flowers and fruits per meter length of shoot (Cesoniene, 2008) in the year applied.

Furthermore, the importance of gibberellins in stimulating stem elongation of kiwifruit vines was indicated by using the gibberellin biosynthesis inhibitor, prohexadione-Ca (Mike Clearwater, personal communication). With Prohexadione-Ca (Ca-pro) at 250 mg L^{-1} they observed a significant reduction in mean shoot length compared with untreated control vines of kiwifruit 'Hort16A'. Except for these few reports, how exogenous plant growth regulators modify architecture of kiwifruit is largely unknown and no one has ever sprayed exogenous plant growth regulator to understand the role of endogenous hormones in vegetative growth. Thus, the main objective of this Chapter was to use exogenous growth regulators to elucidate the role of endogenous growth hormones involved in vigorous growth of kiwifruit vines.

The use of gibberellin biosynthesis inhibitor prohexadione-Ca reduced shoot growth in apple (Miller, 2002) by reducing internode length indicated the importance of gibberellins in stimulating stem elongation. Reduction in length of shoots can be achieved by blocking specific dioxygenases involved in the biosynthesis of gibberellins. The timing of the foliar sprays for apple was found to be critical for effective response (Quinlan and Richardson, 1983; Richardson and Quinlan, 1986). The optimum rate and use pattern of growth inhibitor vary across locations, and management practices (Evans et al., 1996). For example, foliar sprays of paclobutrazol (PBZ) at full bloom +21 days, when average length of shoot was 16.7 cm long (Richardson and Quinlan, 1986) were effective in controlling tree growth and inducing fruit production in young, previously non-bearing McIntosh apple trees on vigorous rootstocks (Estabrooks, 1993).

For kiwifruit, it is largely unknown how exogenous plant growth regulators modify architecture, therefore, an experiment was designed using foliar sprays of hormone active substances such as gibberellins, cytokinins, NAA in comparison with untreated control for kiwifruit 'Hort16A' and 'Hayward'. With the knowledge how paclobutrazol reduced vegetative growth of apples, a trial was conducted on kiwifruit 'Hayward'. In Chapter 4, NPA was applied in lanolin paste to 'Hort16A', therefore, in this experiment

NPA was given as a foliar spray to 'Hayward' to elucidate the effect of auxin transport on growth of 'Hayward' vines.

5.2 Materials and methods

5.2.1 Experimental plant material

The experiment was conducted during the 2009 (January to April) growing season at the Plant Growth Unit, Massey University, Palmerstone North. The plant material used was obtained from aerial layering of 'Hayward' and from rooted stem cuttings of 'Hort16A'.

5.2.1.1 *Aerial layering*

During January 2008, bases of one-year-old shoots were girdled by removing a 10 mm ring of bark and then treated with aqueous solution of 1000 mg L^{-1} IBA in 25% ethanol. All girdled portions were wrapped in moist sphagnum moss and covered with plastic film to hold the moss in place. In order to provide aeration, small holes were pierced through the plastic film, which was then covered with tinfoil to prevent evaporation due to solar radiation.

Prolific callus formation was observed in 'Hayward' (Figure 5.1) but only 1% of the treated shoots developed roots by May, 2008. Aerial layering of 'Hort16A' was unsuccessful and no callus formed.

Figure 5.1. Portion of *Actinidia deliciosa* 'Hayward' shoot after aerial layering (Jan, 2008) showing callus with a few roots produced by the end of the growing period (May, 2008).

Shoots of 'Hayward' with callus formation were cut from parent vines after winter i.e., after dormancy (October, 2008) and transplanted in 10 L pots with long term potting medium. They then slowly developed roots and were ready to be used as experimental material by Jan, 2009.

Because 'Hort16A' failed to form callus in response to aerial layering, the plant material for this experiment was derived from stem cutting material as previously explained in Chapter 4 (Section 4.2.1.1.1). Therefore, the plant material used in this experiment was produced either from aerial layering ('Hayward') or from stem cuttings ('Hort16A'). These plants were transplanted into 40 L polythene growing bags, cut back to a healthy basal bud during December, 2008 and three consecutive shoot length measurements were taken (3rd Jan to 17th Jan, 2009) before application of treatments on 17th Jan, 2009.

5.2.2 Growing medium and irrigation

In this experiment, the medium contained bark/pumice mix (70:30), 50 g super-phosphate, 50 g lime, 150 g dolomite and 300 g 14 months slow release fertiliser with trace elements (Osmocote, Scotts, USA) per 100 L. The irrigation system consisted of 19 mm polytube line placed under the plant row. For each bag (40 L), a 4 L hr^{-1} pressure compensator dripper was used. To each compensator, a 1.5 m length of flexible PVC line (3mm internal diameter) was connected and a stake drip emitter was attached to the end of the PVC line. A single stake drip emitter was placed into the medium near the centre of each polythene growing bag. Irrigation was scheduled daily for one hour in the morning and evening using an automated irrigation controller (Hunter, Smart Valve Controller, USA).

5.2.3 Treatments and concentrations

Actinidia chinensis 'Hort16A' and *Actinidia deliciosa* 'Hayward' primary shoots that developed from one healthy bud were used to conduct this experiment. Four treatments common to 'Hort16A' and 'Hayward' and two extra treatments to 'Hayward were applied. The four common treatments (foliar sprays) applied to 'Hort16A' and 'Hayward' were:

1. Control
2. Gibberellins (GA_3 + GA_{4+7})
3. Cytokinins (BAPSoL)
4. Auxins (NAA)

Two additional treatments (foliar sprays) to 'Hayward' were:

5. Anti-gibberellin (PBZ)
6. Auxin transport inhibition (NPA)

For gibberellins, 400 mg L^{-1} $GA_4+_{7,}$ (Novagib®, Fine Agrochemicals Ltd., Worcester, UK) and 400 mg L^{-1} GA_3 (Sigma, product number 48880 5G-F) together (800 mg L^{-1}); for cytokinin treatment, 400 mg L^{-1} BAP (BAPSoL™, Gro-Chem, New Zealand, Ltd) and for auxin treatment, 200 mg L^{-1} synthetic auxin naphthalene acetic acid (NAA)

were sprayed onto the entire primary shoot including young parts of the plant for both 'Hort16A' and 'Hayward'. Additionally for 'Hayward', an anti-gibberellin (4000 mg L^{-1} paclobutrazol (Payback ^{TM}, Nufarm limited) and an auxin transport inhibitor (400 mg L^{-1} naphthylphthalamic acid (NPA) were sprayed. Distilled water was used as foliar sprays for control plants. GA_3 and NPA were made water soluble by dissolving them in 5 ml absolute ethanol and made up to the required volumes with distilled water.

5.2.3.1 *Solution preparations:*

Aqueous solution of gibberellins GA_3+GA_4+$_7$, of 800 mg L^{-1} concentration was made by dissolving 0.1 g GA_3 in 5 ml ethanol and 10 ml GA_4+$_7$ was added and diluted up to 250 ml with distilled water. Similarly for other chemicals such as 400 mg L^{-1} BAP, 5 ml BAPSol (20 g/1000 ml); 200 mg L^{-1} NAA, 0.05 g NAA in 5 ml ethanol; 400 mg L^{-1} NPA, 0.1 g NPA in 5 ml ethanol; 4000 mg L^{-1} PBZ , 4 ml payback^{TM}, (250 g/1000 ml) were diluted to 250 ml with distilled water.

5.2.3.2 *Application of treatments*

Plants were sprayed to the point of runoff using 500 ml pressure sprayers. Adjacent plants were covered with plastic sheets during the application of chemicals to prevent contamination from spray drift. The treatments (GA_3 + GA_{4+7}, BAPSoL and NAA) began in the 3rd week of January, 2009 for both cultivars and, PBZ and NPA for 'Hayward' a week later. Gibberellins and BAP applications were repeated fortnightly and NAA, NPA, PBZ were applied only three times (Jan, 17th, Feb 17th and March 17th, 2009) during the growing season. There was no spraying of hormones on March 1st due to continuous bad weather with heavy rain and wind during late February and early March, 2009.

5.2.4 Measurement of vine growth

The length and node number of primary shoots were measured fortnightly from January to April, 2009. For kiwifruit, the shoot that developed from the bud on the plant has been called the primary shoot and the shoots that developed from the axillary buds on primary shoot have been termed sylleptic axillary shoots (SAS). The number, position, length and node number of sylleptic axillary shoots were also measured during the

growing period. During spring of the following growing season (Aug-Oct, 2009), bud break and the number of flowers were measured for each vine. The percentage of bud break was calculated, excluding the number of buds on primary shoot already broken during the previous growing season.

5.2.5 Experimental design and statistical analysis

In this experiment, there were two cultivars: 'Hort16A' and 'Hayward' and four treatments: GAs, BAP, NAA and untreated control replicated only four times because of lack of rooted material. Thus, there was a factorial arrangement (2 × 4) of treatments and the experimental design was a randomised complete block design (RCBD). Due to windy weather during 2009, two vines of gibberellin treatment for both cultivars were broken and, as a result there were only two replicates of GA treatment for analysis. Data were checked in SAS for homogeneity of variance and for a normal distribution and analysed using the GLM procedure of SAS. Firstly, data were analysed for main effects (cultivar and hormone treatment) and also for interaction between cultivar and hormone treatment. Means were separated using the LSD test at 5% level of significance and pre-planned comparisons of some treatment means were made using least square means (lsmeans tests). For 'Hayward' data were analysed for all six treatments using one-way ANOVA to compare the effect of PBZ and NPA with control. When data were not normally distributed, the raw data were subjected to an appropriate transformation before analysis of variation (ANOVA).

5.3 Results

Three days after spraying NAA to 'Hort16A' and NAA and NPA to 'Hayward', plants exhibited epinasty as was observed in the experiments of the previous Chapter (Section 4.4.1).

5.3.1 Treatment effects on the length of primary shoot

5.3.1.1 *Treatment effects (GA, BAP and NAA) on the length of primary shoot of 'Hort16A' and 'Hayward'*

There were no significant cultivar effects or cultivar × hormone treatment interactions on the length of the primary shoot. However, there was a significant (*P=0.0001*) effect

of hormone treatment. NAA significantly reduced the mean primary shoot length compared with the control (Table 5.1). There was no difference in the length of primary shoots treated with GA or BAP compared with the control. Hormone effects on primary shoots of both cultivars were pooled together and growth curves calculated (Figure 5.2 A&B). Two shoots for each cultivar with GA treatment were broken after four weeks (17^{th} Feb, 2009) of initial treatment (17^{th} Jan, 2009). There were no significant differences between GA and the control in primary shoot length after removing broken shoots (Figure 5.2A), nor with all four replicates (Figure 5.2B).

Table 5.1. Effect of exogenous auxin (NAA), benzylaminopurine (BAP) gibberellins (GA_3+GA_{4+7}) and untreated control for 'Hort16A' and 'Hayward' on the mean primary shoot length, node number and internode length (cm) at the end of the first growing season, April, 2009 (n=2 for gibberellin treatment (i.e., broken shoots removed) and n=4 for all other treatments for each cultivar). For cultivars, means are averaged over hormone treatment and for hormone treatment means are averaged over cultivars. There were no significant interactions. Data for SAS number and length were log transformed to meet the assumptions of ANOVA.

Main effects	Primary shoot			Sylleptic axillary shoot			
	Mean length (cm) (**A**)	Mean node number	Mean internode length (cm)	Mean number	Mean total length (cm) (**B**)	Average length (cm)	Total shoot length (cm) (A+B)
Cultivar							
'Hort16A'	238.64 a	38.7 a	6.21 b	2.31 a	52.0 a	8.3 a	287.76 a
'Hayward'	246.64 a	32.7 a	7.34 a	0.6 a	15.8 a	4.8 a	248.00 a
LSD	69.18	8.76	0.96	1.97	58.4	10.6	89.4
P value	*0.7*	*0.1*	*0.007*	*0.1*	*0.2*	*0.6*	*0.3*
Hormone effect							
Control	274.76 a	39.6 a	6.91 b	0.6 a	13.13 b	2.0 b	282.88 a
GA	342.76 a	36.0 a	10.1 a	3.13 a *P=0.06*	104.6 a	19.3 a	362.63 a
BAP	269.76 a	39.4 a	6.6 b	1.36 a	9.8 b	2.8 b	272.88 ab
NAA	143.36 b	28.6 a *P=0.06*	6.18 c	1.0 a	8.13 b	1.9 b	163.13 b
P value	*0.001*	*0.2*	*0.0001*	*0.2*	*0.05*	*0.05*	*0.01*
LSD	87.6	12.9	1.41	2.78	82.6	16.04	126.48
Interactions	ns (*P=0.62*)	ns (*P=0.48*)	ns (*P=0.18*)	ns (*P=0.9*)	ns (*P=0.7*)	ns (*P=0.9*)	ns (*P=0.6*)

Means within a column for a single main effect sharing the same letter are not significantly different using LSD at *P=0.05*

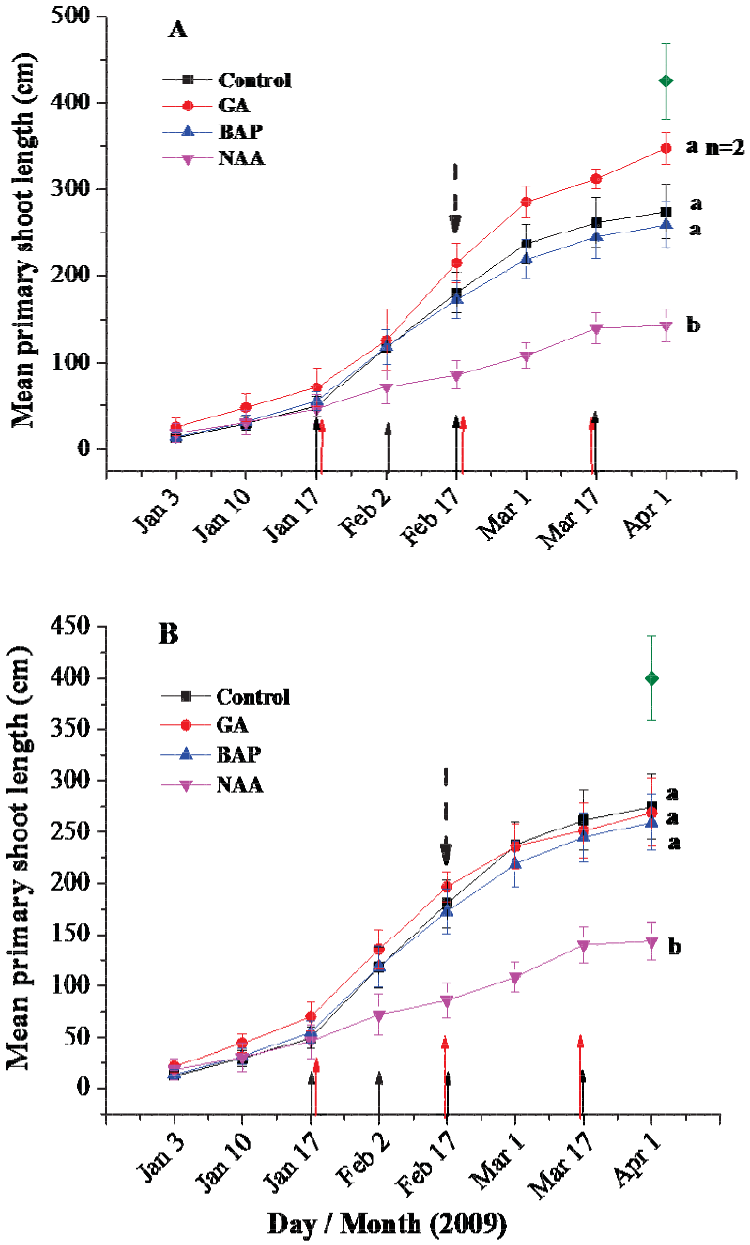

Figure 5.2. Effect of foliar sprays of auxin (NAA), benzylaminopurine (BAP) gibberellins (GA_3+GA_{4+7}), and untreated control on mean primary shoot length of both cultivars pooled together. Figure A with n=4 for GA (i.e., broken shoots removed) and n=8 for all other treatments; Figure B with n=8 for all treatments (i.e., broken shoots included). The black arrows represent the dates of gibberellin and BAP spray and red the NAA sprays. There was no spraying during early March due to bad weather. Means sharing the same letter shown on the right are not significantly different using LSD at *P=0.05*. Vertical bar on the top of the last measurement date represent LSD at *P=0.05*. Black dotted inverted arrow indicates the time when sylleptic axillary shoots were first observed in the gibberellin treated plants.

5.3.1.2 *Treatment effects (GA, BAP and NAA, PBZ and NPA) on the length of the primary shoot of 'Hayward'*

When wind damaged primary shoots were removed from analysis, the increased mean length of primary shoot with GA approached significance at *P=0.09* compared with untreated control (Figure 5.3). There was no significant difference between the lengths of primary shoots treated with NAA, NPA and PBZ. Similarly, the reduced mean primary shoot length with NAA also approached significance at *P=0.09*. With NPA treatment mean length of primary shoot was reduced (*P=0.04*) compared with control (Table 5.2).

5.3.1.3 *Treatment effects on the termination of the primary shoot*

NAA foliar sprays promoted termination of 50% of treated shoots by 17th Feb, 2009 i.e., four weeks after the first spray (17th Jan, 2009) and 100% shoots had terminated by the time of last measurement (April 1st, 2009) for both 'Hort16A' and 'Hayward'. For 'Hort16A', 25% of untreated control primary shoots had terminated by March 1st, 2009 and the remaining shoots grew until 1st April, 2009, whereas for 'Hayward', by April 1st, 75% shoots had terminated. There were no traces of termination for shoots treated with GAs, while 50% BAP treated shoots had terminated on the last day of measurement (1st April, 2009). However, in the gibberellin treatment primary shoot growth rate did slow down from 1st March, possibly due to SAS formation (Figure 5.2A), but full termination had not occurred by the last measurement date (1st April, 2009). For 'Hayward', 100% shoots treated with foliar sprays of PBZ and NPA had terminated by 17th March, 2009 (Figure 5.3).

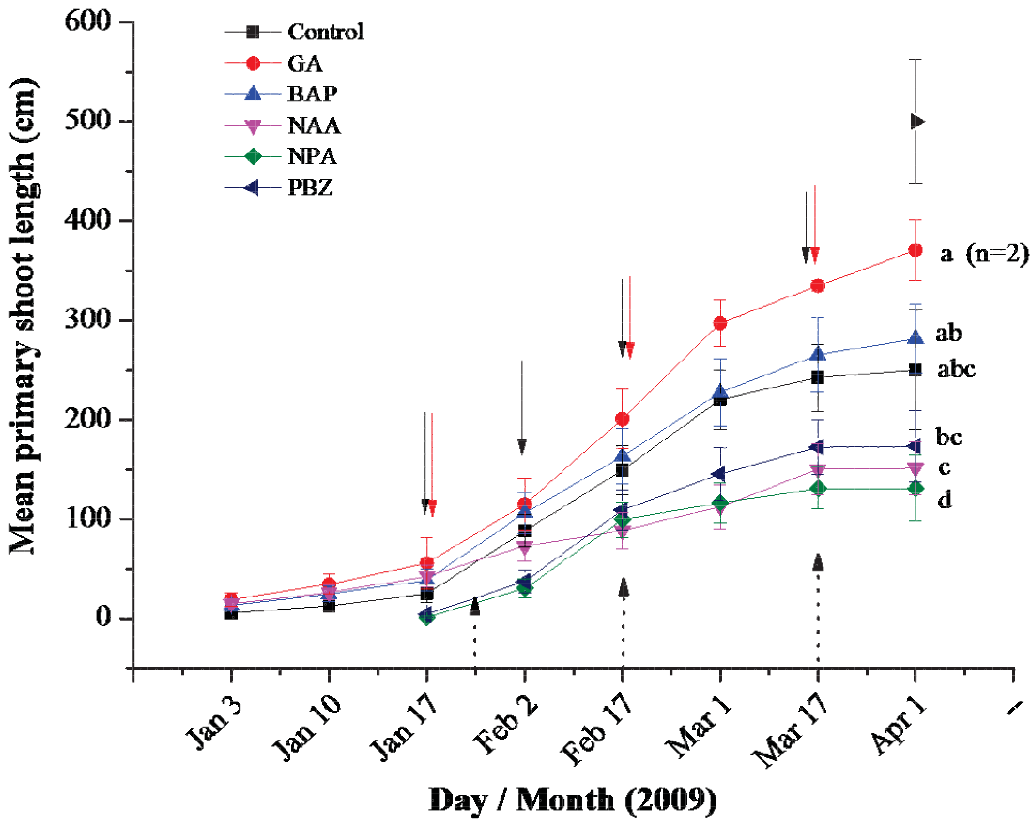

Figure 5.3. Effect of exogenous auxin (NAA), benzylaminopurine (BAP) gibberellins (GA_3+GA_{4+7}), auxin transport inhibitor (NPA) and gibberellin biosynthesis inhibitor (PBZ) on the mean primary shoot length of 'Hayward'. The black solid arrows represent the dates of gibberellins and BAP sprays and red the NAA, and black dotted arrows NPA and PBZ. During early March due to bad weather there was no spraying. Foliar sprays of NAA were given three times (Jan 17th, Feb 17th and March 17th); PBZ and NPA sprays were given three time (Jan 22nd, Feb17th and March 17th). Means sharing the same letter shown on the right are not significantly different using LSD at *P=0.05* (n=2 for GA treatment (i.e., broken shoots removed) and n=4 for all other treatments). Vertical bar on the top of the last measurement date represents LSD at P=0.05.

5.3.2 Treatment effects on mean node number and internode length of primary shoot

5.3.2.1 *Treatment effects (GA, BAP and NAA) on mean node number and internode length of primary shoot of 'Hort16A' and 'Hayward'*

There were neither main effects (cultivar and hormone treatment) nor interactions for mean node number of the primary shoot (Table 5.1). There was a significant effect of cultivar and hormone on mean internode length. For 'Hort16A', the mean internode length was shorter (*P=0.007*) compared with 'Hayward'. With gibberellin treatment the mean internode length was longer (*P=0.0001*) compared with control, BAP and NAA. For NAA, the internodes were shorter compared with all other treatments.

5.3.2.2 *Treatment effects (GA, BAP and NAA, PBZ and NPA) on mean node number and internode length of primary shoot of 'Hayward'*

For 'Hayward', primary shoots treated with NPA had a lower mean node number compared with the control at *P=0.05*. The primary shoots treated with BAP had a higher mean node number compared with NAA, PBZ and NPA at *P=0.02, 0.05, 0.005* respectively (Table 5.2). For 'Hayward', there was no significant difference in mean node number between control, BAP and GA-treated primary shoots.

There was a strong positive correlation between primary shoot length and node number for 'Hort16A' with R^2 between 0.77-0.99 (Figure 5.4) and 'Hayward' with R^2 between 0.88-0.99 (Figure 5.5). For 'Hayward', GA treatment increased internode length significantly compared with all other treatments (*P=0.0001*). Mean internode lengths for primary shoots treated with BAP, NAA, NPA and PBZ were statistically similar (Table 5.2).

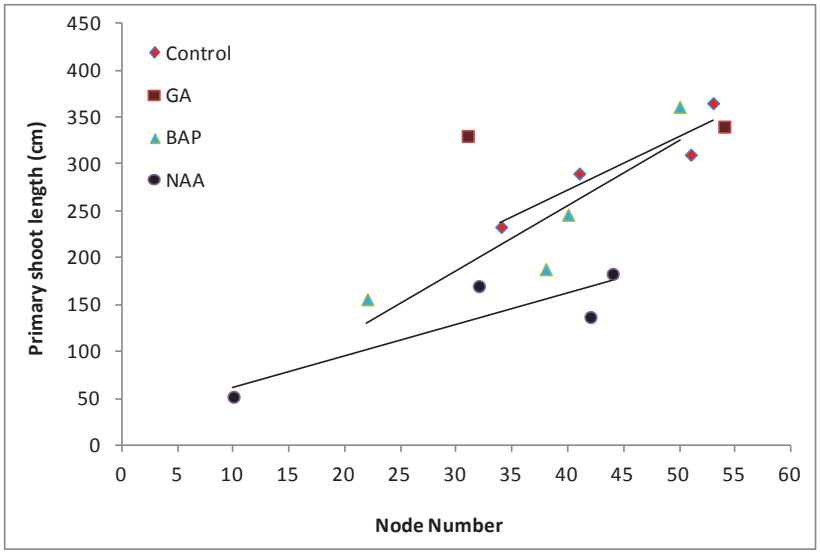

Figure 5.4. Effect of exogenous auxin (NAA), benzylaminopurine (BAP) gibberellins (GA_3+GA_{4+7}), on correlation between node number and primary shoot length of 'Hort16A' (n=2 for GA treatment (i.e., broken shoots removed) and n=4 for all other treatments). The internode lengths can be compared from the regression line of data on each graph by dividing a y-value by its corresponding value of x in the graph. Trendline was not given for GA treatment as only two data points were available.

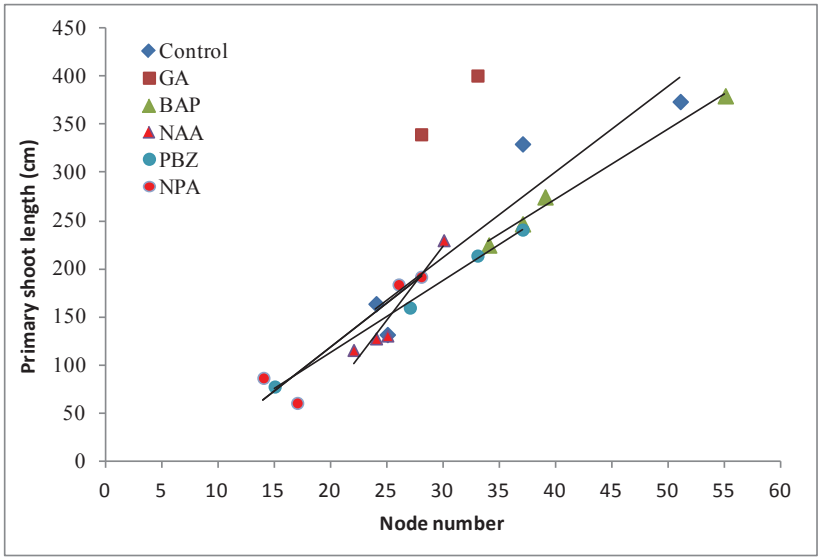

Figure 5.5. Effect of exogenous auxin (NAA), benzylaminopurine (BAP) gibberellins (GA_3+GA_{4+7}), auxin transport inhibitor (NPA) and an anti-gibberellin (PBZ) on correlation between node number and primary shoot length of 'Hayward' (n=2 for GA treatment (i.e., broken shoots removed) and n=4 for all other treatments). The internode lengths can be compared from the regression line of data on each graph by dividing a y-value by its corresponding value of x in the graph. Trendline was not given for GA treatment as only two data points were available.

5.3.3 Treatment effect on sylleptic axillary shoot formation

5.3.3.1 *Treatment effects (GA, BAP and NAA) on mean number and total length of sylleptic axillary shoots (SAS) of 'Hort16A' and 'Hayward'*

There was no significant difference between cultivars for mean number (*P=0.1*) and total length (*P=0.2*) of SAS (Table 5.1). For hormone treatment, there was an increase in SAS number with GA foliar sprays at *P=0.06*. Only the two replicates that were not broken due to wind were taken to detect the gibberellin effect on axillary bud release. For mean total length of SAS, the hormone treatment effect showed differences which approached significance at *P=0.06.* With GA treatment, the mean total length of SAS was higher compared with control (*P=0.06*). The average length of SAS with gibberellins was also more (*P=0.06*) compared with those of untreated control plants. There was no effect of NAA on the formation of SAS and their mean total length compared with the control (Table 5.1).

5.3.3.2 *Treatment effects (GA, BAP and NAA, PBZ and NPA) on mean number and total length of sylleptic axillary shoots (SAS) of 'Hayward'*

For the number of sylleptic axillary shoots, 'Hayward' exhibited significant differences among treatments (Table 5.2). For 'Hayward', gibberellin treatment increased sylleptic axillary shoot formation significantly compared with the control, NAA, PBZ and NPA (*P=0.001*) and with BAP (*P=0.006*). For 'Hayward, the mean total length of SAS was higher with GA treatment compared with the control, NAA, NPA and PBZ (Table 5.2), but less so with BAP (*P=0.06).*

5.3.4 Treatment effect on the total shoot length (primary plus sylleptic secondary shoots)

5.3.4.1 *Treatment effects (GA, BAP and NAA) on the total shoot length (primary plus sylleptic axillary shoot) of 'Hort16A' and 'Hayward'*

There were no significant cultivar effects or cultivar × hormone treatment interactions (Table 5.1) on the total shoot length. There were differences among hormone treatments at *P=0.01*. With NAA foliar spray, the total shoot length was reduced compared with the control at *P=0.04* and GA at *P=0.002*. The effect of NAA approached significance (*P=0.06*) compared with BAP foliar sprays for the mean total shoot length.

5.3.4.2 *Treatment effects (GA, BAP, NAA, PBZ and NPA) on the total shoot length (primary plus sylleptic secondary shoots)*

For 'Hayward', gibberellin treatment significantly (*P=0.007*) increased the total shoot growth compared with the control (Table 5.2). The reduced total shoot length of the NAA treatment approached significance (*P=0.09)*, whilst NPA significantly reduced (*P=0.04*) total shoot growth compared with the control. There was no effect of BAP and PBZ on the total shoot length compared with the control.

Table 5.2. Effect of foliar sprays of auxin (NAA), benzylaminopurine (BAP) gibberellins (GA_3+GA_{4+7}), auxin transport inhibitor (NPA) and an anti-gibberellin (PBZ) on the mean primary shoot length, node number and length; mean sylleptic axillary shoot number, length and mean total shoot length [primary shoot plus sylleptic axillary shoot in (cm)] for 'Hayward' at the end of their first growing season (April, 2009). Data for SAS number and length were log transformed to meet the assumptions of ANOVA.

	Primary shoot			Sylleptic axillary shoot		
Hormone treatment	Mean primary shoot length (cm) (A)	Mean node number	Mean internode length (cm)	Mean number	Mean total length (cm) (B)	Mean total shoot length (cm) (A+B)
Control	250.0 abc	34.2 ab	7.1 b	0.0 b	0.0b	250.0 b
GAs	370.6 a	30.5 ab	12.1 a	2.0 a	97.0 a	467.6 a
BAP	281.6 ab	41.2 a	6.8 b	0.5 b	5.3 ab	286.1b
NAA	151.2 bc	26.3 bc	6.87 b	0.0 b	0.0 b	151.2 bc
PBZ	173.2 bc	28.0 bc	6.0 b	0.0 b	0.0 b	173.2 bc
NPA	131.0 d	21.2 c	6.9 b	0.0 b	0.0 b	131.0 c

Means sharing the same letter in a column are not significantly different using LSD at *P=0.05*

5.3.5 Treatment effect on the spread of bud break and flowering for 'Hort16A' and 'Hayward' in the following spring

Bud break was spread from the last week of August to first week of October inclusive (25^{h} Aug to 5th Oct, 2009) for both cultivars. However, for 'Hort16A', bud break started one week earlier than for 'Hayward' and was completed by 8th Sept, 2009 (Figure 5.6). To calculate percentage bud break, the nodes present on the primary shoot excluding number of SAS (buds already broken in the previous season) were taken into consideration. For 'Hort16A', plants treated with BAP bud break was very compact with 81% (31 buds) occurring in the first week and a further 7% occurring over the next two weeks (Figure 5.6), whereas for the control plants less than 36% (21 buds) bud break was observed in the first week, which increased to 64% in the two subsequent weeks. With NAA treatment, the percentage of bud break was 33% (16 buds), while the plants treated with GAs exhibited no bud break until 1st Sept, 2009 (Figure 5.6). Later on bud break for GA treatment slowly increased. Separately, bud break on the sylleptic axillary shoots that formed with GA treatment was 45% (21 buds) and for BAP, 42% (6.5 buds) were observed (data not shown). For the final number of buds broken, BAP

treatment significantly increased (*P=0.008*) bud break compared to GA whereas, GA reduced (*P=0.03*) compared to the control and there was no difference between bud break with the control and NAA treatment (Figure 5.6).

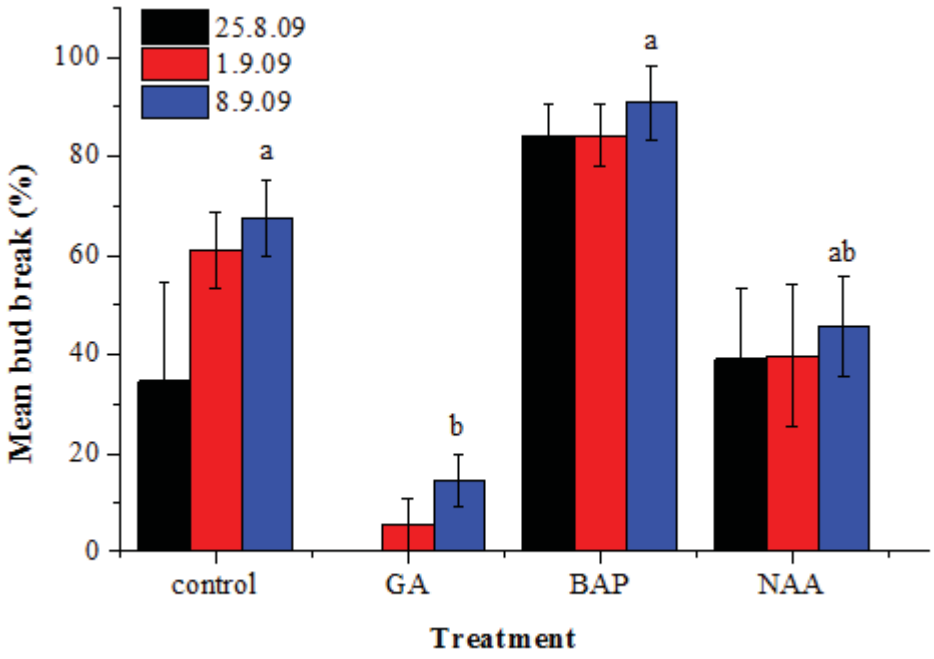

Figure 5.6. Effect of foliar sprays of auxin (NAA), benzylaminopurine (BAP) gibberellins (GA_3+GA_{4+7}) and untreated control vines of 'Hort16A' on the mean bud break percentage during spring of the second growing season (2009). Percentage values were square root transformed before analysis. Means sharing the same letter at the top of the last bar column are not significantly different using LSD at *P=0.05*. Bars represent the standard error.

For 'Hayward', bud break was spread from the 1st week of Sept to the 1st week of October, 2009 (Figure 5.7). Similar to 'Hort16A', the percentage of bud break was highly reduced (*P=0.0001*) with GA treatment compared with the control. It was also reduced for NAA compared with control, BAP and PBZ. There was an increase (*P=0.02*) in the percentage bud break for NPA treated primary shoots compared with the control vines (Figure 5.7).

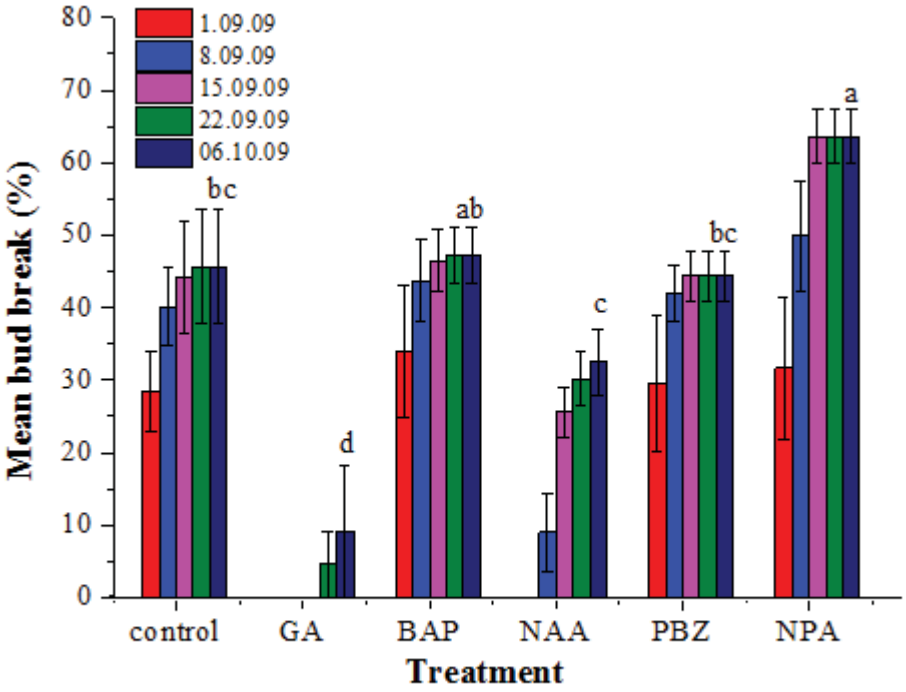

Figure 5.7. Effect of exogenous auxin (NAA), benzylaminopurine (BAP) gibberellins (GA_3+GA_{4+7}), auxin transport inhibitor (NPA) and gibberellin biosynthesis inhibitor (PBZ) and untreated control vines of 'Hayward' during spring of the second growing season (2009). Percentage values were square root transformed before analysis. Means sharing the same letter for the final date are not significantly different using LSD at *P=0.05*. Bars represent the standard error.

5.3.5.1 *Treatment effect (GA, BAP and NAA) on the final percentage of bud break for 'Hort16A' and 'Hayward' in the following spring (Oct, 2009)*

For 'Hort16A', the final percentage of bud break was significantly higher compared with 'Hayward' (Table 5.3). There were no cultivar × hormone interactions. There was a significant hormone treatment effect (*P=0.0002*). For BAP, the percentage of bud break was higher compared with NAA and GA. Gibberellin foliar sprays reduced spring break compared with the control. NAA did not affect spring bud break compared with the untreated control (Table 5.3).

5.3.5.2 *Treatment effect (GA, BAP, NAA PBZ and NPA) on the final percentage of bud break for 'Hayward' in the following spring*

For 'Hayward', NPA increased the percentage spring bud break (P=0.02) compared with untreated control plants (Table 5.4; Figure 5.7). When means were averaged over the cultivars, there was a significant increase with BAP foliar sprays (Table 5.3) compared with NAA. However, BAP foliar sprays did not increase bud break compared with other treatments for 'Hayward' (Table 5.4) except for NPA. The effect of GA for both cultivars was similar in reducing spring bud break, compared with the control. The percentage bud break was reduced for NAA (P=0.02) compared with BAP treatment. There was no significant difference between the control and PBZ in the percentage bud break, but NPA had greater (P=0.01) bud break percentage than PBZ (Table 5.4)

5.3.6 Treatment effects on the first occurrence of vine flowering

5.3.6.1 *Effect of GA, BAP and NAA for 'Hort16A' and 'Hayward' on total number of flowers produced in the following spring*

There was a significant cultivar main effect on the flowers produced in the following spring (October, 2009) (Table 5.3). The number of flowers that developed for 'Hort16A' was more than 'Hayward' (P=0.001). There was no flower formation with GA treatment (Table 5.3). The BAP foliar sprays significantly increased flower formation compared with GA at P=0.01. However, NAA did not reduce flower formation compared with the control. There was a strong trend that untreated control vines produced more flowers (P=0.07) than GA. Among hormone treatments, BAP increased mean number of flowers compared with GA.

5.3.6.2 *Effect of GA, BAP, NAA, PBZ and NPA for 'Hayward' on the number of flowers produced in the following spring*

Plants treated with gibberellins failed to flower in the following season. NPA significantly increased the number of flowers (P=0.02) compared with the control and GA foliar sprays (Table 5.4). Compared with NAA, NPA increased flower number at P=0.04. With PBZ, the mean number of flowers produced was similar with NPA foliar sprays.

Table 5.3. Effect of auxin (NAA), benzylaminopurine (BAP) and gibberellins (GA_3+GA_{4+7}) applied to 'Hort16A' and 'Hayward' in summer (Jan-April, 2009) on mean percentage bud break and mean number of flowers during spring of the next growing season (October, 2009). There were no significant interactions in data. Main effects of cultivar and hormone treatment are averaged over hormone treatment and cultivar, respectively. Percentage values were square root transformed before analysis. Data for mean flower number were log transformed to meet the assumptions of ANOVA.

Main effect	Mean no. of nodes	Mean % bud break	Mean number of flowers
Cultivar			
'Hort16A'	38.7 a	56.5 a	13.18 a
'Hayward'	32.7 a	34.3 b	0.60 b
LSD	8.76	11.67	6.9
P-value	*0.1*	0.0006	0.0001
Hormone treatment			
Control	39.5 a	54.9 ab	9.30 ab
GA	35.3 a	21.8 c	0.00 b
BAP	39.3 a	62.7 a	13.13 a
NAA	28.6 a	42.2 b	6.30 ab
LSD	12.96	16.51	9.7
P-value	*0.2*	0.0002	0.06

Means sharing the same letter in each column for each main effectare not significantly different using LSD at P=0.05.

Table 5.4. Effect of auxin (NAA), benzylaminopurine (BAP) gibberellins (GA3+GA4+7), auxin transport inhibitor (NPA) and gibberellin biosynthesis inhibitor (PBZ) applied to 'Hayward' in summer (Jan-April, 2009) on mean percentage bud break and mean number of flowers during spring of the next growing season (October, 2009). Percentage values were square root transformed before analysis. Data for mean flower number were log transformed to meet the assumptions of ANOVA.

Hormone Treatment	Mean no. of nodes	Mean spring bud break (%)	Mean flower number per vine
Control	34.2 ab	45.7 bc	0.0 b
GAs	30.5 ab	7.90 d	0.0 b
BAP	40.8 a	50.9 ab	1.3 ab
NAA	22.7 bc	32.6 c	0.5 b
PBZ	28.0 bc	44.4 bc	3.8 ab
NPA	21.2 c	65.0 a	5.3 a

Means sharing the same letter in each column are not significantly different using LSD at P=0.05.

5.4 Discussion

An objective of this experiment was to elucidate the role of endogenous growth hormones involved in vigorous vegetative growth of kiwifruit vines by using exogenous growth regulators to manipulate shoot architecture. Three days after spraying NAA to 'Hort16A' and NAA and NPA to 'Hayward', plants exhibited epinasty as was observed in the experiments of Chapter 4 (see 4.4.1). The appearance of epinasty three days after NPA application was assumed to be due to auxin transport inhibition. This downward bending of leaves (Figure 4.9) may be due to ethylene production (Leather et al., 1972; Bradford and Dilley, 1978; Harbage and Stimart, 1996) as a result of auxin accumulation (Van Onckelen et al., 2003). Epinasty was also found in apple after NPA application to the rootstock stem (van Hooijdonk, 2009) and in pigeon pea after NAA application (Hammerton, 1975).

5.4.1 Hormone treatment effect (GA_3+GA_{4+7} and BAP) on vegetative growth of 'Hort16A' and 'Hayward' kiwifruit vines

5.4.1.1 *Primary shoot*

Gibberellins GA_3+GA_{4+7} at 800 mg L^{-1} did not stimulate shoot growth as the mean primary shoot length for control vines was similar with those treated with GA_3+GA_{4+7} for 'Hort16A' (Figures 5.2) and for 'Hayward' (Figure 5.3). However GA_3+GA_{4+7} appeared to reduce node number (Table 5.1), thus increasing internode length. Considerable evidence is available in the literature that GA_1 is the native gibberellin that controls internode elongation in a number of plant species (Ingram et al., 1984; Spray et al., 1984; Kobayashi et al., 1989). Ross et al., (1992) found a correlation between log of GA_1 concentration in pea apices and the length of internodes elongating within the apical region for pea genotypes Le and le. Therefore, with GA treatment for kiwifruit vines it is likely that there were higher levels of bio-active GA_1 compared with untreated controls, resulting in a significant increase in internode length (Table 5.1). In contrast, Chapter 2 of this thesis reported increase in mean node number for apple trees with gibberellin treatment (Table 2.6) and also in Chapter 6, there was a significant increase in the mean node number with GA 500 mg L^{-1}. Therefore, the concentration used in this experiment (800 mg L^{-1}) may be considered as supra-optimal. For apples, gibberellins stimulated apical meristem to produce more nodes and the sub-apical meristem to increase internode length (Figure 2.6), whereas for kiwifruit, gibberellin at the concentrations used appeared to increase internode elongation but not node number. However, it is not known whether this effect is due to differences between apple and kiwifruit, or simply due to the concentrations used. An alternative hypothesis is that in this experiment the response to gibberellins was being limited by the size of the root system of the rooted kiwifruit cuttings.

Benzylaminopurine foliar sprays did not increase the length of primary shoots, as also found for BAP applied to scion on 'M.9' dwarfing rootstock (van Hooijdonk et al., 2010). Similar to these results, BAP did not increase the mean length of sylleptic secondary shoots compared with untreated control trees (Elfving, 1984; Miller and Eldridge, 1986). In addition, Jasinski et al., (2002) found that *KNOX* genes in

Arabidopsis promoted meristem function partly through repression of GA biosynthesis, and also found that *KNOX* function was mediated by cytokinin (CK), a growth regulator that promotes cell division and meristem function. Their results indicated that cytokinin activity is both necessary and sufficient for stimulating GA-catabolic gene expression, thus, reinforcing the low-GA regime by *KNOX* protein in the central zone of the shoot apical meristem (SAM). Therefore, *KNOX* may act as general orchestrator of growth regulators at the central zone (CZ) of the shoot apex of *Arabidopsis* by simultaneously activating CK and repressing GA biosynthesis, thus, promoting meristematic activity (see Chapter 3; section 3.1.1.3). Thus, for the overall stability and maintenance of the meristem an intricate balance between cytokinin and GA, and *KNOX* gene expression is essential (Traas and Vernoux, 2002). Therefore, for Arabidopsis, there is high cytokinin and low GA in the central zone for meristematic activity and in the peripheral zone high auxin and GA for leaf development. Therefore, foliar sprays of BAP applied to the shoot apex may not be involved in shoot extension as the shoot extension involves in addition of nodes by apical meristem (IAA and GA) and elongation of internode by sub-apical meristem (GA).

5.4.1.2 *Sylleptic axillary shoots*

Within the first fortnight (Jan 17th - Feb, 2nd) after applying GA_3+GA_{4+7} and BAP as foliar sprays, axillary bud release was stimulated for both 'Hort16A' and 'Hayward'. The number of SAS increased further with GA foliar sprays after Feb 17th possibly because additional sylleptic axillary shoot formation was stimulated by the release of apical dominance (Grant and Ryugo, 1982) in two out of the four replicates where the tip broke off. However if only the replicates with unbroken shoots were analysed it was clear that gibberellins were indeed stimulating axillary bud release, a response normally associated with cytokinins. Moreover, if a SAM is active for a longer period by applying GA, more nodes are added to the primary shoot, and there would be a possibility of having more axillary buds to be broken to form SAS. In order to find whether GA alone could stimulate activation of axillary bud, it would need to apply GA to axillary buds. Also, other cytokinins that are more aggressive, like Thidiazuron

(TDZ), would be worthy of testing to understand the regulation of syllepsis by cytokinin and/or GA in vines.

The sylleptic axillary shoots that developed for GA-treated kiwifruit vines also grew longer than in the control plants for both 'Hayward' and 'Hort16A' (Table 5.1) and as a result the total shoot length (primary and sylleptic axillary shoots together) was significantly higher compared to all other treatments. In order to better understand the role of gibberellins in the vigorous growth of kiwifruit vines and also to elucidate probable endogenous GA concentrations, a dose-response curve experiment was conducted (Chapter 6).

In the case of apples, 'M.9' rootstock reduced sylleptic axillary shoot formation on the scion and a mixture of BA and GA_3+GA_{4+7} (Jaumien et al., 1992) and GA_{4+7} (van Hooijdonk, 2009) sprays increased branching, as shown also in Chapter 2 of this thesis. The involvement of gibberellins in release of axillary buds was also observed in certain other species such as pea: in germinating pea seedlings, a higher concentration of gibberellins was chromatographically demonstrated after the decapitation and amputation of one cotyledon, which was associated with growth of axillary bud of amputated cotyledon (Sebanek, 1965). This indicated that increased axillary bud growth of a plant with amputated cotyledons is connected with an earlier increase in the activation of endogenous gibberellins. A new bioregulator, cyclanilide®, which appears to interfere with auxin transport and action (Pederson et al., 1997and, temporarily interrupts apical dominance increased the formation of well developed feathers for sweet cherry (Elfving et al., 2006). Additionally, young 1-year-old sweet cherry trees, when treated with GA_3, GA_4 and GA_{4+7}, increased axillary shoot formation and, in one trial GA_{4+7} proved to be as effective in stimulating branching as 6-benzyladenine (Elfving et al., 2011). Three reasons for increased sylleptic secondary shoots for GA treatment could be postulated here:

(1) increased duration of primary shoot growth extension and greater number of nodes on the primary shoot;

(2) Gibberellins may help cytokinin-activated axillary buds continue growth rather than becoming latent without forming a shoot;

(3) Gibberellins may be directly involved in hormonal mechanism of the release of axillary buds.

Although, BAP stimulates sylleptic axillary shoot formation on the primary shoot of apple trees on 'M.9' dwarfing rootstocks (van Hooijdonk et al., 2010), it did not stimulate sylleptic axillary shoot formation in kiwifruit (Table 5.1 and 5.2). BAP application along with gibberellins increased the length of sylleptic axillary shoots in apples (Jaumien et al., 1992), but, gibberellins alone appeared to increase the number and length of sylleptic axillary shoots for apples (see Chapter 2; Table 2.9) and for kiwifruit vines (Table 5.2), although for kiwifruit vines, they were very slender compared with the sylleptic axillary shoots of other treatments. Although, cytokinins are usually associated with activation of axillary buds and gibberellins with their continued growth, in kiwifruit gibberellins appeared to be more directly involved in activation. The mode of action of GA in overcoming apical dominance to promote SAS is not known. However, Elfving et al., (2011) stated that stimulation of axillary bud to produce SAS by applied GA is likely the result of interaction with apical dominance control system. While endodormancy is the tree's inability to start bud break even with moderate temperatures, ecodormancy is the lack of bud break when conditions are not favourable for growing (Tersoglio and Naranjo, 2009). The initial activation of lateral bud in the spring and their failure to continue their growth to develop into long shoots may be due to rapid re-establishment of apical dominance (i.e., paradormancy), which was manifested as a deficiency of GA required to stimulate cell elongation (Elfving et al., 2011). Therefore, gibberellins may help cytokinin-activated axillary buds to continue growth rather revert back to an inactivate state. However, in kiwifruit it was observed that 400 mg L^{-1} BAP did not promote as many sylleptic axillary shoots as 800 mg L^{-1} $GA_3 + GA_{4+7}$ did (Table 5.1). Axillary bud growth was prevented by apical bud. In apical dominance, although all the nutritive constituents required for growth are present, all hormonal factorsmay not be present until apical bud is suppressed (Martin, 1987). Consequently, it can be assumed as Elfving et al., (2011) stated, that in response to applied GAs, the synthesis of a chemical compound that is critical for overcoming paradormancy may be synthesised *de novo.* That critical compound may be cytokinin as the application of cytokinin to the inhibited bud released axillary buds from apical

dominance (Sachs and Thimann, 1967; Catalano and Hill, 1969; Letham, 1969; Schaeffer and Sharpe Jr, 1969; Coenen and Lomax, 1997). Although gibberellins generally have been found to enhance apical dominance (Brian et al., 1959; Bradley and Crane, 1960), in this study, and also other studies (Marth et al., 1956; Elfving et al., 2011) observed a stimulation of axillary bud activation. Moreover, Ali and Fletcher, (1970) mentioned that there was a sequential role of auxin, cytokinin and gibberellin on apical dominance and the effectiveness of any one of these hormones in either inhibiting or releasing the bud from inhibition, depended upon the physiological stage of the bud, that is, whether it is undergoing mitosis or not. However, further research is needed to elucidate the mode of action of GA-induced sylleptic axillary shoot formation.

5.4.1.3 *Spring bud break and flower formation*

Overall, the GA_3+GA_{4+7} treatments for both cultivars reduced spring bud break, whereas for the control and BAP, foliar sprays increased spring bud break in the following growing season (Table 5.3). The consequence of expression of GA-biosynthesis genes was studied in bud break and suggested different efficiencies of GA_3 and GA_4 in the process of bud break (Rinne et al., 2011). They mentioned, as bio-active GAs are needed for shoot elongation, bud burst is dependent on sufficient GA levels as *Populus* tree with lowered levels of bio-active GAs delayed bud burst. This needs further work and it is still an open question whether GA biosynthesis is activated during chilling and after release from the dormancy (Cooke et al., 2012). In this study, for 'Hort16A' more than 80% and for control 64% bud break was observed. Moreover, with BAP foliar sprays the bud break was very compact (Figure 5.6) with 80% in the first week and a further 10% occurring over the next two weeks. Generally, dormancy bud break is often limited by inadequate winter chilling (Linsley-Noakes, 1989) and, with hydrogen cyanamide (HC) applied to dormant vines, the amount of bud break and flowering were increased (Henzell and Briscoe, 1986a). With HC, this increase was greatest at the warmest sites (46% average increase at KeriKeri) and less at cooler sites (25% at TePuke). Year to year variation in natural bud break and flowering may be slightly reduced by HC application but it does not protect the vine by nullifying any

effect of winter temperatue. Winter temperature had a major impact on the bud break of vines treated with HC as it does not negate any effect of spring temoeratures (McPherson et al., 2001). In this study, there was a very compact higher bud break i.e., 80% in the first week itself. Highly synchronised bud break was observed with BAP foliar sprays for kiwifruit 'Hort16A' vines. This result may look trivial, as the experimental material used was only young two-year old rooted stem cutting with small root system. However, if each of these vines is compared to a single cane of the mature vines in the orchard, the number of buds broken for these vines (for example 31 buds) multiplied by the number of canes of the matured vines in the orchard gives a larger effect, which may have a major impact on fruit yields. As BAP is not toxic and registered to be used, it is recommended that further studies on trials using BAP foliar sprays to evaluate its effect on bud break in the orchard are needed.

Cytokinins, in spite of their central role in regulation of cell division, have not been studied extensively in respect to the dormancy and bud break (Cooke et al., 2012). Levels of trans-zeatin riboside and dihydrozeatin hydraside were increased as the rate of primordia initiation increased in *Picea glauca* bud development, although a causal role could not be ascribed (Kayal et al., 2011). However, their role in cell division and the extensive crosstalk between auxin, cytokinin and gibberellins in regulation of meristem activity suggest that they are all very important in this process (Durbak et al., 2012).

5.4.1.4 *Flower formation for both cultivars*

Overall, GA_3+GA_{4+7} treatment inhibited flower formation for 'Hort16A', and for 'Hayward' there were no flowers for GA and control, whereas BAP foliar sprays increased them in the following growing season (Table 5.3). There are many published papers on apples showing that flowering was inhibited by applied GA_3 and/or GA_{4+7} mixtures (Guttridge, 1962; Dennis Jr and Edgerton, 1966; Tromp, 1973; Luckwill, 1974; Tromp, 1982). This effect appeared to be a direct effect of gibberellins rather than simply a stimulation of vegetative growth since when apple trees were treated with Succinic acid 2, 2-dimethylhydrazide (SADH), flowering was not noticeably enhanced although the shoot growth was significantly reduced (Looney et al., 1977). There are

supporting reports to say growth retardants (SADH) increased flowering with no significant reduction in shoot growth (Luckwill, 1970; Tromp, 1973; Naito et al., 1983). Moreover, physiology of biennial flowering in apple and pears trees was reported to be due to gibberellins present in seeds that inhibited flower initiation (Hoad, 1977). In grapes, a specific balance between gibberellins and cytokinins control inflorescence development (Srinivasan and Mullins, 1981), where high gibberellin levels induce tendril formation and suppress inflorescence development, and higher levels of cytokinin cause the tendril primordial to convert to an inflorescence primordia. In citrus, low temperatures induced flowering by a decrease in endogenous GAs (Goldschmidt et al., 1997). In the present study on 'Hort16A' and 'Hayward', foliar sprays of GA_3+GA_{4+7} completely inhibited flowering, which could be related to several months persistence of the chemical in the treated organs, which may be similar to fruit seed-inhibition of flowering that supposedly led to biennial bearing (Hoad, 1978; Pharis and King, 1985) in fruit trees. It was reported that stem elongation and flowering are not controlled by the same gibberellins (Goldschmidt et al., 1997); not all GAs are inhibitory to flowering and some gibberellins have been shown to promote flower formation. In rice, large amounts of GA_4 and other non-13-hydroxy GAs were found in generative organs (Takahashi and Kobayashi, 1991), which are absent in vegetative organs. Therefore, it was logical to deduce that certain structural features of the gibberellin molecule, presumably the number and sites of hydroxylation, determine the development of vegetative or reproductive structures (Goldschmidt et al., 1997). There are reports that indicated gibberellins may promote flowering in certain plants. A combination of GA_4 and zeatin promoted flowering of apple trees (Looney et al., 1985). It was found that for cherry plants only hydroxylated gibberellins with double bond in the C-1,2 position was highly inhibitory (Oliveira and Browning, 1993). Correspondingly, in citrus there was consistent promotion of flowering with a 16, 17-dihydro-GA_5 (Ben-Tal and Erner, 1997). Although, in herbaceous angiosperms, stem elongation is a part of flowering there seems to be antagonism between flowering and shoot elongation in woody fruit trees. Although, gibberellins are always associated with stem elongation its role in flowering is reversed. Therefore, precise genetic-physiological association needs to be clarified when it comes to the role of GAs in

flowering. In citrus, flower intensity is inversely correlated to stem elongation, as flowers are born on short generative shoots (Goldschmidt and Monselise, 1970).

Generally excessive stem elongation reduces flower formation after GA treatment and failed to increase yield (Stuart and Cathey, 1961) in wheat, potatoes, turnips, carrots peas (Morgan and Mees, 1958). Gibberellins at 800 mg L^{-1} in this experiment increased total vegetative growth of 'Hayward' (Table 5.2) and inhibited flower formation, but further experiments would be required to distinguish between a direct effect of gibberellins on inhibiting flowering compared to an indirect effect on shoot growth.

The production of flowers in woody plants proceeds in three phases, which may be separated by varying amounts of time (Snelgar and Manson, 1992). First the meristem undergoes the process of evocation (induction). Second phase is flower differentiation, where flower primordia become visible and complete morphological development of primordia into floral organs. In many deciduous fruit trees these two phases are separated by a period of winter dormancy and a certain amount of chilling may be required before differentiation can occur. In some species such as apples and grapes, the lapse between evocation and differentiation is short and, the buds enter winter with differentiated floral organs. In kiwifruit, evocation occurs in summer (Davison, 1990) and flower primordia are not visible until a few days before bud burst in the following spring (Polito and Grant, 1984). This long period between evocation and floral initiation may make kiwifruit vines particularly susceptible to the premature cessation of flower development. Many fruit trees produce an excess of flowers, making flower or fruit thinning necessary to avoid over cropping. Kiwifruit vines are unusual in that they produce only 30-50 flowers/m^2 of canopy and consequently, in some areas, virtually all flowers must be retained to ensure commercially acceptable crop load (Ferguson, 1984). The timing of evocation is related to the maturation of the shoot. Girdling during January or March resulted in a higher bud break and more flowers. Girdling shoots would be expected to increase the availability of carbohydrate within a shoot (Snelgar and Thorp, 1988). They reported girdling may not influence evocation directly, but rather via the accumulation of carbohydrate in the shoot, which may affect flower initiation, or reduce flower abortion in the following spring. In contrast to girdling, defoliation reduced flower number. Ryugo (1985) reported that treatments which reduce

the nutrient supply available to the developing bud during spring reduced flowering (Snelgar and Manson, 1992). Therefore, the delay of spring bud break and inhibition of flower formation with GA treatment may be related to the unavailability of carbohydrate in the shoot.

5.4.2 NAA treatment effect on the growth of 'Hort16A' and 'Hayward' kiwifruit vines

NAA treatment reduced primary shoot growth, node production and internode length compared with untreated control vines (Table 5.1) and also promoted early cessation of growth compared with the control. In the literature, it was reported that application of NAA can affect endogenous cytokinin and IAA. For example, when NAA was applied in kiwifruit petiole culture, there was a decline in cytokinin concentrations (Palni et al., 1988), and when BA was added cytokinin oxidase activity was increased (Kaminek and Armstrong, 1990). Also, IAA levels were decreased when NAA was added, which is in agreement with the results of Epstein and Ludwig Muller, who observed that in the early stages of growth of *Arabidopsis thaliana* seedlings, NAA reduced the amount of free IAA and IBA (Epstein and Ludwig Müller, 1993). Therefore, the effect of exogenous plant growth regulators NAA and BA can be related to modifications in natural auxin and cytokinin levels in kiwifruit tissues (Centeno et al., 1996). Not only that, NAA application greatly altered the metabolism and levels of IAA in soybean hypocotyls cultured *in vitro* (Liu et al., 1997). Synthetic NAA is also able to interfere with the polar auxin transport and disturbed the endogenous IAA amounts by altering its transport (Lomax et al., 1995). Therefore, the reduction in the growth of a kiwifruit plant treated with NAA could be due to the disturbance in auxin transport or in natural auxin levels. NAA is able to interfere with basipetal auxin transport even when cultured tissues are entirely in direct contact with the medium (Smulders et al., 1988). Therefore, applying foliar sprays of NAA may overload the system, possibly because kiwifruit tissue is more sensitive to auxin.

The level of IAA was estimated to be 17.0 ng per g fresh weight in callus growing explants of soybean on the medium with NAA and kinetin together, which was much

higher than that of the treatments with NAA or kinetin alone (Liu et al., 1997). This accumulation was found probably to be due to the inhibition of IAA conversion by NAA or kinetin. Beffa et al., (1990) reported that several synthetic auxins (NAA and 2, 4-D) inhibited the auxin oxidase activity of extracts from *Zea mays* L.cv LGII apical root segments. Liu et al., (1997) also reported that the exogenous NAA caused the accumulation of endogenous IAA through reducing the activity of IAA oxidase. Thus, due to more accumulation of natural auxin in kiwifruit stem, the growth was reduced presumably, because kiwifruit tissue is more sensitive to auxin.

5.4.3 NPA treatment effect on the growth of 'Hayward' kiwifruit vines

Of all the treatments, NPA reduced total shoot length significantly compared with the control, GA and BAP (Figure 5.3). It reduced primary shoot length, node number and significantly compared with control, GA and BAP treatments (Table 5.2). Interestingly, NPA treatment of the primary shoot did not promote sylleptic axillary shoot formation for 'Hayward', which was contrary to the results described in Chapter 4, where NPA was applied to the cutting stem in a band of lanolin for 'Hort16A'. Therefore, the difference in NPA response may be the cultivar or the method of treatment application. Foliar sprays of NPA reduced the total shoot length of 'Hayward' (Table 5.2) and NPA in lanolin applied to base of 'Hort16A' stem did not reduce the total growth compared with the control (Figure 4.15). Alternatively the mechanism may be different for 'Hayward'. However, foliar sprays of NPA for kiwifruit had a similar effect to stem applied NPA for apple trees (van Hooijdonk et al., 2010).

One of the most important developmental signals in shoot-root communications is auxin, indole-3-acetic acid (IAA) that affects many developmental processes such as cell division, cell expansion, development of vascular tissues and roots (Ljung et al., 2001). The decrease in IAA in specific tissues of *Arabidopsis* when treated with NPA was probably by feedback inhibition of IAA biosynthesis (Ljung et al., 2001). Auxins in the shoot move from cell to cell from apex to base unidirectionally. Auxins are transported into and out of cells through influx and efflux proteins respectively. These efflux carrier proteins are sensitive to synthetic inhibitors such as N-naphthylphthalamic

acid (NPA) and tri-idobenzoic acid (TIBA). Therefore, non-availability of free IAA due to NPA foliar sprays could be the reason for reduced primary shoot and node number in the NPA treated 'Hayward' shoots affecting both apical and sub-apical meristems (Table 5.2).

In this experiment, the significant increase in number of flowers produced with NPA for kiwifruit 'Hayward' shoots (Table 5.3) exhibited a sort of similarity to 'M.9' rootstock. For the apple scion on 'M.9' rootstock had more floral buds compared with that on 'MM.106, (van Hooijdonk, 2009) and also found that the treatments that caused earlier termination such as 'M.9' produced more floral buds. The untreated control 'Hayward' shoots failed to produce flowers and when the shoots were treated NPA significantly increased number of flowers. The most likely candidates in controlling the shift from vegetative to generative bud development in floral induction besides carbohydrates and florigen are plant hormones (Hegele et al., 2005). Feeding cytokinins into the xylem of apple spurs promoted flowering (Skogerbo, 1992).The flower promoting effect of single branch girdling in apple might also account for the buildup of cytokinin pool in the wood and bark (Hegele et al., 2005). Therefore, the reduced primary shoot length and node number and early termination of growth with NPA treatment in the year applied for kiwifruit 'Hayward' may be coincided with the low IAA and GA and elevated cytokinins levels in the bud during next spring, which might be favourable conditions for floral induction. Therefore, the NPA treatment due to auxin inhibition might produce cytokinins, which were stored in the bark and wood, might serve as a source to build up this high ck/(auxin + GAs) ratio, which seemed to be essential for successful floral induction (Hegele et al., 2005).

5.4.4 Paclobutrazol treatment effect on 'Hayward'

Paclobutrazol 4000 gm L^{-1} used for 'Hayward' did not reduce primary shoot length, node number and internode length compared with those of the untreated control. However, PBZ reduced primary shoot length and mean internode length compared with GA (Figure 5.3, and Table 5.1). Application of PBZ to young stems and shoot tips of apple shoots also reduced shoot extension growth and leaf production (Quinlan and

Richardson, 1983). According to Steffens and Wang (1985), growth reduction by PBZ was due to lack of gibberellin biosynthesis, since PBZ does not block the action of either endogenous or applied gibberellins. In gibberellin biosynthesis, paclobutrazol prevents the oxidative step from ent-kaurene to ent-kaurenoic acid without affecting the formation of ent-kaurene (Hedden and Graebe, 1985). Rademacher, (2000) suggested that practically the effect of growth inhibitors depended on plant responsiveness, uptake and translocation. To suppress gibberellin biosynthesis, a threshold concentration of PBZ must be maintained (Lever, 1985) in the shoot apex. Quinland and Richardson (1983) showed that the concentration of PBZ was reduced as a result of distance covered by transport due to rapid shoot extension growth in apple trees. As kiwifruit shoots extend more rapidly than apple shoots, (on average 3-7 mm day^{-1} for apple trees (Chapter 2); 20-40 mm day^{-1} for kiwifruit observed during this study), one can assume that the growth inhibiting substance PBZ was more rapidly depleted. In this Chapter, a significant decrease in the total shoot length of kiwifruit vines even with a high concentration (PBZ 4000 gm L^{-}) was not found. This may be because PBZ does not block gibberellin biosynthesis effectively in kiwifruit. It was also reported that placlobutrazol (PBZ) was effective in increasing ABA levels (Tafazoli and Beyl, 1993) in *Actinidia arguta,* a woody sub-tropical vine and also delayed bud break. In 'Hayward', there was no delay in bud break with PBZ and the percentage of bud break was also similar compared with the control and BAP treatment (Table 5.3). For 'Hayward', PBZ also increased flower formation compared with control vines (Table 5.3). It was observed that low rates of PBZ appeared to stimulate growth more than the control (Estabrooks, 1993), which was also observed using PBZ 600 gm L^{-} (Chapter 6). Therefore, reasons for PBZ 4000 mg L^{-1} not being effective in reducing total shoot length for kiwifruit 'Hayward' could include:

1) Frequency of sprays given in this experiment was insufficient to completely block the step from ent-kaurene to ent-kaurenoic acid;

2) The effect of PBZ was decreased as the gap between two dates of successive sprays was long (one month). It was reported in two-year old apple seedlings (Sansavini et al., 1986a) and in *Eucalyptus globules* (Hetherington et al., 1992) that sequential

sprays were needed to avoid a resumption of growth as it cancelled the previous retardation before the end of the season.

3) The gibberellin pathway in kiwifruit may have alternative steps for synthesising ent-kaurenoic acid.

5.5 Summary

The final mean length, node number and internode length of the primary shoot of 'Hort16A' was not significantly affected by the gibberellin foliar sprays. For 'Hayward', although there was no significant effect of gibberellins on the final mean length and node number of primary shoot, there was a significant increase in the internode length (Table 5.1). Gibberellin also increased the mean total vegetative growth for 'Hayward' by increasing sylleptic axillary shoot number. Additionally, gibberellin foliar sprays significantly reduced spring bud break. On the other hand, the anti-gibberellin PBZ also did not significantly reduce the mean total vegetative growth, but, NAA reduced total vegetative growth significantly compared with the control (Table 5.2 and 5.3). Therefore, from these results it can be suggested that kiwifruit shoots may be highly sensitive to auxin levels. There was a significant overall reduction in vine growth with NAA for both cultivars and with NPA for 'Hayward'. The reduction in vigour due to NAA foliar sprays suggests that the shoot tissue perhaps may have sufficient IAA levels to function and the extra auxin with NAA foliar sprays may become supra-optimal. The non-availability of free IAA due to NPA treatment could be the reason for the reduced primary shoot and node number in the NPA treated 'Hayward' shoots affecting apical and sub-apical meristems (Table 5.2). However, in order to confirm this result, this experiment needs to be repeated with more replicates. Therefore, the reduction of vigour with NAA and NPA is important information as it could fill a gap in our understanding of the biology of kiwifruit vegetative vigour.

The over-all objectives of the experiments in Chapter 4 and 5 were to investigate whether hormonal mechanism of vigour reduction operating for apples also operate in kiwifruit vines. It was understood that decreased auxin supply from shoot to root system (Lockard and Schneider, 1981) decreased root growth and synthesis of root-produced

cytokinins and gibberellins (van Hooijdonk, 2009) in apple trees. In support of this hypothesis, the 'M.9' rootstock reduced scion vigour and final dry mass of the root system (Chapter 2). In addition, the 'M.9' rootstock reduced the number and length of sylleptic axillary shoots formed on the primary shoot and promoted their early termination, which supports the hypothesis that the root-produced cytokinins and gibberellins were reduced. In contrast, for kiwifruit, NPA when applied to the base of the stem in lanolin paste for 'Hort16A'did not affect growth In Chapter 4, for kiwifruit, NPA even though presumably restricted auxin supply to root system (see Chapter 4 Section 4.3; Figure 4.10); there was no effect on sylleptic axillary shoot (SAS) formation (Figure 4.13) and presumably no effect on cytokinin. There was also no effect on the dry weight of the total root system. The growth of 'Hort16A' was similar to the untreated control when NPA was applied to the base of the stem in lanolin paste. This suggests that restricting shoot-root transport of IAA has little effect on the growth of kiwifruit shoot and that IAA supply to the root system is non-limiting, or that stimulation of root-produced hormones by IAA is non-limiting. On the other hand, NPA when applied as foliar sprays, significantly reduced shoot length of 'Hayward'. Thus kiwifruit showed similarities to apples in vigour reduction when applied as foliar sprays. Moreover, foliar sprays of NAA reduced shoot growth, which supports the above statement that kiwifruit shoot tissue had enough IAA to function and that the extra IAA overloaded the tissue resulting in the reduction of growth.

One reason that the results of this Chapter did not show significant gibberellin effect compared with the control, may be because replication was insufficient to reduce the error term and increase the treatment effect. Therefore in order to further investigate the levels of endogenous gibberellins in kiwifruit vine, a gibberellin dose/response growth curve experiment was designed (Chapter 6).

It was also found that in kiwifruit, unlike in apple, NPA increased sylleptic axillary shoot formation (Chapter 4) and moreover in this Chapter, GA foliar sprays increased sylleptic axillary shoot formation compared with BAP foliar sprays. This result gave further impetus to the need to investigate kiwifruit response to gibberellins more closely (Chapter 6).

From the poor response obtained to gibberellin and PBZ, it was hypothesized that endogenous gibberellin levels may be high. Therefore another experiment was designed to understand the role gibberellins (GAs) may have in stimulating shoot vigour and to investigate whether inhibition of gibberellin biosynthesis could decrease shoot vigour (Chapter 6). These results will give an insight into the roles of gibberellins in vegetative vigour and the steps at which gibberellin biosynthesis could be inhibited to reduce vigour of kiwifruit vines.

Chapter 6
Vegetative growth of 'Hayward' kiwifruit vines in response to gibberellins and anti-gibberellins

6.1 Introduction

In Chapter 4, it was shown that a reduced supply of auxin to the root system of 'Hort16A' kiwifruit vines by applying naphthylphthalamic acid (NPA) to the base of the stem did not affect the total growth, although, presumably reduced IAA transport to the root system. In contrast, for composite apple trees, auxin restriction by applying NPA to the rootstock stem, reduced primary shoot growth and sylleptic axillary shoot formation on the scion and promoted early termination of growth (van Hooijdonk et al., 2010). In Chapter 5, foliar sprays of NPA on 'Hayward' significantly reduced the mean number and length of SAS and mean total shoot length of the vine (Table 5.2). Kiwifruit vines in their response to foliar sprays of NPA, showed similarity with apple trees with stem applied NPA. Moreover, in Chapter 5, gibberellins GA_3 + GA_{4+7} at 800 mg L^{-1} did not increase primary shoot length of 'Hort16A' and 'Hayward' significantly (Table 5.1), but increased mean total shoot length of 'Hayward' by increasing the number and length of SAS (Table 5.2). Therefore, in order to elucidate the biological importance of gibberellins on vigour and architecture of kiwifruit vines, it was thought to be important to obtain a dose/response growth curve.

As it has been postulated that decreased basipetal transport of indole-3-acetic acid (IAA) within the phloem and cambial cells of the rootstock stem reduced vigour of scions grafted onto dwarfing rootstocks (Lockard and Schneider, 1981), as low levels of IAA to the root system may limit root growth, and differentially affect the synthesis of root derived cytokinins and gibberellins (GA_{19}) and their transport in xylem vasculature to the scion. In support of this hypothesis, Kamboj et al., (1999a) reported lower total concentrations of zeatin (Z) and zeatin riboside (ZR) in the xylem sap of 'Fiesta' scion on 'M.9' rootstock, and van Hooijdonk et al., (2011) reported lower concentrations of GA_{19} in the xylem sap of 'RG' scion grafted on 'M.9' rootstock. The lower cytokinin concentration correlated with fewer growing points on the primary shoot and lower GA

concentration with a higher proportion of them being terminated earlier, resulting in less total shoot growth on a dwarfing rootstock. In contrast, in Chapter 4, applying NPA in lanolin paste to the base of the kiwifruit cuttings did not reduce growth compared with the control (Table 4.2), as it did in apple (van Hooijdonk, 2009). The number of SAS produced by apple scion on vigorous rootstock with NPA applied on the rootstock stem was significantly less, which coincided with lower cytokinin and a high portion of them being terminated earlier with lower GA concentration, (van Hooijdonk, 2009) compared with untreated control. Therefore, for kiwifruit, vigorous vegetative growth with long non-terminating shoots, high levels of gibberellins may be involved. In order to understand the role of gibberellins and at which step of gibberellin biosynthesis could be stopped, anti-gibberellins paclobutrazol (PBZ) and prohexadione-Ca (Ca-Pro) were used in this study.

Different anti-gibberellins block gibberellin biosynthesis at different stages to reduce the levels of bioactive gibberellin GA_1 and subsequently growth (Figure 6.1). Paclobutrazol prevents the oxidative step from ent-kaurene to ent-kaurenoic acid without affecting the formation of ent-kaurene (Hedden and Graebe, 1985) (Figure 6.1). Prohexadione-calcium prevents the oxidative step of two oxoglutarate-dependant dioxygenases that catalyse the 3-beta hydroxylation of GA_{20} to GA_1 and the conversion of inactive GA_{20} into highly active GA_1, (Evans et al., 1999; Rademacher, 2000). Thereby reducing levels of highly bioactive GA_1 and accumulating its precursor GA_{20} (Rademacher, 2000) (Figure 6.1). In Chapter 5, it was observed that PBZ 4000 mg L^{-1} did not reduce the total shoot growth of kiwifruit 'Hayward' vines compared with control (Table 5.3). So it was decided to test PBZ at lower concentrations and also the anti-gibberellin prohexadione-calcium on field grown vines. Knowing which growth inhibitor reduces the vegetative vigour of kiwifruit vines would provide insight into which step in the gibberellin biosynthetic pathway could be altered to reduce vine vigour of kiwifruit.

Prohexadione-Ca (Regalis®) is known to be less toxic and less persistent in the environment compared with chloromequat, paclobutrazol and diaminozide (Evans et al., 1996; Owens and Stover, 1999). Therefore, now Regalis® a formulation of Prohexadione-calcium is being used to reduce vegetative growth, improve fruit quality

and control fire blight in apples and pears. Prohexadione-calcium does not exhibit antibacterial activity against fire blight but increases host resistance by reducing plant vigour (Norelli et al., 2003). Trials in Michigan in 1999 and 2000 demonstrated the ability of Prohexadione-calcium to control fire blight under severe natural infection conditions. When applied at petal fall, it was observed that there was 60-75% level of control of disease under natural conditions of infection.

The biological half-life period of Ca-Pro in an actively growing apple tree is in the range of 10 to 14 days (Rademacher et al., 2005). In addition, its half-life in microbially active soils is 24 hours. Therefore, the best way to apply Ca-Pro is by foliar spray. Prohexadione-calcium (Ca-Pro) a gibberellin biosynthesis inhibitor with low toxicity and has been registered as a growth retardant for apples in North America (Apogee®) and Europe (Regalis®) (Miller and Tworkoski, 2003) and it also reduces vegetative growth in cultivars of pears (Costa et al., 2001; Smit et al., 2005) and apples (Medjdoub et al., 2004). This growth inhibitor is being commercialised as an anti-lodging agent for rice, cereals and turf grasses grown for seed production and also groundnuts (Rademacher and Bucci, 2002). Rates of 125 mg L^{-1} to 250 mg L^{-1} have typically been sufficient to provide effective vegetative control of vigorous trees. As Ca-pro, 250 mg L^{-1} had already been used on kiwifruit vines (Mike Clearwater, personal communication) it was decided to use 250 mg L^{-1} and 350 mg L^{-1} in this experiment.

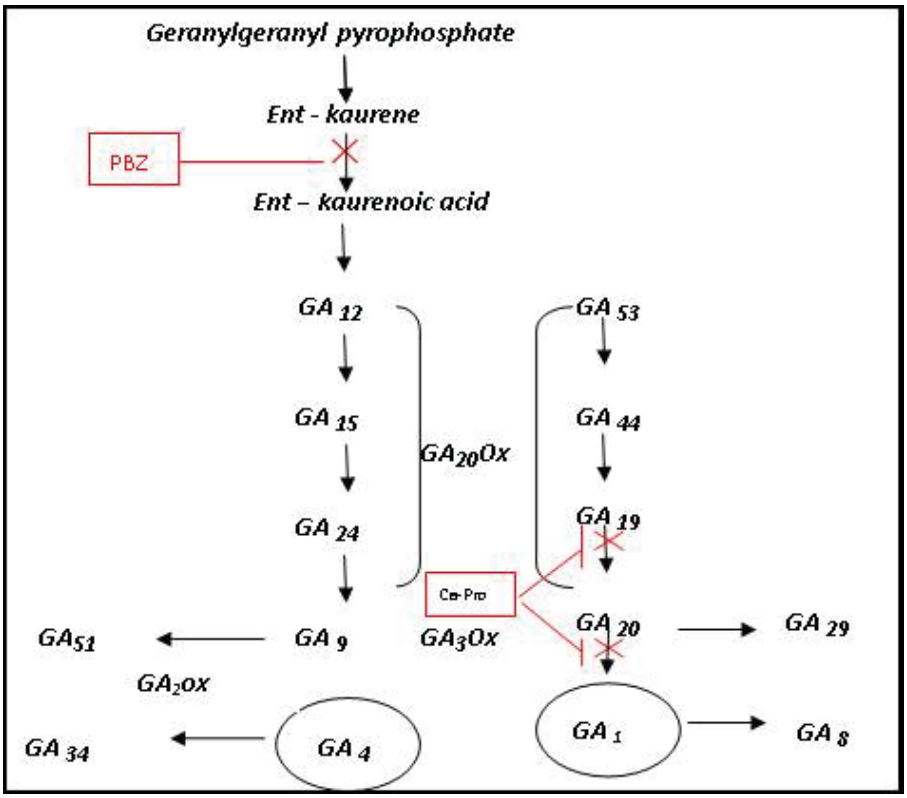

Figure 6.1. Scheme of gibberellin biosynthetic pathway showing the steps at which anti-gibberellin PBZ and Ca-Pro inhibit gibberellin biosynthesis. PBZ inhibiting early step of GA biosynthesis and Ca-Pro the last reactions catalysed by GA deoxygenases source :(Fagoaga et al., 2007). GA20ox and GA3ox are the activating enzymes and GA2ox is the deactivating enzyme. GA_1 and GA_4 are bioactive enzymes. The inhibition by PBZ and Ca-Pro are pointed with an 'X' mark.

Therefore, in summary, the objectives were:

1. To produce a dose\response growth curve to elucidate the biological importance of gibberellins on shoot vigour of kiwifruit 'Hayward'.

2. To obtain an insight into which growth retardant may be effective and which step in gibberellin biosynthesis may be affected.

6.2 Materials and methods

6.2.1 Experimental plant material

The experiment was conducted in an experimental orchard at Massey University, Palmerston North (lat. 40.2ºS, long 175.4°E), New Zealand. Mature 'Hayward' kiwifruit vines grafted onto seedling rootstocks trained onto a T-bar trellis system were used. The male to female vine ratio was 1:5. The irrigation system consisted of Dan-Modlar micro-sprinklers attached to 19 mm polyethylene tubing. Nine canes per vine on eight different vines were used for nine treatments i.e., one cane for each treatment on eight vines. At the onset of spring bud break in October, five healthy emerging shoots per cane were used for each treatment (Figure 6.2).

6.2.1.1 *Experimental treatments*

To produce a dose/response growth curve and to elucidate the effect that gibberellins may have in stimulating shoot vigour, buds on each cane were treated immediately after the spring bud break with five concentrations (0, 500, 1000, 2000 and 4000 mg L^{-1}) of $GA_3 + GA_{4+7}$ as foliar sprays. For control (0 concentration of gibberellin), only distilled water was applied as a foliar spray. For all gibberellin concentrations, GA_3 and GA_{4+7} were at a 1:1 ratio. The first application of $GA_3 + GA_{4+7}$ began at the onset of spring bud break (13th October 2009), and subsequent applications occurred every 14 days until January 12th, 2010 (seven applications). To determine whether inhibition of gibberellin biosynthesis could decrease shoot vigour, foliar sprays of Paclobutrazol (PBZ) or Prohexadione-calcium (Ca-Pro) were given. Paclobutrazol (Payback™ NuFarm Limited) was used at three different concentrations (0, 400, 600 mg L^{-1}) and prohexadione-calcium (Regalis®) at 0, 250, 350 mg L^{-1}. Each anti-gibberellin was applied three times at 14-day intervals beginning on 13th October 2009. Therefore the three sprays of PBZ and Ca-Pro were given on 13th October, 27th October and 10th November, 2009.

Figure 6.2. Selection of experimental kiwifruit material of Actinidia deliciosa 'Hayward' in the Massey University experimental orchard during spring Five emerging shoots per cane and nine canes per vine were selected immediately after spring bud break for application of treatments. Canes were labelled distributing the treatments randomly (other emerging shoots and flowers on the selected canes were removed).

6.2.1.2 *Experimental design and statistical analysis*

After winter pruning, before starting the experiment, canes of approximately the same thickness and with the same number of buds were selected. For each cane, only five proleptic shoots were allowed to grow for the treatment, the remaining being removed. All nine treatments were randomly distributed on each vine. Thus each vine is a block with five internal replicates for each treatment and the experimental design was a randomised complete block (RCBD). All nine treatments were replicated five times on eight vines (5 × 8 = 40 replicates). A goodness of fit test (Shapiro-Wilk) was applied to determine the normality of the data. As data were not normally distributed, non-

parametric ANOVA and multiple LSD were used for mean comparisons. Data were ranked before ANOVA. Ranks are determined by sorting the data into order and replacing each value by its relative position in the order. As a result of ranking the data, larger ranks are associated with larger values and smaller ranks being associated with smaller values.

6.2.1.3 *Measurements of shoot growth*

Length and node number of the lateral shoot (proleptic shoots) were measured once every month. In this Chapter, the shoots that developed from previous season buds on the canes have been called lateral shoots and the outgrowths of axillary buds on these lateral shoots have been termed sylleptic axillary shoots (SAS), i.e., shoots formed from current season buds (Figure 6.3).

Figure 6.3. Diagrammatic representation of proleptic bud (A), proleptic shoot (lateral shoot) (B), and sylleptic axillary shoot (C) on a cane of kiwifruit vine.

Lateral shoots that developed from buds on the canes were classified as short (≤ 25 cm), medium (25–60 cm) and long (> 60 cm). The short lateral shoots were self-terminating with only preformed nodes (nine or less nodes present in the overwintering bud). The medium length lateral shoots were also self-terminating but with more than nine nodes that include both pre and neo-formed nodes. The long lateral shoots were non-terminating with up to 60 neo-formed nodes. On 8th Feb, 2010, shoots were harvested destructively, fresh weights taken and then the shoots oven dried at 80°C to a constant mass and their dry weights recorded on a four-decimal place balance (METTLER

AE200, Switzerland) within 30 seconds of removal from the oven. Leaf areas were measured using a leaf area meter (Li-3100, Li-Cor Inc., USA) and leaf fresh weight and dry weights obtained. The specific weight of leaves was calculated by dividing total dry weight with total leaf area per shoot in g/m^2. The diameters of lateral shoot bases were measured using digital calipers and shoot cross-sectional area (SCA) was calculated as $\pi D^2/4$.

6.3 Results

6.3.1 Treatment effect on shoot length

There was a significant treatment effect on the mean length of lateral shoots developed from the selected buds (F = 21.72, DF = 8, $P<0.0001$). Gibberellins sprayed at 500 and 1000 mg L^{-1} significantly increased the length ($P<0.0001$) of lateral shoots (Figure 6.4). Compared to the control, 500 mg L^{-1} treatment increased length 182%, the 1000 mg L^{-1} 124% and 2000 mg L^{-1} 82%. For the 2000 and 4000 mg L^{-1} treatments, the shoot apex and upper young leaves withered, however, some internode elongation continued until February 8th, 2009.

The lengths of shoots treated with PBZ at 400 and 600 mg L^{-1} were similar compared with the control (Fig. 6.4) The length of shoots treated with Ca-Pro 250 mg L^{-1} were significantly shorter ($P=0.04$) compared with the control, but surprisingly the effect of Ca-Pro at 350 mg L^{-1} was not significantly different from either.

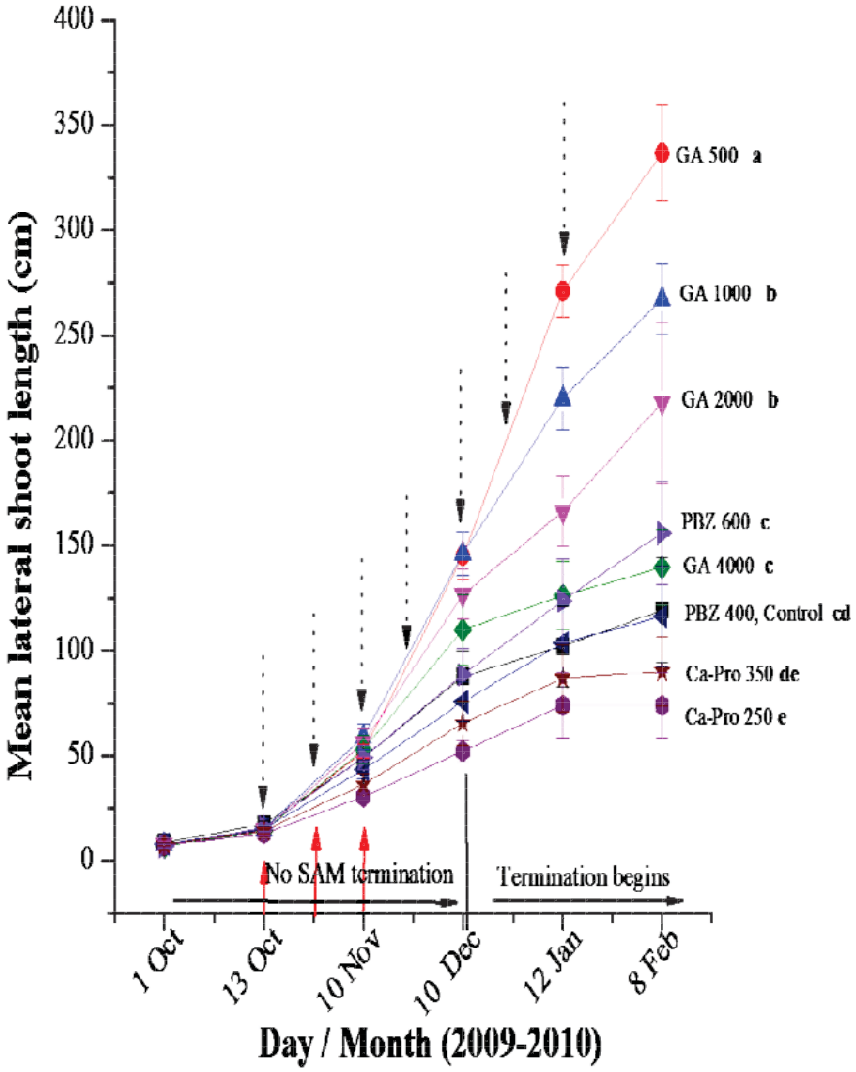

Figure 6.4. Effect of gibberellin treatment at 0, 500, 1000, 2000, 4000 mg L^{-1} and anti-gibberellins PBZ and Ca-Pro at 0, 400, 600 and 0, 250, 350 mg L^{-1} respectively, on mean length of lateral shoots of Actinidia deliciosa 'Hayward'. The black vertical dotted lines represent the dates of treatment gibberellin application (seven applications) and red solid lines represent foliar sprays of anti-gibberellins. Means sharing the same letter shown on the right are not significantly different using LSD at P=0.05. Vertical bars represent the standard error. Black solid line separates the periods before and after initiation of shoot termination.

6.3.2 Treatment effect on shoot type / termination

Lateral shoots that developed from buds on canes were classified as short, medium and long. For control, 7 and 9 short and medium shoots were developed respectively out of a total of 40 shoots, but for the three lower gibberellin concentrations all shoots were

long (Figure 6.5A). With Ca-Pro 250 mg L^{-1}, medium shoots were more (19) compared with long shoots (12) (Figure 6.5A) and also compared with the control (9). With PBZ 400 and 600 mg L^{-1}, the number of long shoot development out of the total 40 shoots was 26 and 28 respectively more than control (23).

Using χ^2 test, number of short, medium and long shoots for control, Ca-Pro 250 and Ca-Pro 350 mg L^{-1} were analysed. For control, the number of long shoots were more ($\chi^2 = 11.69$; $P=0.002$) compared with medium and short (Figure 6.5A). For Ca-Pro 250 mg L^{-1}, the number of medium shoots were more ($\chi^2=6.86$; $P=0.03$) compared with short and medium shoots. Among these three treatments, there was no difference in the number of short shoots produced, except for Ca-Pro 350 mg L^{-1} with trends to increase the number at ($P=0.08$) (Figure 6.6). Number of medium shoots produced for Ca-pro 250 mg L^{-1} was more ($\chi^2 = 6.523$; $P=0.01$) and long shoots were less ($\chi^2 = 5.383$; $P=0.02$) compared with the control (Figure 6.6). For GA 500 mg L^{-1}, there were no short and medium shoots and long shoots were significantly high ($\chi^2=13.2$; $P=0.004$) compared with the control.

With gibberellin 500 and 1000 mg L^{-1} treatment number of long shoots produced were significantly higher compared with all other treatments ($\chi^2=17.92$; $P=0.006$). Ca-Pro 250 mg L^{-1} reduced long shoots ($P=0.01$) compared with the control (Figure 6.5B).

Growth cessation for all short and medium shoots of all treatments started very early and by December, 2009, 48% of shoots had terminated for the control. PBZ 600 mg L^{-1} also began shoot termination very early and 35% shoots had terminated by December, 2009 (Table 6.1). Similarly, 47% shoots treated with Ca-Pro 350 and 27% with 250 mg L^{-1} had terminated. By the time of destructive shoot harvest (8th Feb, 2010), 77% of lateral shoots had terminated for the control. Out of these terminated control lateral shoots, 55% were long (Table 6.1). None of the lateral shoots treated with GA 500, 1000 and 2000 mg L^{-1} had terminated on 8th Feb, 2010. With GA 4000 mg L^{-1} treatment, 60% long shoots stopped further elongation after Jan, 2010. For GA 500, 1000 and 2000 mg L^{-1}, there were no symptoms of growth cessation and elongation of shoot was still continuing on Feb, 2010, the day of destructive shoot harvest (Figure 6.4). After the last foliar application of Ca-Pro 250 mg L^{-1} (Jan 2nd week) more than

80% of the lateral shoots had terminated. However, for Ca-pro 250 mg L^{-1} the highest percentage of shoots had terminated by 12th January, 2010 (Table 6.1).

Table 6.1. Effect of gibberellin treatment (GA_3 + GA_{4+7}) at 0, 500, 1000, 2000, 4000 mg L^{-1} and anti-gibberellins (PBZ and Ca-Pro) at 0, 400, 600 and 0, 250, 350 mg L^{-1} respectively, on the percentage of shoots of Actinidia deliciosa 'Hayward' that terminated from December, 2009 to February, 2010.

Treatment mg L^{-1}	Percentage of shoots terminated		
	December	January	February
Control	48	65	77
GA 500	0	0	0
GA 1000	0	0	0
GA 2000	0	0	0
GA 4000	0	0	60
PBZ 400	0	67	77
PBZ 600	35	50	57
Ca-Pro 250	27	82	90
Ca-Pro 350	47	70	80

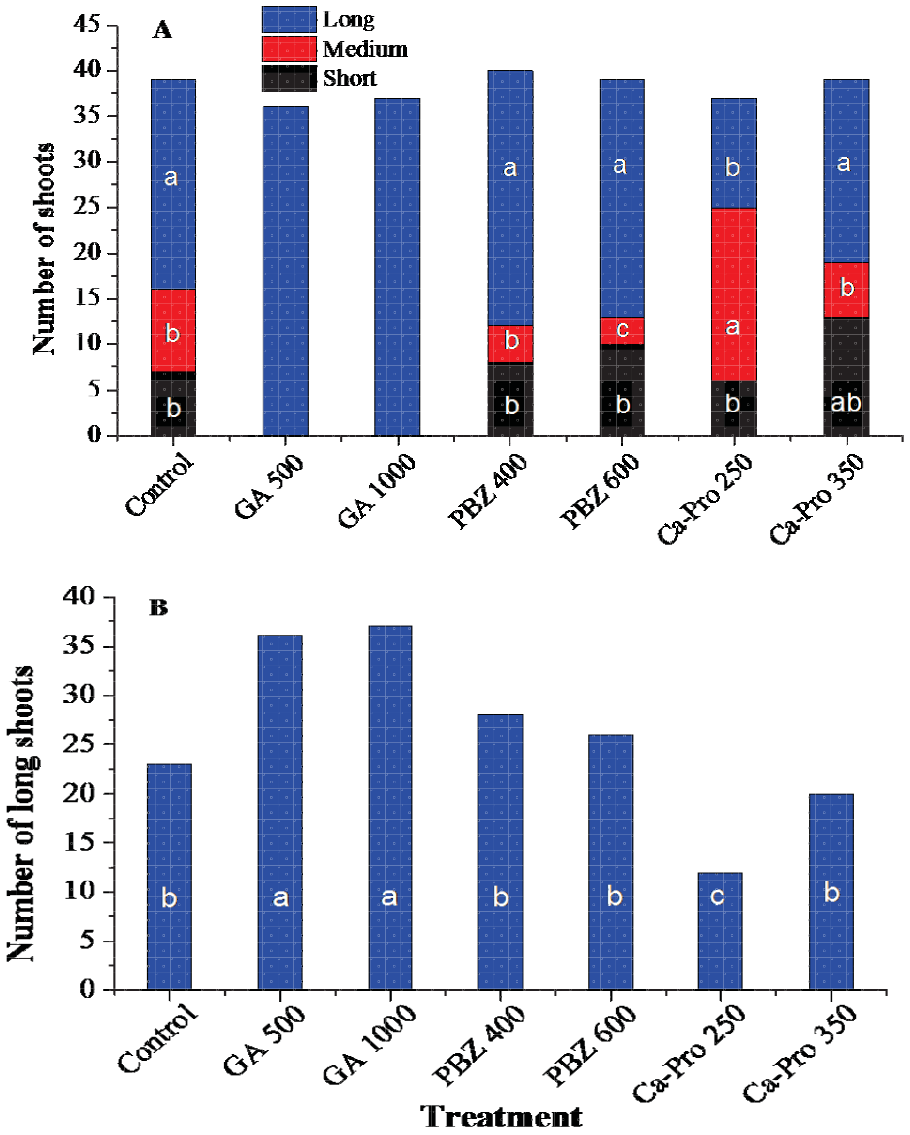

Figure 6.5. Effect of gibberellin treatment at 0, 500, 1000, mg L^{-1} and anti-gibberellins PBZ at 0, 400, 600 and Ca-Pro at 0, 250, 350 mg L^{-1} on the number of shoots (A): short, medium and long proleptic shoots developed from the emerging shoot selected at the onset of spring bud break (October, 2009) for *Actinidia deliciosa* 'Hayward'. 'B' represents the number of long shoots of all treatments. Values within the bar column in 'A' and across bar columns in 'B' sharing the same letter are not significantly different at P=0.05 using χ^2 test.

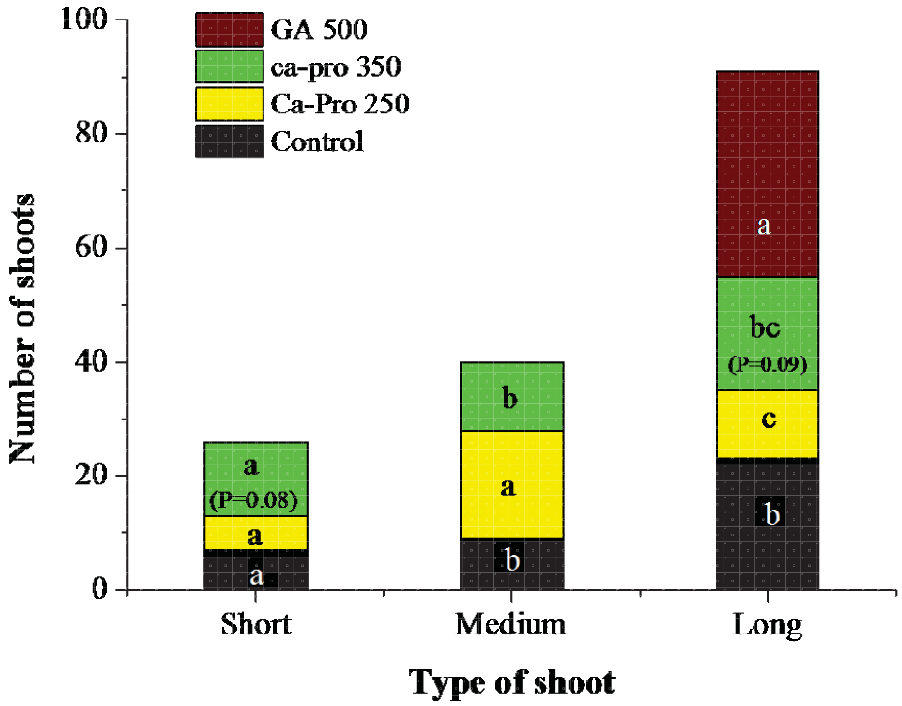

Figure 6.6. Effect of anti-gibberellins Ca-Pro at 0 (control), 250, 350 mg L^{-1} and GA_3+GA_{4+7} 500 mg L^{-1} respectively on the number of shoot types: short, medium and long proleptic shoots developed from the emerging shoot selected at the onset of spring bud break (October, 2009) for *Actinidia deliciosa* 'Hayward'. Values within a bar column sharing the same letter are not significantly different at P=0.05 using χ^2 test.

6.3.3 Dose/response growth curve for length of lateral shoots for different concentrations of GA_3+GA_{4+7}

The growth response for different gibberellin concentrations was compared with that of the control shoots. There was a significant response with gibberellins at 500 and 1000 mg L^{-1} from 10th Jan, 2010 until the day of destructive shoot harvest i.e., 8th Feb, 2010 (Figure 6.7). Shoot growth was impaired at the higher concentrations of 2000 and 4000 mg L^{-1} compared with 500 and 1000 mg L^{-1} GA. In addition, although lateral shoot top portions withered with gibberellins at higher concentrations, internodes elongated and also promoted axillary bud outgrowth. As a result sylleptic axillary shoots developed on the lateral shoots (proleptic).

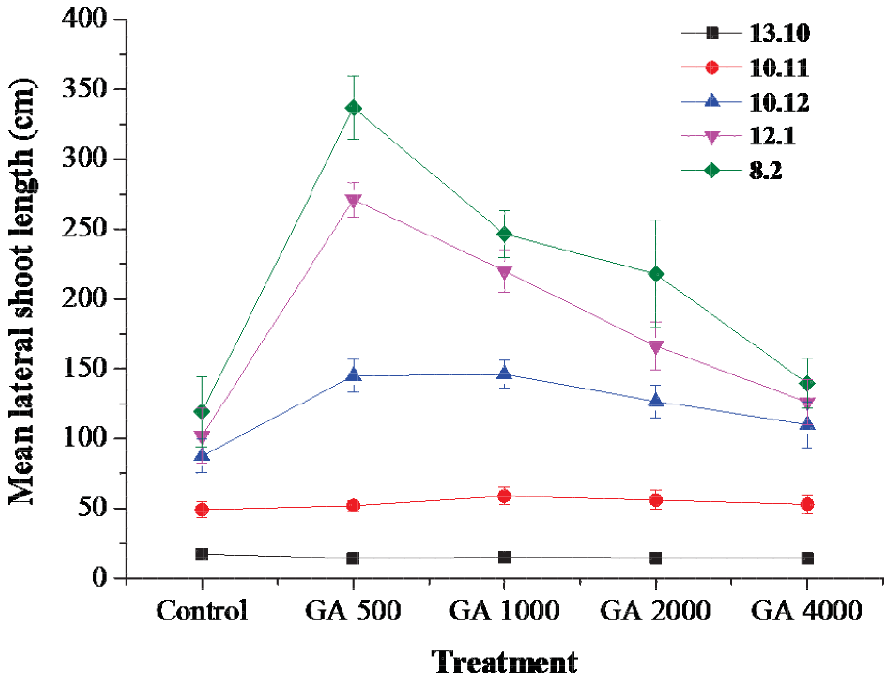

Figure 6.7. Dose/response growth curve for GA_3 + GA_{4+7} applied fortnightly at 0 (control), 500, 1000, 2000, and 4000 mg L^{-1} on mean lateral shoot length of *Actinidia deliciosa* 'Hayward'. Bars represent the standard error. There was a significant response at 500 and 1000 mg L^{-1} GA_3 + GA_{4+7} on the length of lateral shoots.

6.3.4 Treatment effect on shoot cross-sectional area (SCA)

The final mean shoot cross-sectional area of the lateral shoots treated with different concentrations of gibberellin were higher compared with those of the control, PBZ and Ca-Pro treatments (Figure 6.8) at *P= 0.0001*. Ca-Pro 250 and 350 mg L^{-1} reduced SCA at *P= 0.06* and *0.006,* respectively, compared with the control. There was no significant difference between the control and PBZ treatments on the final mean SCA of lateral shoots.

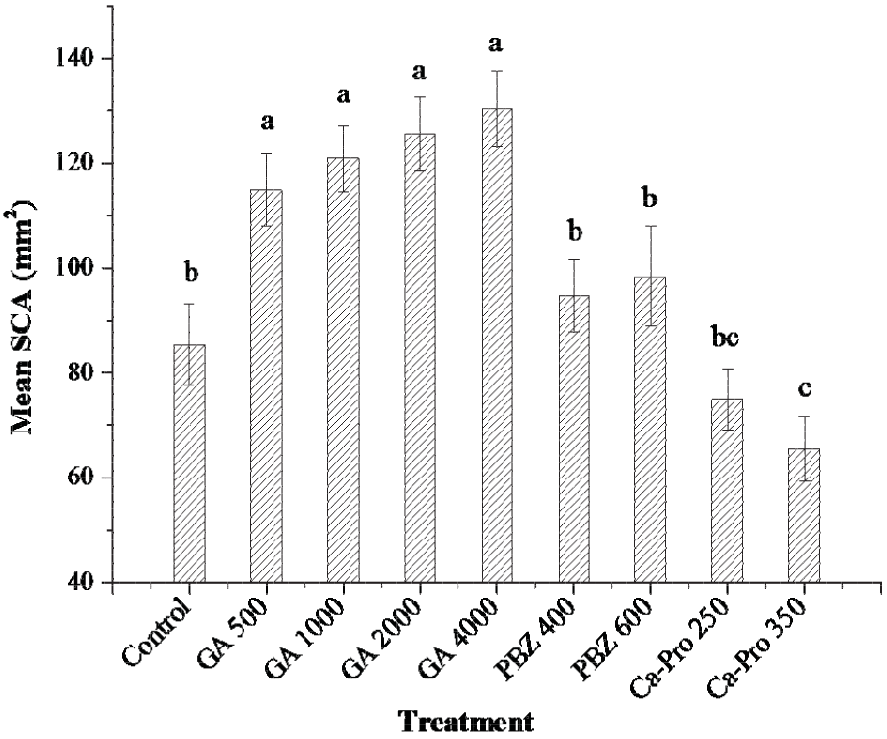

Figure 6.8. Effect of gibberellins GA_3 + GA_{4+7} at 0, 500, 1000, 2000, 4000 mg L^{-1} and anti-gibberellin PBZ at 0, 400, 600 and Ca-Pro at 0, 250, 350 mg L^{-1}, on the mean shoot cross-sectional area (SCA) measured after harvesting (Feb 8th, 2010) shoots of *Actinidia deliciosa* 'Hayward'. Bars represent the standard error.

6.3.5 Dry weight of shoot

The gibberellin treatment increased the dry weight of the shoots. Lateral shoot lengths averaged 107±21cm (equivalent to 19 g dry weight) for control vines in comparison with average lateral lengths 314±23 cm (equivalent to 51 g dry weight) for vines with GA 500 mg L^{-1} foliar sprays. Therefore, dry weight of lateral shoot increased as the SCA and shoot length increased.

6.3.6 Treatment effect on node formation

Lateral shoots treated with gibberellins at 500 mg L^{-1} developed significantly more nodes (*P=0.02)* compared with the control (Table 6.2). There was no significant increase in the mean node number with GA at 1000 mg L^{-1} compared with the control. As the shoot apex plus a few young leaves withered with 2000 and 4000 mg L^{-1}, no new nodes were formed after the second GA treatment (Oct, 30th), however, internode

elongation continued until the end of December for GA 4000 mg L-1, whereas, for GA 2000 mg L^{-1} it continued until the day of harvest. Ca-Pro and PBZ at both concentrations did not affect mean node number compared with the control (Table 6.2). Ca-Pro 250 mg L-1 only reduced node number at *P = 0.1* compared with the control (Table 6.2).

Table 6.2. Effect of gibberellin treatment (GA_3 + GA_{4+7}) at 0, 500, 1000, 2000, 4000 mg L^{-1} and anti-gibberellins (PBZ and Ca-Pro) 0, 400, 600 and 0, 250, 350 mg L^{-1} on the length, node number and internode length of shoots of *Actinidia deliciosa* 'Hayward' from 13th Oct, 2009 to 8th Feb, 2010).

Concentration mg L^{-1}	Mean lateral shoot length (cm)	Mean node number	Mean internode length (cm)
Control			
0	119.2 cd	22 bc	4.2 b
GAs mg L^{-1}			
500	336.6 a	39 a	6.9 a
1000	267.3 b	28 b	7.5 a
2000	217.0 b	-	7.4 a
4000	139.5 c	-	6.7 a
PBZ mg L^{-1}			
400	116.0 cd	23 b	3.9 bc
600	156.0 c	26 b	3.8 bc
Ca-Pro mg L^{-1}			
250	73.9 e	16 c	3.4 c
350	90.0 de	20 c	3.5 c

Means within a column sharing the same letter are not significantly different at P≤0.05 using LSD.

6.3.7 Treatment effects on mean internode length

There was a significant treatment effect on the mean length of internodes (*P=0.0001)*. The mean internode length was significantly greater with gibberellins treatment of all concentrations compared with the control lateral shoots (Table 6.2). For PBZ 400 and 600 mg L^{-1} treatments, there was no significant difference (Table 6.2) and, Ca-Pro treatment internodes were significantly shorter compared with the control at *P=0.05*. There was a strong positive correlation between lateral shoot length and node number for all the treatments (R^2 = 0.7 - 0.9) except for GA 500 mg L^{-1} for which R^2 was 0.19 (Figure 6.9).

Shoots treated with Ca-Pro 250 mg L^{-1} and the control shoots extended their internodes completely (Figures 6.10 and 6.11A) and for GA 500, internodes were still elongating (Figures 6.10and 6.11B) on the day of destructive shoot harvest. There was a noticeable effect of Ca-Pro on shoot morphology by reducing internode length (Figures 6.6 and 6.7A).

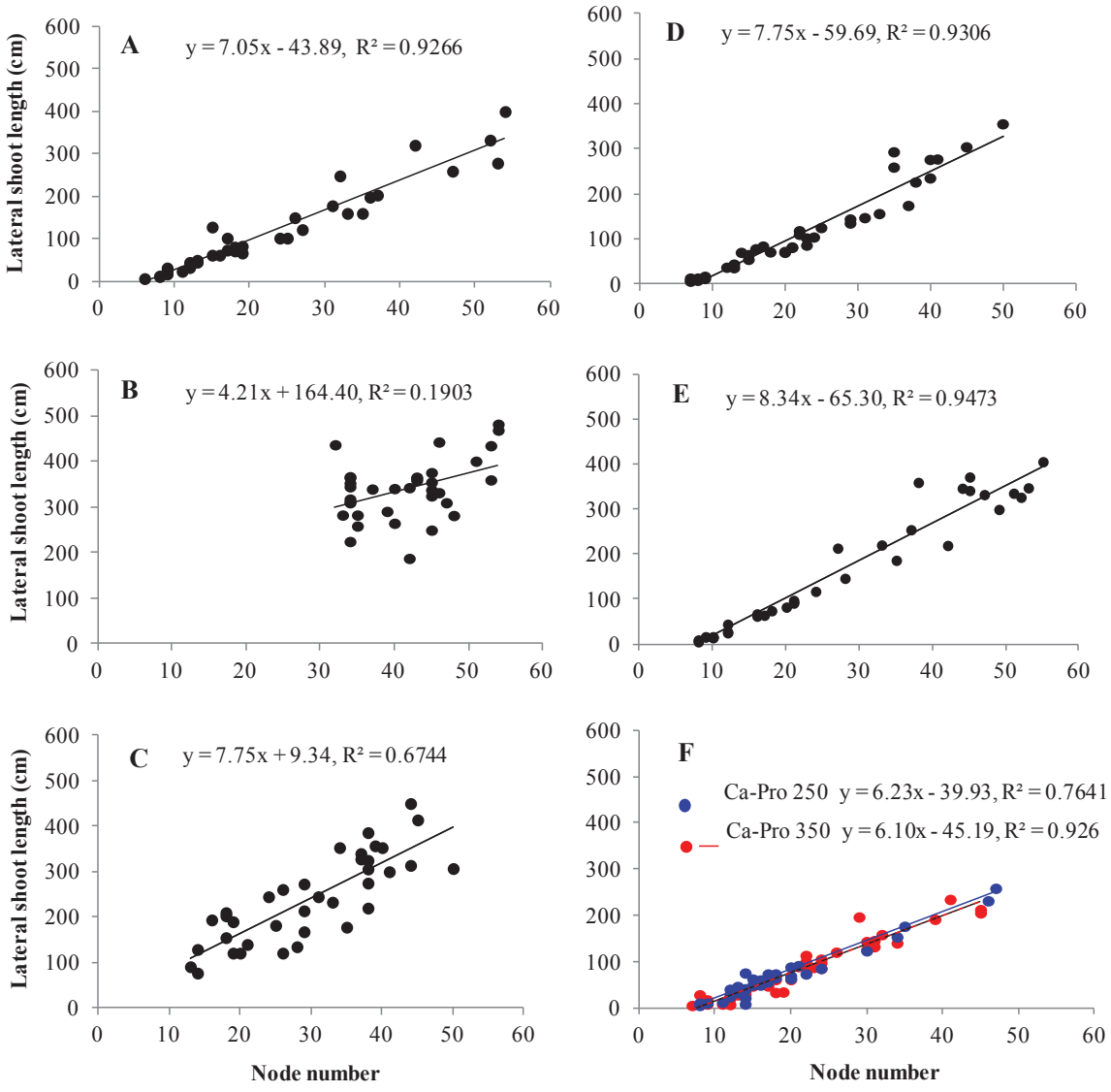

Figure 6.9. Relationship between node number and lateral shoot length of *Actinidia deliciosa* 'Hayward' vines in response to foliar sprays of gibberellin (GA_3 + GA_{4+7}) at 0 (A), 500 (B), 1000 mg L^{-1} (C) or anti-gibberellins PBZ 400 (D), 600 (E) and Ca-Pro 250 and 350 mg L^{-1} (F) applied fortnightly from October, 2009 to February, 2010.

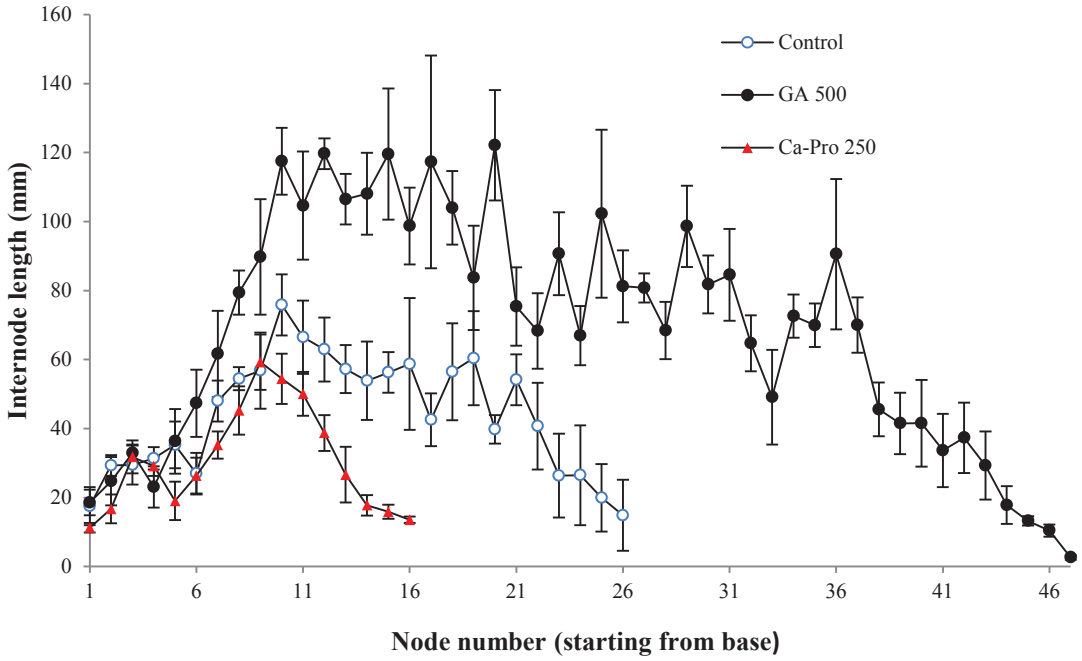

Figure 6.10. Internode length of the control, GA (GA_3 + GA_{4+7}) 500 mg L^{-1} and Ca-pro 250 mg L^{-1} treated shoots of *Actinidia deliciosa* 'Hayward' measured on the destructive shoot harvest (8th Feb, 2010). Values are means ± SE, n=5 shoots for each treatment. Internodes of shoots treated with Ca-pro 250 mg L^{-1} and untreated control are completely elongated (growth was fully terminated); for GA 500 mg L^{-1} shoots still exhibiting elongation.

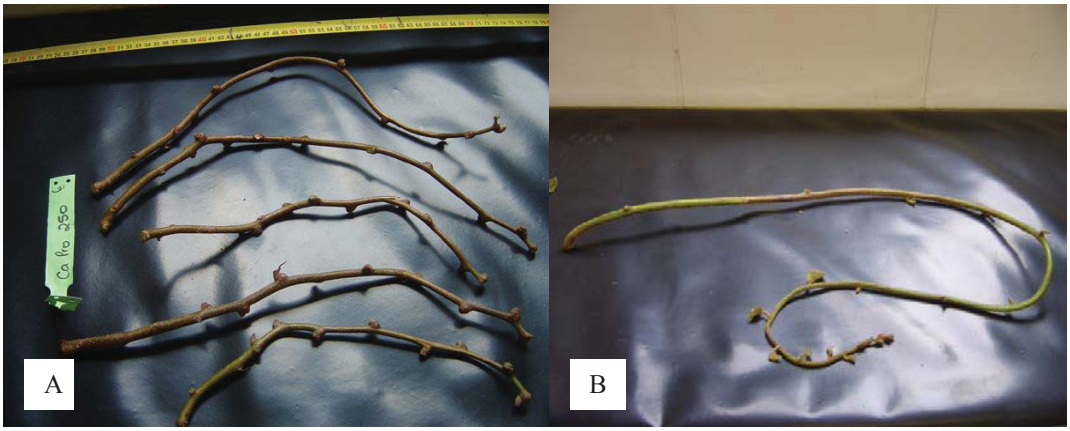

Figure 6.11. Shoots of *Actinidia deliciosa* 'Hayward' treated with Ca-pro 250 mg L^{-1} (A) and a shoot treated with GA_3+GA_{4+7} 500 mg L^{-1} (B) on the final harvest day (8th Feb, 2010) displayed to measure internodes. Internodes of shoots treated with Ca-pro 250 mg L^{-1} are fully elongated (growth terminated); for GA 500 mg L^{-1} shoots still exhibiting elongation.

6.3.8 Treatment effect on leaf specific weight (SLW)

Shoots treated with GA 1000, 2000, 4000 mg L^{-1} had higher specific leaf weight (g/m^2) compared with GA 500 mg L^{-1} (Figure 6.12). Shoots treated with PBZ 600, PBZ 400, Ca-Pro 250 and 350 mg L^{-1} foliar sprays did not differ significantly from the control (Figure 6.12). SLW of control shoots was greater (*P=0.03*) compared with those treated with GA 500 mg L^{-1}. Of all the treatments, GA 2000 and 4000 mg L^{-1} produced leaves with the highest SLW, which was greater than PBZ 400, 600 mg L^{-1} and Ca-Pro 250 and 350 mg L^{-1} (*P≥0.001*). For the shoots treated with GA 4000 mg L^{-1}, the SLW was significantly more compared with those treated with GA 1000 mg L^{-1} (*P=0.03*).

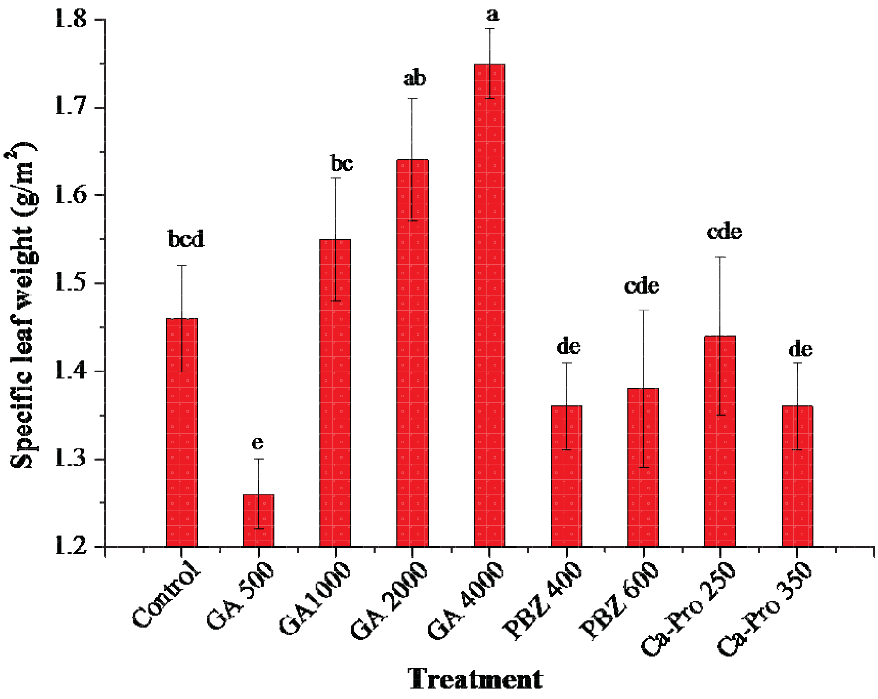

Figure 6.12. Effect of gibberellins (GA_3 + GA_{4+7}) at 0 (control), 500, 1000, 2000 and 4000 mg L^{-1}; anti-gibberellins: PBZ and Ca-Pro 0, 400, 600 and 0, 250, 350 mg L^{-1}, applied fortnightly from October, 2009 to February, 2010 on mean specific leaf weight of *Actinidia deliciosa* 'Hayward'. Vertical bars are the standard error. Columns with same letter are not significantly different at P≤0.05 using the multiple LSD test.

6.3.9 Treatment effect on the number and length of sylleptic axillary shoots formed on lateral shoots

Axillary bud release was promoted on lateral shoots (proleptic shoots) treated with gibberellins at high concentrations (Figure 6.13). The number of axillary buds activated on the shoots treated with 2000 and 4000 mg L^{-1} were 25 and 35 respectively, compared with five for control shoots and ten for 1000 mg L^{-1} GA. Gibberellin treatment at 500 mg L^{-1} reduced axillary shoot formation compared with the control. PBZ 400 and 600 mg L^{-1}, Ca-Pro 350 mg L^{-1} and GA 500 mg L^{-1} reduced sylleptic axillary shoot formation compared with the control and all other gibberellin treatments. Ca-Pro 250 mg L^{-1} inhibited axillary shoot formation and only a few axillary buds were released with Ca-Pro 350 mg L^{-1} with an average length similar to the controls (Figure 6.14). GA 2000 mg L^{-1} promoted longer sylleptic axillary shoots than GA 4000 mg L^{-1} however, GA 4000 mg L^{-1} promoted a higher number of axillary shoots compared with GA 2000 mg L^{-1} (Figures 6.7 and 6.8).

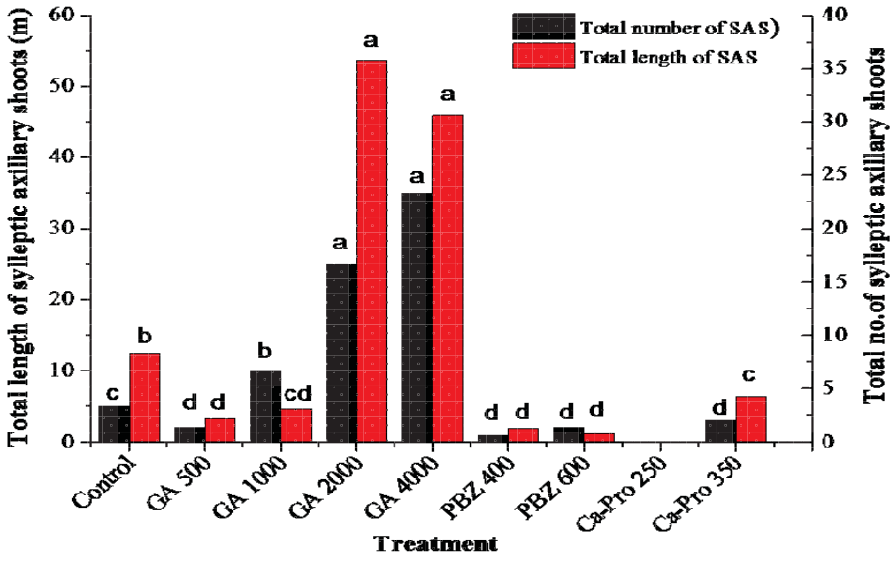

Figure 6.13. Effect of gibberellins (GA_3 + GA_{4+7}) at 0, 500, 1000, 2000, 4000 mg L^{-1}, anti-gibberellins; PBZ at 0, 400, 600 and Ca-pro at 0, 250, 350 mg L^{-1}, on number and total length of sylleptic axillary shoots (SAS) formed on the proleptic shoots of *Actinidia deliciosa* 'Hayward'. Columns with the same colour and the same letter are not significantly different at P≤0.05 using the multiple LSD test.

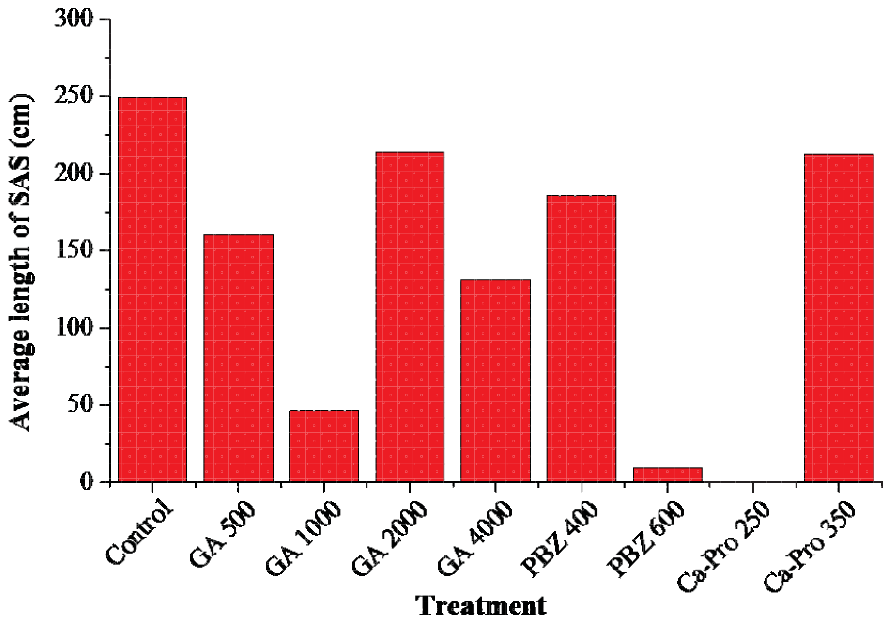

Figure 6.14. Effect of gibberellins (GA_3 + GA_{4+7}) at 0, 500, 1000, 2000, and 4000 mg L^{-1}, anti-gibberellins 0, 400, 600 PBZ and 0, 250, 350 mg L^{-1} Ca-pro, respectively, on the average length of sylleptic axillary shoots formed on the treated lateral shoots of *Actinidia deliciosa* 'Hayward' during 2009-2010.

6.4 Discussion

6.4.1 Effect of gibberellins on vegetative growth

6.4.1.1 *Growth of lateral shoots*

The apical meristem, which is involved in node formation and sub-apical meristem in node elongation (see Chapter 3, Section 3.1.1.1) were stimulated with gibberellins at 500 and 1000 mg L^{-1} and produced more nodes and elongated lateral shoots. With 2000 and 4000 mg L^{-1} the shoot apex was damaged, but either the sub-apical meristem was stimulated and/or cell elongation in the internodes increased. According to the dose/response growth curve (Figure 6.7), the shoot apical meristem of kiwifruit was highly responsive to 500 and 1000 mg L^{-1} and 2000 and 4000 mg L^{-1} was supra-optimal. The impairment of growth with GA 2000 and 4000 mg L^{-1} may not simply due to supra-optimal gibberellin levels but also due to the NovaGib formulation (unknown).

However, these high concentrations still stimulated internode elongation, either through an effect on sub-apical meristem activity and/or cell elongation. For gibberellins at 500 mg L^{-1} concentration, there was a significant increase in the mean node number and length. Therefore, gibberellins at this concentration could activate both apical and sub-apical meristems and increase mean node number and length (Table 6.2). Although node number increased with shoot length, with GA 500 mg L^{-1} there was no correlation (Figure 6.9B) as the internode length was not consistent. The reason for not having correlation with GA 500 mg L^{-1} maybe because shoots had not finished extending internodes by the time of destructive harvest of shoots. For example, the shoot with 35 nodes has shoot length ranging from 200-450 cm (Figure 6.9B). Therefore, with the same node number a few shoot were longer due to longer internode, thereby the mean internode length with GA 500 mg L^{-1} was significantly longer (Table 6.2) compared with the control. With gibberellins at 1000 mg L^{-1}, there was a significant increase in the mean length of lateral shoot and no increase in the mean node number (Table 6.2), but the individual shoot length increased as the node number increased with a good correlation (R^2=0.67) between node number and shoot length (Figure 6.9C). In Chapter 5 also, it was observed gibberellins at 800 mg L^{-1} did not increase the mean node number when compared with the control, but in fact, reduced it (see Chapter 5; Table 5.1). Therefore, even 800 mg L^{-1} for potted two-year-old kiwifruit vines may be slightly above optimal levels. It appeared the gibberellins at slightly higher than optimum concentration (800 and 1000 mg L^{-1}) stimulated sub-apical meristem rather than apical meristem. On the other hand, gibberellins at higher concentrations 2000 and 4000 mg L^{-1} even in the absence of apical meristem stimulated sub-apical meristem and increased the mean lateral shoot length. The significant increase in SCA of lateral shoot with gibberellins (Figure 6.8) may be due to increased cambial activity, as it was reported that cambial activity was increased with gibberellin in woody plants, (Bradley and Crane, 1957) in angiosperms (Digby and Wareing, 1966) and *Pisum sativum* stems (Arney and Mancinelli, 1966).

Ross et al. (2000) reported that IAA from the apical bud activates gibberellin biosynthesis in the elongating internode. Activity of applied gibberellins on the elongation of internode without interaction with exogenous auxins was observed in this

experiment, supporting the findings of Barratt and Davies (1997) with pea plants. The commonly held belief was that excised segments are not gibberellin responsive and that they require IAA to cause an elongation response (Brian and Hemming, 1958; Tanimoto et al., 1967; Ockerse, 1970). Contrary to this common belief, Baratt and Davies observed expansion segments of all genotypes of pea responding to gibberellins. It was also found that GA treated segments have a three-fold increase in endogenous IAA content. Therefore, GA response may be correlated with endogenous IAA content. It was also found that the GA response in the internode elongation correlated with the age of the internode decreasing as the endogenous IAA levels decreased (Barratt and Davies, 1996). In this study, although there was no intact apical bud for the supply of auxins in the high GA treatments, GA promoted elongation of the internodes. Therefore it is possible that internode elongation with GA concentration at 2000 and 4000 mg L^{-1} may be due to:

(1) Availability of endogenous auxin during expansion of internode;

(2) Gibberellins might have increased endogenous IAA levels during the period of expansion, or

(3) Only gibberellins are required for internode elongation in kiwifruit stem tissue.

In celery plants, foliar sprays of gibberellins appeared to increase the content of endogenous auxin and gibberellins (Kato and Ito, 1962). It was also mentioned that the increase in IAA levels was due to a decrease in IAA oxidase activity due to GA treatment (Galston and Purves, 1960). Therefore, elongation of internodes with gibberellins at 2000 and 4000 mg L^{-1} even in the absence of functional shoot apical meristem (SAM), may be due to increase in endogenous IAA levels due to exogenous gibberellins.

6.4.1.2 *Sylleptic axillary shoots (SAS)*

In this study, as GA concentration increased the mean number of axillary bud released was significantly increased (Figure 6.13). The increased GA concentrations also

damaged the apical part of the shoot. The number of SAS produced for GA 4000 and 2000 mg L^{-1} foliar sprays of gibberellins was higher compared with GA 1000 mg L^{-1}, that in turn produced more compared with GA 500 mg L^{-1}. GA 500 mg L^{-1} gave the greatest lateral shoot elongation (Figure 6.4; Table 6.2), but did not increase SAS formation. As this concentration was not supra-optimal it was less likely to have damaged shoot tips, hence no SAS formation. In Chapter 2 and 5, it was observed that gibberellin foliar sprays promoted axillary bud activation and produced more sylleptic axillary shoots in composite apple trees on dwarfing rootstocks and kiwifruit vines respectively. In this chapter, gibberellins at very high concentration (2000 and 4000 mg L^{-1}) promoted axillary bud activation, presumably due to loss of apical dominance because of damaged shoot apex. Although the number of axillary shoots at these high concentrations of gibberellins was high, their average length was similar to those of control shoots. Therefore, the average length of axillary shoots for the untreated control and gibberellins at 2000 mg L^{-1} was approximately similar (Figure 6.14). In this study, GA at supra-optimal concentrations could promote SAS if apical dominance was removed due to damage to the apex. With high GA concentration, shoot apical tissue was damaged and SAS growth promoted. The removal of apical dominance could have increased axillary bud activation and the additional supply of GA stimulated elongation.

6.4.2 Effect of Anti-gibberellins

6.4.2.1 *Effect of paclobutrazol (PBZ) on vegetative growth*

For kiwifruit vines, when PBZ at 400 and 600 mg L^{-1} was applied to young stem and shoot tips, there was no significant reduction in growth compared with the control (Figure 6.4). PBZ 4000 mg L^{-1} in Chapter 5 also did not significantly reduce the total shoot length ($P=0.1$) of recently rooted kiwifruit 'Hayward' cuttings (Chapter 5; Table 5.2).

Paclobutrazol (PBZ), an anti-gibberellin reduced growth of a broad range of species (Hedden and Graebe, 1985; Lever, 1985). It is a triazole derivative and has been shown to inhibit growth on apple trees (Tukey, 1982; Quinlan and Richardson, 1983; Williams,

1984). It controlled vegetative growth and had no direct chemical effect on fruit size (Williams, 1984). The use of PBZ showed considerable promise for controlling excessive vegetative growth without seriously reducing fruit size or quality in apples and pears (Williams and Edgerton, 1982). According to Steffens and Wang (1985), growth reduction by PBZ was due to lack of gibberellin synthesis, since, PBZ did not block the action of either endogenous or applied gibberellins. In gibberellin biosynthesis, paclobutrazol prevents the oxidative step from ent-kaurene to ent-kaurenoic acid (Figure 6.1) without affecting the formation of ent-kaurene, (Hedden and Graebe, 1985) by inhibiting kaurene oxidase, a cyt P-450 oxidase, thus blocking the oxidation of kaurene to kaurenoic acid (Dalziel and Lawrence, 1984). The effect of growth inhibitors depends on plant responsiveness, uptake and translocation (Rademacher, 2000). To suppress gibberellin biosynthesis, a threshold concentration of PBZ must be maintained (Lever, 1985) in the shoot apex. Quinland and Richardson (1983) showed that the concentration of PBZ was reduced as a result of distance covered by transport due to rapid shoot extension growth. As kiwifruit shoots extend more rapidly than apple shoots (on average 3-7 mm day^{-1} for apple trees (Chapter 2) 20-40 mm day^{-1} for kiwifruit observed during this study), one can assume that the growth inhibiting substance PBZ was more rapidly depleted.

In studies with peach seedlings grown in nutrient solutions, it was observed that in leaves and young stems the physiologically active PBZ breaks down rapidly, though it was stable in the nutrient solution (Early and Martin, 1988). Therefore, the breakdown of PBZ is rapid and is highest in the most physiologically active areas of the plant, the leaves and the young stem. Given this explanation, the reason that at 400 and 600 mg L^{-1} PBZ did not reduce shoot length of kiwifruit in mature vines may be because of lower concentration and/or higher degree of breakdown in young and physiologically active parts of vine, the leaves and young stems, or may be due to accumulation of ent-kaurene, which might have led to the synthesis of bioactive gibberellins by a different pathway. As a result there was no difference between control shoots and shoots treated with PBZ 400 and 600 mg L^{-1} (Figure 6.4). The concentration used in this experiment was much less compared with that used in the experiments in Chapter 5. With this high concentration, PBZ 4000 mg L^{-1} there was no reduction in the growth compared with

the untreated control vines significantly (see Chapter 5; Table 5.2). Lateral shoots treated with foliar sprays of PBZ 600 mg L^{-1} started termination very early but, there was no significant difference in the final shoot length compared with the control, therefore, it may be suggested that PBZ needed to be applied multiple times to arrest the re-growth due to its rapid degradation (Rademacher, 2000).

6.4.2.2 *Effect of Prohexadione-Calcium (Ca-Pro)*

Prohexadione-calcium (calcium 3,5-dioxo-4 propionyl-cyclohexane-carboxylate) reduces vegetative growth by blocking two oxoglutarate-dependant dioxygenases that catalyse the 3-beta hydroxylation of GA_{20} to GA_1 and the conversion of inactive GA_{20} into highly active GA_1, (Evans et al., 1999; Rademacher, 2000), thereby reducing levels of highly bioactive GA_1 and accumulating its precursor GA_{20} (Rademacher, 2000). Apple trees treated with 250 mg L^{-1} Ca-Pro showed a reduction in the final length of shoots, internodes and node number, and endogenous hormone analysis showed reduction in gibberellin and auxin concentrations (Ramirez, 1998). This re-inforces the idea given in a previous paragraph that gibberellins may increase endogenous auxin levels. It was also mentioned, in such treated apple shoot apices, GA_9, GA_{20}, and GA_{51} were detected compared to the bio-active gibberellins GA_1, GA_4 and GA_7 in control trees. This suggests that reduction in the shoot growth for Ca-Pro 250 mg L^{-1} treated shoots of kiwifruit vines may be due to the lack of bio-active GA_1 (Figure 6.4 and Table 6.2) acting on the sub-apical meristem. Although there was no significant effect of Ca-Pro 250 and 350 mg L^{-1} on the mean node number, they both reduced the mean internode length significantly and, with Ca-Pro 250 mg L^{-1} there was significant decrease in the mean shoot length (Figure 6.4). Moreover, the correlation between node number and lateral shoot length for Ca-Pro 250 and 350 mg L^{-1} was good with R^2 value 0.74 and 0.94 respectively (Figure 6.9F). The early termination of shoots of kiwifruit with Ca-Pro treatment (Table 6.1) may also be due to the absence of biologically active gibberellins. Therefore, for kiwifruit, with Ca-Pro 250 mg L^{-1} treatment, the internode elongation was significantly reduced and the production of neoformed nodes was reduced (16) compared with the control (22) leading to early termination (Table 6.2). As

young leaves are the source of IAA supply (Ljung et al., 2001), and when neoformation of leaves was slow, the IAA supply to the SAM to produce leaf primordia may be reduced, leading to termination of growth. Thus, anti-gibberellin Ca-Pro acted mostly on sub-apical meristem. However, hormonal analysis would be recommended to ascertain the reason for early termination of kiwifruit shoot with Ca-Pro foliar sprays. Therefore, from these results, it was understood that if biosynthesis of biologically active gibberellins was inhibited by Ca-Pro, lateral shoot length was reduced by reducing mean internode length. As Ca-Pro affect the late stages of gibberellin biosynthesis (Figure 6.1), if the genes that control late stages of gibberellin biosynthesis could be suppressed, reduction in the vigour of kiwifruit vines may be possible by gene manipulation.

6.5 Summary

The results of this experiment indicated that high vegetative vigour of kiwifruit vines depended on sufficient amounts of bioactive gibberellins stimulating the activity of both the apical and sub-apical meristem. This effect of gibberellins affected node number, internode length, date of termination, type of shoot: short, medium and long and, number of sylleptic axillary shoots.

The prolonged growth of lateral shoots with gibberellin foliar sprays compared with control shoots showed that for the continuity of apical and sub-apical meristem activity, there is a requirement of gibberellins. Whether the shoot was predestined to form short shoots and terminate with only preformed nodes or whether it develops into a long shoot, was not known as the emerging shoot for this experiment were selected soon after the bud burst. In general, for a kiwifruit vine, about 40% develop as short shoots, 40% as medium and the remaining as long shoots. However, with gibberellin foliar sprays all shoots developed into long shoots (Figure 6.5). Presumably foliar sprays of gibberellins on potential short and medium shoots stimulated both apical and sub-apical meristems to produce more nodes and longer internodes.

In this study, the anti-gibberellin PBZ did not reduce growth compared with the control, but Ca-Pro 250 mg L^{-1} did. There was a significant reduction in the length of lateral shoots and axillary bud release and, early termination of lateral shoots with Ca-Pro 250

mg L^{-1}. Thus, the vigorous growth of kiwifruit shoots may be due to bioactive gibberellins. Eighty per cent of the shoots treated with Ca-Pro 250 mg L^{-1} terminated very early (12th Jan, 2010) and 100% shoots treated with gibberellin foliar sprays (500 and 1000 mg L^{-1}) had not shown any symptoms of termination on 8th February, 2010. From this it can be suggested that early termination with anti-gibberellin Ca-Pro 250 mg L^{-1} may be due to lack of stimulation of the apical meristem by bioactive gibberellins. However, this could be confirmed only after hormone analysis of xylem sap of the kiwifruit stem, which is recommended for future studies.

Chapter 7 General discussion and conclusions

7.1 The role of gibberellins in vigour control in apple

7.1.1 Introduction

An important objective of this thesis was to elucidate how gibberellins are involved in vigour control of composite apple trees. The published literature revealed that the reduction in scion vigour imposed by dwarfing rootstocks may result from modified transport of growth hormones (Rogers and Beakbane, 1957; Lockard and Schneider, 1981; Webster, 1995; Kamboj et al., 1997; van Hooijdonk et al., 2010). The stem of dwarfing rootstocks reduced the velocity of basipetal transport of radio-labelled IAA (Soumelidou et al., 1994a) and basipetal auxin supply from apical meristem to the root system is of central importance in regulating rootstock-induced scion dwarfing (van Hooijdonk, 2009). The dwarfing effect of 'M.9' rootstock could be mimicked by applying an auxin transport inhibitor, NPA, to the stem of normally vigorous 'Royal Gala' rootstock (van Hooijdonk, 2009). For example, the decreased IAA supply to root system, either by 'M.9' rootstock (Figure 7.1) or by NPA application, reduced mean primary shoot length and sylleptic axillary shoot formation and increased the proportion of shoots that terminated growth early in the growing season. Promotion of earlier shoot termination of the scion and formation of fewer SAS, imposed by 'M.9' or NPA applied to the rootstock stem, could be reversed with foliar sprays of BAP and gibberellins, which either prevented shoot termination, or reinstated sylleptic axillary shoot formation, respectively. This evidence suggests that dwarfing of scion may be controlled by shoot-root-shoot signalling of endogenous hormones, as suggested by van Hooijdonk (2009) - IAA from shoot to root and cytokinin and gibberellin from root to shoot in the xylem sap. Although it appears there is an important role for gibberellins in stimulating the vegetative growth of apple, its role in rootstock-induced dwarfing of the scion grafted onto a dwarfing rootstock has been questioned by scientists at East Malling (Webster, 2004; East Malling, 2005), albeit, previously it was reported that exogenous GA_3 stimulated apple shoot apical meristem (SAM) to increase node production and the proportion of annual shoots that were actively growing late in the

season (Luckwill and Silva, 1979). In support of the hypothesis that scions on a dwarfing rootstocks are deficient in gibberellins, the SAM of apple scions on dwarfing rootstocks are widely reported to increase the proportion of shoots that terminate growth early (Swarbrick, 1929; Colby, 1935; Tubbs, 1951; Avery, 1969; Robitaille and Carlson, 1976). Therefore, the main objective of this thesis was to further evaluate whether gibberellins may be important in vigour control of composite apple trees grafted onto rootstocks of different vigour (Chapter 2 and 3) and to elucidate whether similar shoot-root-shoot hormonal signalling mechanisms suggested for apple were applicable to vigour regulation in kiwifruit vines (Chapter 4, 5 and 6) as summarised below (Figure 7.1 and 7.2). The role of gibberellins was mainly studied through its activity on apical and sub-apical meristem of shoots involved in the production of new metamers and internode elongation respectively and also in the process of termination of active growth.

7.1.2 Dwarfing rootstock and gibberellin effect

7.1.2.1 *Primary shoot*

Growth on dwarfing rootstocks (‘M9RG’ and ‘M9M9’) in the first year during spring to mid-summer was found, rather surprisingly to be fast compared with the scions on vigorous rootstocks (‘RGRG’ and ‘RGM9’) (Figure 2.2). The longer primary shoots produced on ‘M.9’ during this period (Oct 16th, 2007 to Jan 12th, 2008) was due to longer internodes rather than an increase in node number, which was due to more sub-apical than apical meristematic activity. During this initial period, IAA transport from the shoot may not be decreased, and as a result gibberellin supply may be adequate to stimulate cell division in the internode for elongation, but insufficient to stimulate apical meristem activity for the production of new metamers. This view is supported by the observations that the bark of ‘M.9’ metabolises more IAA than all vigorous rootstocks (Martin and Stahly, 1967; Gur and Samish, 1968) and the rootstock stem reduced polar transport of radio-labelled IAA (Soumelidou et al., 1994a; Kamboj, 1996), particularly as shoot growth slowed late in the season (Kamboj et al., 1997). Therefore, the longer primary shoots in the initial period was presumably due to sufficient gibberellin supply from root to shoot, because IAA transport from shoot to root may not be reduced

during spring to midsummer. Similar faster growth of 'RG' scion on 'M.9' rootstocks during the early period of growth was also reported under the same climatic conditions of Manawatu, New Zealand (van Hooijdonk, 2009).

From 12th January 2008 onward, there was a clear decrease in the growth rate of primary shoot and plastochron for scions on dwarfing rootstocks, resulting in a decrease in mean length of the primary shoot (Figures 2.8A and B, 2.9A and B and 2.10A and B). Thus by the end of the first year growth from grafting, the length of primary shoots of scions on 'M.9' rootstocks reduced significantly, which was partly due to a slower rate of node production and partly due to early termination. However, the growth of 'RG' scion on 'M.9' rootstock may vary depending on the location (e.g. Manawatu versus Hawkes Bay).

There is a high degree of plasticity in the growth of 'RG' primary and secondary shoots on 'M.9' rootstock during the first year of growth from grafting. For example, studies conducted by Seleznyova et al., (2007) reported that there was no difference in the architecture of 'RG' scion on 'M.9' and 'MM106' rootstocks at growth cessation in the first year of growth from propagation. The authors found that the growth of scion on 'M.9' rootstock was reduced in year two of growth from grafting, after increased flowering of the scion. However, van Hooijdonk, (2009) reported that 'M.9' rootstock significantly decreased the mean total shoot length and node number of the 'RG' apple scion by the end of the first year of growth from grafting. He also mentioned that the first occurrence of rootstock-induced dwarfing of the scion was a vegetative change, followed by flowering on the scion in the spring of year two. It justified that these differences in the growth of scion on 'M.9' rootstock was due to a warmer climate in Hawke's Bay. On the other hand, in another experiment, very similar results to the results of Seleznyova (2007) were mentioned regarding primary shoot length by van Hooijdonk in Manawatu, however, total growth was reduced, which was different from Seleznyova results. In the results reported in Chapter 2, it was observed that the newly grafted 'RG' scion on 'M.9' decreased the total shoot length significantly compared with that growing on 'RG' rootstock from the middle of year one from grafting. Thus these studies together indicate that the growth of 'RG' primary shoot on the 'M.9' rootstock exhibits a high degree of plasticity within the first year of growth from grafting.

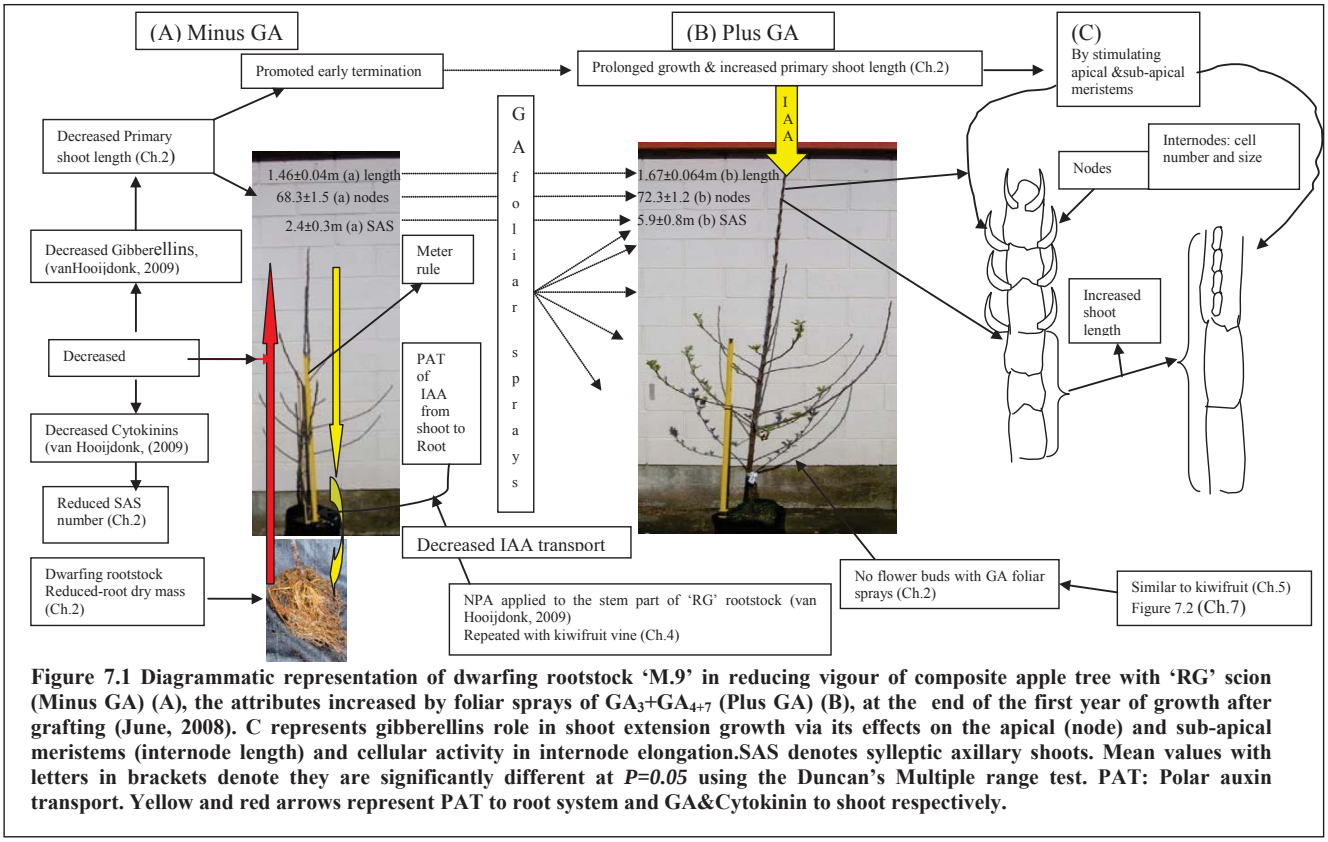

Figure 7.1 Diagrammatic representation of dwarfing rootstock 'M.9' in reducing vigour of composite apple tree with 'RG' scion (Minus GA) (A), the attributes increased by foliar sprays of GA_3+GA_{4+7} (Plus GA) (B), at the end of the first year of growth after grafting (June, 2008). C represents gibberellins role in shoot extension growth via its effects on the apical (node) and sub-apical meristems (internode length) and cellular activity in internode elongation.SAS denotes sylleptic axillary shoots. Mean values with letters in brackets denote they are significantly different at *P=0.05* using the Duncan's Multiple range test. PAT: Polar auxin transport. Yellow and red arrows represent PAT to root system and GA&Cytokinin to shoot respectively.

Even though growth rate was initially faster on a 'M.9' rootstock, the large decrease later in the season resulted in significantly short primary shoots with less branching (SAS formation) (Figure 2.8). Slower mean growth rate of primary shoot for scions on 'M.9' rootstocks was observed even before the first sign of primary shoot termination (15/4/08) (Table 2.7).

Previous study provided the information that there were trends that, from February onwards, root growth was slower for 'M.9' compared with 'RG' rootstock and that,-slowing of root dry mass gain of 'M.9' root system was preceded by a large reduction in the basipetal transport of diffusible IAA from the primary shoot apex (van Hooijdonk, 2009). In this study (see Chapter 2; Section 2.4.5), it was reported that final mean dry weight of total root system of composite apple tree 'M9RG' was significantly reduced compared with that of 'RGRG' (Figure 2.18 and Table 2.14), which supports the view that the basipetal IAA transport from shoot to root may be reduced. These results were similar to previous findings that final dry weight of root system was reduced for 'M.9' (van Hooijdonk et al., 2011). Therefore, the decrease in the final shoot growth of scion on a dwarfing rootstock could be correlated with decreased root dry mass, which presumably resulted in decreased root-produced cytokinins and gibberellins from the 2nd week of January to the end of the growing season. Moreover, the reason for the initial faster growth of the scion on the dwarfing rootstock may be due to sufficient supply gibberellins from root to shoot as there was no response to GA (Nov) foliar sprays (Nov 2007-Jan 2008) (Figure 2.11) during this early growth period. Thus scions on dwarfing rootstocks during the initial period (October, 2007 to 2nd week of January, 2008) may have enough gibberellins to stimulate cell division in the sub-apical meristem to increase internode length. Greater effect of GA application during late growth (from midsummer; 12th January to 15th May) may indicate that scions on dwarfing rootstocks were GA deficient. Several reports are available on the effect of GA to stimulate shoot extension growth of apple shoots (Robitaille and Carlson, 1976; van Hooijdonk et al., 2010). GA_{19} appeared to be an important transport form present in the xylem sap of apple (Motosugi et al., 1996), which could be converted to bioactive GA_1 (Yamaguchi, 2008) by shoot apices of the scion. The levels of GA_{19} increased with increasing rootstock vigour (van Hooijdonk et al., 2011). Furthermore, they found lower levels of GA_{19}; coincide with a higher percentage of shoots that terminated growth earlier.

It would be reasonable to measure root growth and root-produced hormones, gibberellins periodically to correlate with the scion growth on a dwarfing rootstock in comparison with that on a vigorous one. It would be very useful to study the relationship between root growth of different rootstocks under different environmental conditions and rate of growth of the shoot. It would also be useful to observe rates of IAA transport and see if that correlates with IAA oxidases and phenols in the rootstock bark. Although, a few studies on apple root-growth are available (Cripps, 1970; Psarras et al., 2000), as apple root-growth and its seasonal patterns were affected by rootstock genotype (Rogers, 1939) and presumably would differ across different climatic regions (Psarras et al., 2000), it would be useful to study root-growth during the early growth of the scion on a dwarfing rootstock under the same climatic condition where faster growth was observed (Manawatu, New Zealand). However, it would be necessary to check root shoot ratios long term since it takes several years for allometric ratios to become established.

Although the levels of endogenous gibberellins were not quantified in this study, the significant response of primary shoot growth to gibberellin foliar sprays during late growth (from midsummer; 12th January to 15th May), in stimulating SAM activity in increasing the final node number and sub-apical meristem activity in increasing internode length significantly and, thereby increasing the mean primary shoot length (Figure 2.11; Table 2.13, 2.14; Figure 7.1), showed that the transport of gibberellin from root to shoot was lower for scion on dwarfing rootstock without GA foliar sprays. In Chapter 3, it was reported that gibberellins increased shoot length by stimulating both apical (node production) and sub-apical (internode elongation) activity so that cell number as well as cell length increased. Consequently, deficiency of bioactive gibberellins in SAM for scion on 'M.9' rootstock might have led to the early termination of shoots on the scion (Table 2.7).

7.1.2.2 *Primary shoot termination*

As indicated in Figure 7.1, reduced total length of the shoots of scions on dwarfing rootstocks without GA foliar sprays due to effects on both the primary and sylleptic shoots, was partly due to a slower growth rate from midsummer and partly due to early termination (Figure 2.8A&B; Table 2.7). The termination of shoot apical meristems on

the primary shoots was delayed with gibberellins (GA_3+GA_{4+7}) foliar sprays (2.4.1.3 and Table 2.7). Thus, gibberellin foliar spray effect for 'RG' scions prolonged the primary shoot growth by activating both apical and sub-apical meristem, which increased both node number and internode length (Figure 7.1). The bigger effect of gibberellins for 'RG' on 'M.9' rootstock showed that the scion on 'M.9' was GA deficient. In Chapter 3, the role of gibberellins in internode elongation regarding its contribution for cell division and/or cell elongation was observed. Shoot apical meristem requires proper balance between lateral organ initiation from its peripheral zone (PZ) and indeterminate growth at its centre (CZ) (Chapter 3, Section 3.1.1.3).

Therefore, the early termination seemed to be associated with reduced gibberellin which was also associated with reduced supply of IAA as GA is thought to be involved in the regulation of IAA synthesis (Law, 1987). Moreover, other hormones such as abscisic acid (ABA), which inhibit root growth (Pilet, 1977) and stem elongation (Robitaille and Carlson, 1971) and inhibit the response of plant to some growth hormones especially gibberellin (Kefeli and Kadyrov, 1971) and also inhibits polar auxin transport in excised tissue (Basler and McBride, 1977). Therefore, ABA involvement in the dwarfing effect of apple rootstock may be possible because, high concentrations of ABA-like substances were found in stems of some dwarfing apple rootstocks (Kamboj,et al., 1999b), during shoot growth cessation and terminal bud formation.

7.1.2.3 *Sylleptic axillary shoots (SAS)*

The 'M.9' rootstock reduced the formation of sylleptic axillary shoots (SAS) on the newly grafted 'RG' scion when compared with 'RG' rootstock control (Table 2.9). These results, similar to previous studies (van Hooijdonk, 2009), found that 'M.9' decreased the formation of SAS formation during the first year of growth from grafting. This decreased formation of SAS by 'M.9' rootstock in the first year of growth from grafting is a very important step that leads to reduced total shoot growth for scion on 'M.9' rootstock compared with 'RG' rootstock control. Moreover, all dwarfing rootstock types did not affect branching pattern in the first year (Fazio and Robinson, 2008) as the 'Brookfield Gala' scion grafted on 'Geneva 16' (G.16) dwarfing rootstock

developed many more secondary shoots by the end of the first year of growth in a tree nursery compared with the same budded on 'M.9' rootstock. But in this study, 'M.9' rootstock was observed to reduce SAS and hence final tree architecture.

As the node number of SAS increased shoot length increased regardless of the rootstock (see the slopes of regression lines in Figures 2.15 A, C and E for 'M9RG' and B, D and F for 'RGRG'). Thus, irrespective of the rootstock type, sylleptic shoots with similar node number had a very similar shoot length. With GA, the architecture of 'RG' scion on 'M.9' rootstock was changed and became similar to the scion on 'RG' rootstock with or without GA. This result with the 'RG' scion was in direct contrast to that with a 'M.9' scion (see Section 7.2.2).

7.1.2.4 *Termination of SAS*

The sylleptic shoots of untreated scions had terminated very early during January, 2008 even before primary shoots had terminated (Table 2.10) but, with gibberellin foliar sprays the growth of sylleptic shoot apical meristem was prolonged, thus forming longer sylleptic shoots with more nodes and longer internode as the growth continued until the end of April, 2008 (Figure 2.15). Thus, the effect of gibberellins on both primary and secondary shoot was similar in affecting node formation and internode elongation. The total scion growth for 'RGRG' was significantly higher compared with 'M9RG' (Figure 2.17) and similar to 'M9RG' treated with gibberellins. Therefore, as the vigour reduction of scion on 'M.9' rootstock was reversed by foliar sprays of gibberellins, the scion vigour control on dwarfing rootstocks may be due to gibberellin deficiency. The root system dry weight with GA foliar sprays was decreased compared with that of without GA foliar sprays. Although it is well known that dwarf genotypes are often gibberellin deficient mutants (Ross et al., 1997), it is usually claimed that the effect of dwarfing rootstocks cannot be explained simply as a gibberellin deficiency. In this work, using exogenous gibberellins the evidence is clear that lack of gibberellins is a major factor affecting growth of SAM and the time of termination.

7.1.3 Growth of 'M.9' as a scion - gibberellin effect

7.1.3.1 *Primary shoot*

Interestingly, it was found that not only the rootstock but also the scion-rootstock interaction was responsible for scion vigour control for composite apple trees. In Chapter 2, there was no difference in primary shoot length (Table 2.13), node number and number of SAS and the time of termination for 'M.9' scion on dwarfing ('M9M9') or on a vigorous rootstock ('RGM9') (Figure 2.10 and 11; Table 2.13). During early growth, the shorter primary shoots of 'M.9' scion on 'RG' rootstock with shorter internodes compared to those on dwarfing rootstock (Figure 2.2) indicates that even if transport of IAA from 'M.9' rootstock was rapid early in the season, nevertheless the primary shoot growth of 'M.9' scion was slow, maybe because of its inability to convert root produced gibberellins (GA_{19}) to bioactive gibberellins (GA_1). This showed that the growth of 'M.9' scion was not changed due to vigorous rootstock, but the intrinsic nature of 'M.9' was to grow slowly, presumably because of poor gibberellin inter-conversion rates. However caution needs to be exercised in the interpretation of these results as observations were made using one year-old composite apple trees, but vigorous rootstocks may grow slowly in the first year of development and may take a few years to express their full vigour potential.

During late growth i.e., from midsummer to the end of the growing season (12th Jan to June 5th 2008), the growth rate of scion 'M.9' appeared to slowly increase on 'RG' rootstock (Figure 2.2). However, at the end of the season the mean primary shoot length of 'M.9' on 'RG' rootstock was not significantly different from that of 'M9M9' (Table 2.4). This indicates that whether IAA transport was slow or fast, the 'M.9' scion may have little ability to convert the root produced gibberellins (GA_{19}) to bioactive GA_1. The crucial step for all these changes in gibberellin levels and in the architecture is auxin transport from shoot to root. Therefore, it would be worthwhile to compare rates of IAA transport from shoot to root and check whether that correlates with IAA oxidases and phenols. Although, Soumelidou, (1994a) assessed auxin transport capacity in relation to the dwarfing effect of apple rootstocks and found the lower, the technique used was paper chromatography of the ethanol extract obtained at the end of the

transport period. Therefore, it is recommended to quantify IAA transport from 'M.9' scion to 'RG' rootstock periodically to ascertain whether GA_{19} levels in xylem sap correlates with IAA transport and whether 'M.9' scion has capacity to convert them into bioactive gibberellins (GA_1).

Finally, results of this thesis on rootstock-induced scion dwarfing support the findings of van Hooijdonk (2009) who found basipetal auxin transport as the mechanism behind dwarfing in apple. Therefore, the stem part of the rootstock plays the first role in decreasing IAA transport, but how much the root system of the dwarfing rootstock plays a role in the dwarfing mechanism is not known. In order to know the importance of shoot versus root in rootstock dwarfing mechanism, a 'RG' scion could be grafted directly onto the root system of a dwarfing rootstock 'M.9' without any piece of stem above the root system, although it may be rather difficult to obtain a graft union.

7.1.3.2 *'M.9' Scion and gibberellin effect on primary shoot termination*

The vigorous 'RG' rootstock could not prolong primary shoot growth of 'M.9' scion (Table 2.7). This shows the probable intrinsic nature of 'M.9' scion in maintaining its dwarf nature. The significant rootstock × gibberellin interaction for 'RG' scion on 'M.9' rootstock ('M9RG' composite trees) compared with 'RGRG' helps confirm that scions on 'M.9' rootstocks were gibberellin deficient, whereas the 'RG' rootstock supplied enough gibberellins to 'RG' scions for 'RGRG' (see Chapter 2; Section 2.4.4.2; Table 2.13). For the 'M.9' scion, there are two likely reasons for the slow growth on a vigorous rootstock:

1) Only low levels of auxin were transported from the scion to the rootstock and therefore there was a reduction in root produced gibberellins.

2) Gibberellin precursors from the vigorous rootstock may not have been converted to bioactive gibberellins in 'M.9' scions, which may be due to lack of *GA_{20} oxidase* enzymes (Bulley et al., 2005). However, it is important to note that the slow growth of the 'Greensleeves' scion used by Bulley et al., could be reversed with GA_3 unlike the slow growth of an 'M.9'.

When gibberellins were applied, the 'M.9' scions were still shorter compared with 'RG' scions and the effect of gibberellins was less for 'M.9' scions compared with 'RG' scions (Table 2.12&2.13; Table 2.4). Even with gibberellin foliar sprays, the growth of 'M.9' shoot on both rootstocks was slow and terminated early. This again, shows probably the intrinsic natures of 'M.9' scion. GA foliar sprays were not effective, which may be due to low levels of IAA in the shoot of 'M.9' scion. The 'M.9' scion, which was assumed to be acting like the 'Greensleeves' scion in Bully's study with suppressed gene encoding *GA_{20} oxidase* did not behave in a similar way with gibberellin foliar sprays. The dwarfed 'Greensleeves' scion, was reversed with the application of GA_3 (Bulley et al., 2005). All these observations may lead to new avenues for further future research.

1) As the mechanism by which the rootstock bark of 'M.9' may act to reduce the basipetal auxin transport to the root system still remains largely unknown, it would be worthy of investigating IAA efflux carriers at the plasma membrane (Estelle, 2001) and the rate of IAA metabolism of various rootstocks under different growing environments, which may also explain the plasticity of the growth of primary shoot of the scion on dwarfing rootstock in the first year from grafting (Seleznyova et al., 2007; 2008).

2) The identification of genes encoding for dioxygenases enzymes, required for the conversion of GA precursors to bioactive gibberellins in the SAM of 'M.9' scion is needed, as the genetic expression in the SAM might have repressed gibberellin signalling by synthesising *GA_2 oxidase* at the base of the apical meristem which prevented GA transport from the nearest young leaves to apical meristem (Sakamoto et al., 2003);

3) As the effect of gibberellins was less for 'M.9' scions compared with 'RG' scions, it can be assumed that GA was not effective when IAA levels in the shoot of 'M.9' scions were low. Therefore, it would be interesting to apply GA and IAA together to the 'M.9' shoot.

7.1.3.3 *'M.9' Scion and gibberellin effect on formation of SAS*

For 'M.9' scions, mean number of spurs increased and mean number of SAS reduced, when compared with 'RG' scions, on both the rootstocks (Table 2.8). Although 'M.9' scion on 'RG' rootstock produced fewer SAS, the effect of 'RG' root system on sylleptic axillary shoot formation was still observed. With 'RG' rootstock, there was an increase in the mean number of SAS (*P=0.1*) for 'M.9' scion compared with 'M.9' on 'M.9' rootstock and gibberellin foliar sprays increased the number of SS for 'M.9' scions on both rootstocks (Table 2.9). The effect of gibberellin foliar sprays was more for 'M.9' scion on 'RG' compared with that on 'M.9' rootstock. However, the mean total shoot length for 'M.9' scions on both rootstocks was not significantly different when treated with gibberellin foliar sprays (Table 2.13). This was because of early termination of sylleptic shoots of 'M.9' scion even with GA foliar sprays (Table 2.10), suggesting that gibberellins could not stimulate SAM of SAS.

7.1.3.4 *'M.9' Scion and gibberellin effect on termination of SAS*

Axillary buds on primary shoot of 'M.9' scion on both rootstocks were released from apical dominance and produced spurs, which were short (< 25mm). These spurs terminated early without neo-formation of nodes and only small extension of internodes. In addition to decreasing the mean number of sylleptic shoots that formed per scion (Table 2.9), the 'M.9' scions decreased the final mean total shoot length (Figure 2.17), as more than 80% SAS terminated even after gibberellin treatment (Table 2.10). However, there was a good correlation between shoot length and node number (Figure 2.16A&B). Therefore, with gibberellin treatment, there was extension growth of sylleptic axillary shoots by the addition and elongation of nodes. However, maybe due to decreased IAA diffusion from SAM of 'M.9' shoot, foliar sprays of gibberellins did not stimulate apical and sub-apical meristem and prolong growth by adding new metamers and elongating internodes as much as for 'RG' scions but may also be due to differences in GA oxidase system.

Therefore, from the growth of primary shoot and its termination for composite apple trees resulted from reciprocal grafts between ‘M.9’ and ‘RG’ scions and ‘M.9’ and ‘RG’ rootstocks, it was shown that not only rootstock was responsible for dwarfing, but also the type of scion to which it was grafted. Thus as per the previous studies, it may be confirmed auxin supply from shoot was the critical point in shoot-root hormonal communication in dwarfing mechanism, which in turn decreased the root-produced cytokinins and gibberellin that reduced sylleptic axillary shoot formation and their further growth respectively. This observation also reinforced the ideas that: (1) ‘M.9’ stem tissues of the ‘M.9’ rootstock (the stem part of the rootstock) was important in promoting dwarfing (2) root system of the rootstock was responsible for producing gibberellin precursors, which need to be changed to bioactive gibberellins in the shoot after being transported to shoot through xylem.

7.1.4 Rootstock and gibberellin effect on formation of flower buds

The mean number of flower clusters per scion in the spring of the second year (October, 2008) after grafting (August, 2007) was greater for ‘RG’ scions on ‘RG’ rootstocks compared to ‘RG’ scions on ‘M.9’ rootstocks (Figures 2.20 and 2.21). Previous studies confirmed that the dwarfing apple rootstock increase the proportion of buds that are floral during the spring of year two (Hirst and Ferree, 1995; Seleznyova et al., 2005, 2007, 2008). The cessation of shoot growth was recognised as a prerequisite for the formation of flowers (Luckwill and Whyte, 1968; Swarbrick, 1929). Similarly, there was an increased floral precocity of the scion on ‘M.9’ rootstock (van Hooijdonk, 2009). In this study although there was a significant reduction in the total shoot length of shoot for ‘RG’ scion on dwarfing rootstock with early termination, yet increased floral precocity was not observed. The number of floral clusters produced for ‘RG’ on M.9’ rootstock was less compared witth ‘RG’ on ‘RG’ rootstock. Surprisingly, the vigorous composite apple tree ‘RGRG’ produced significantly more flowers compared with ‘M9RG’ (Figure 2.20 and 2.21).

Thus, the results of this study were interesting in that, it was found that dwarfing of the scion did not correlate with precocity of the scion with greater precocity being observed

in the vigorous composite tree ‘RGRG’. The vigorous growth of ‘RG’ scion on ‘RG’ was assumed to be due to the availability of sufficient gibberellins. In contrast, exogenous gibberellins in this study inhibited flowering. There were also reports, that showed application of GA_3 and/or a mixture of GA_3 and GA_{4+7} inhibited flowering in apple trees (Luckwill, 1970; Tromp, 1973, 1982; Luckwill, 1974). However, not all gibberellins are inhibitory and reportedly short-lived, rapidly metabolised GA_4 is not inhibitory (Looney et al., 1985). If exogenous gibberellins (GA_3+GA_{4+7}) inhibited flower formation for all composite trees (M9M9, RGM9; M9RG, RGRG), the reason for increased flower clusters for ‘RGRG’ would then be another type or form of gibberellin or another hormone that affected flower formation. As there were also reports that applied zeatin promoted flowering in apple (Ramirez and Hoad, 1981), ‘RG’ scions on ‘RG’ may have higher levels of cytokinin. While a mixture of GA_3+GA_{4+7} inhibited flowering in apples, it was also found more specifically that there is growing evidence that native GAs, of which 25 may occur in apple (Ramirez and Hoad, 1981; Koshioka et al., 1985) differ in their effect on flowering initiation. Thus, another reason for higher number of flower clusters for ‘RG’ scions on ‘RG’ rootstocks may be the concentration of native apple gibberellin such as GA_4. As the spur leaves promoted flower bud formation (Davis, 1957) it was suggested that leaves may act as a source of endogenous hormones for flower initiation. The cytokinin, zeatin was predominant in xylem sap from dwarfing rootstocks ‘M.9’ and zeatin riboside in xylem sap from more vigorous rootstocks ‘MM.106’ (Kamboj et al., 1999a). Research at East Malling has shown that floral clusters on ‘M.9’ dwarfing rootstocks open their flowers more slowly than those on rootstocks of similar vigour ‘P.16, ‘Bud.9’, ‘Mac 9’ and ‘P.2’. They also observed some flower cluster formed on the ‘M.9’ trees begin to swell and then degenerate and shrivel (Webster, 1995). In addition, in the present work ‘M.9’ scions on both rootstocks increased the mean proportion of total buds per scion that flowered compared with ‘RG’ scion on ‘M.9’ rootstock. In order to understand rootstock physiology on flowering further work is needed with a wider range of rootstocks. Repeating the experiment with higher number of replicates and checking for consistency in the results and analysing the rootstock and rootstock-scion interaction on flowering would provide useful information regarding rootstock physiology on flowering.

7.2 Comparison of hormonal signalling of apple and kiwifruit

7.2.1 Auxin –shoot growth

The basipetal transport of IAA from shoot to root proved to be an important endogenous signal regulating scion vigour for apples. The architectural changes that NPA or dwarfing rootstock brought for composite apple trees on a vigorous rootstocks were not similar to those that were brought by NPA on kiwifruit stem regarding total shoot length (see Chapter 4, Section 4.3.1.5; Figure 4.14). In Chapter 4, application of NPA to the base of stem below the first node of one-year-old kiwifruit rooted stem cuttings, significantly decreased mean final primary shoot length without affecting mean node number. For kiwifruit, NPA application with root restriction decreased mean primary shoot length, with decreased node number and internode length (Table 4.1). In particular, NPA caused the SAM on the primary shoot to grow slowly and the primary shoots were shorter due to a combination of both node number and internode length as neither of them were significantly different alone (Table 4.1). However, the number of sylleptic axillary shoots formed on the primary shoot during the first year of growth was not reduced as was the case for composite apple trees (Figure 7.1 and 7.2). The complete absence of SAS for root restricted plants suggested that cytokinins were insufficient to promote axillary bud activation on the primary shoot. However, with NPA, although auxin supply to root system restricted, there was no effect on SAS formation compared with the control. Presumably in kiwifruit vines, restriction of IAA to root system had no effect on cytokinins. For apple, BAP foliar sprays increased SAS formation (van Hooijdonk, 2009) whereas for kiwifruit it did not (Table 5.1 and 5.2). Additionally, for kiwifruit, there was no difference in the mean dry mass of the root system at the end of the first growing season with NPA (see Chapter.4; Section 4.3.1.6) unlike apple trees, NPA treatment reduced mean root dry mass, which was similar to that of ‘M.9’ when compared to the ‘RG’ rootstock (van Hooijdonk, 2009). Therefore, for composite apple trees, NPA treatment appeared to promote very similar changes to the ‘M.9’ rootstock in both scion architecture and root growth. Therefore, the reduced scion vigour for composite apple tree on a dwarfing rootstock in Chapter 2, maybe by restriction of IAA to the root system, which resulted in a smaller root mass and a lower supply of root-produced hormones. In contrast, for kiwifruit, restriction of IAA to the root system caused by NPA did not bring any change to root mass or total shoot growth.

Possibly, the IAA transport was not reduced sufficiently to reduce root development. Thus, these results provide new information that, for kiwifruit vine growth, restriction of IAA to root system is not effective and strategies that result in apple tree dwarfing may be ineffective in kiwifruit. Thus the mechanism underlying a potential dwarfing rootstock for kiwifruit may be quite different from that for apple. Conversely, foliar sprays of NPA and NAA significantly reduced the total growth of kiwifruit vine compared with the control (Table 5.1; see Chapter 5; section 5.3.4.2).

There may be different possible explanation to explain the effect of auxin restriction for kiwifruit vines:

1) Root produced hormone synthesis does not require much auxin stimulation from the root.

2) NPA is not effective in blocking auxin transport in kiwifruit, but morphological indications, such as epinasty and axillary bud stimulation below the application of NPA, indicate this is unlikely.

3) Excess amounts of IAA are produced by the shoot system so that sufficient auxin reaches the root even if NPA is present. So possibly in Chapter 5, NAA sprays resulted in supra-optimal auxin levels while NPA sprays resulted in sub-optimal auxin levels

4) Another possible explanation for stem applied NPA being not effective in increasing cytokinins levels in the xylem sap to increase SAS formation compared to the control, is that NPA is known to inhibit root growth and cause root death (Brunn et al., 1992), which could have reduced their cytokinin biosynthetic capability.

5) Alternatively, other auxin transport inhibitors such as TIBA, which acts differently within the cell, compared to NPA, should be tested before concluding that auxin transport inhibitors are not effective when applied to the stem of kiwifruit.

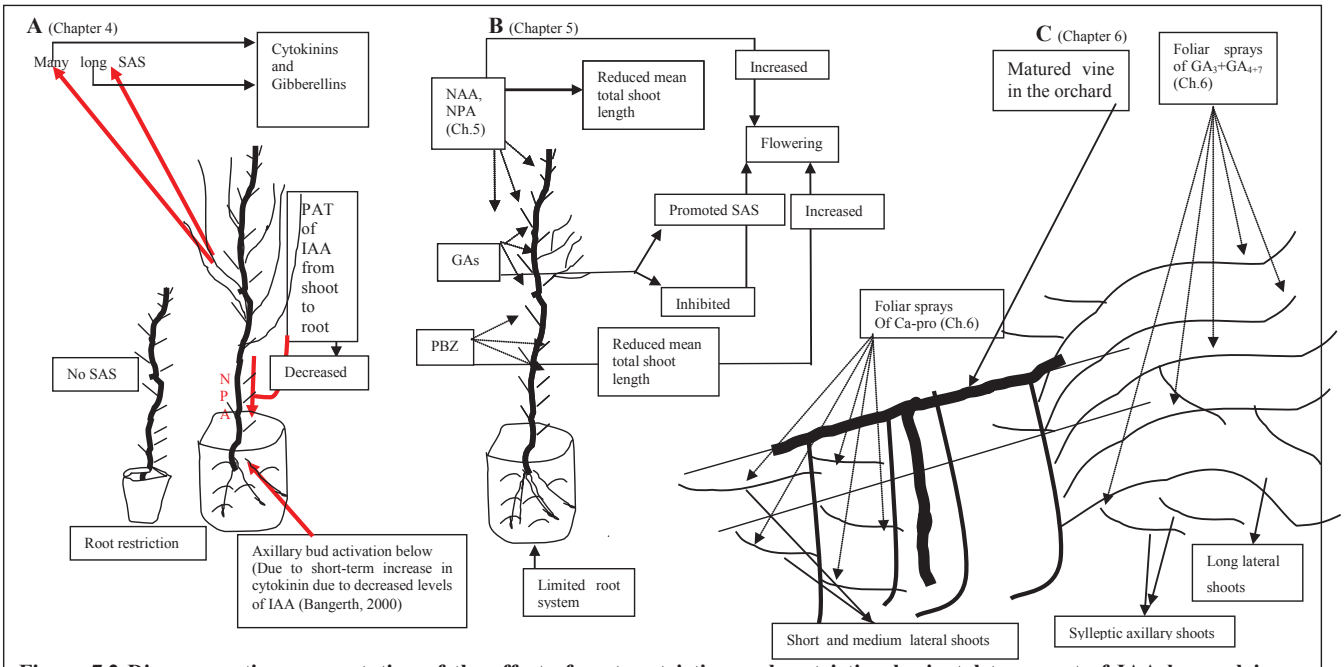

Figure 7.2 Diagrammatic representation of the effect of root restriction and restricting basipetal transport of IAA by applying NPA to base of stem for potted one-year-old rooted stem cuttings of kiwifruit 'Hort16A' (A); effect of foliar sprays of NAA, NPA PBZ and gibberellins (GA_3+GA_{4+7}) on potted rooted kiwifruit stem cuttings (B); gibberellins (GA_3+GA_{4+7}) and Ca-Pro on length of lateral shoots of matured kiwifruit vines in the orchard 'C'. SAS denotes sylleptic axillary shoot.

On the other hand, NAA reduced the total vegetative growth significantly compared with the control (Table 5.1 and 5.2). There was a significant overall reduction in vine growth with NAA for both cultivars. In the literature, it was reported that IAA levels were decreased when NAA was added to the medium (Epstein and Ludwig Muller, 1993), who observed that in the early stages of growth of *Arabidopsis* seedlings, NAA reduced the amount of free IAA and IBA. Exogenous hormone treatment (NAA) in tissue culture decreased endogenous hormone levels such as natural auxins and cytokinin levels in kiwifruit tissues (Centeno et al., 1996). For 'Hayward', NPA foliar sprays reduced growth. May be non-availability of free IAA due to NPA treatment or change in the endogenous hormone levels could be the reason for the reduced primary shoot length and node number and internode length for the NPA treated 'Hayward' shoots affecting both apical and sub-apical meristems (Table 5.2). However, in order to confirm these results, this experiment needs to be repeated with more replicates and an investigation into possible toxic side-effects of NPA and NAA sprays to the whole plant. Over-all the reduction in vigour with NAA and NPA is important information as it could fill a gap in our understanding of the physiology of kiwifruit vegetative vigour.

7.2.2 Gibberellins - shoot growth

For 'RG' scions on 'M.9' rootstocks, gibberellin foliar sprays increased the total shoot growth (Figure 7.1) and the scion appeared similar to 'RG' rootstock control (Table 2.12). For 'RG' scion on 'M.9' rootstock gibberellin (400 mg L^{-1} GA_3+GA_{4+7}), increased the final mean length of primary shoot, node number, internode length and number and length of SAS, delayed termination; thereby increased the total shoot length which became similar to 'RG' scion on 'RG' rootstock with or without GA treatment. In Chapter 5, with GA_3+GA_{4+7} at 800 mg L^{-1} the final mean length and node number of the kiwifruit primary shoot of 'Hort16A' was not significantly increased compared with control. For 'Hayward', although there was no significant effect of gibberellins on the final mean primary shoot length and node number, there was an increase in the internode length (Table 5.1). However, there was a significant

increase in the mean total shoot length for 'Hayward' because gibberellins increased sylleptic axillary shoot number and length.

In Chapter 6, GA_3+GA_{4+7} at 500 and 1000 mg L^{-1} significantly increased the growth of lateral shoots (see Chapter 6; Section 6.3.1 and Figure 6.4). The prolonged growth of lateral shoots with gibberellin foliar sprays compared with the control shoots showed the continual activity of apical and sub-apical meristem of lateral shoots. When selecting emerging shoots (Chapter 6; Section 6.2.2 and Figure 6.2) it was not known whether the resulting shoot was predestined to form short shoots and terminate with only preformed nodes, or develop into medium or long shoots. However, with gibberellin foliar sprays all lateral shoots developed into long shoots (Table 6.1). In general, for an untreated control vine, 40% of the shoots were short, 40% medium and remaining were long shoots. Presumably foliar sprays of gibberellins were effective on short and medium shoots because the hormone stimulated both apical and sub-apical meristems to produce more nodes or longer internodes respectively (Figure 7.2).

For sylleptic axillary shoot formation in the case of apples, BAP (van Hooijdonk, 2009), in this study for apples (Chapter 2) and for kiwifruit (Chapter 5&6) only GA_3 + GA_{4+7} were required. It was observed that 400 mg L^{-1} BAP did not promote as many sylleptic axillary shoots as 800 mg L^{-1} GA_3 + GA_{4+7} for kiwifruit rooted stem cuttings (Table 5.1). Gibberellins at 2000 and 4000 mg L^{-1} promoted SAS formation for kiwifruit matured vines in the orchard because of damaged shoot tip. As there was an increase in number of SAS with GA treatment, it appeared that GA activated axillary bud. Possibly, gibberellins might have helped cytokinin-activated axillary buds continue growth rather than returning to an inactive state. Further study is required with careful observation of stages of bud development, activation and extension of axillary shoot to ascertain whether gibberellins activated or extended the growth of SAS.

In apical dominance, axillary bud growth is prevented by the apical bud although all nutrients required for growth are present (Martin, 1987). Consequently, it can be assumed as Elfving et al., (2011) stated, that in response to applied GAs, the synthesis of the chemical compound that is critical for overcoming paradormancy may be

synthesised *de novo*. That critical compound may be cytokinin, as the application of cytokinin to the inhibited bud released axillary buds from apical dominance (Sachs and Thimann, 1967; Catalano and Hill, 1969; Letham, 1969; Schaeffer and Sharpe Jr, 1969). Although gibberellins generally have been found to enhance apical dominance (Brian et al., 1959; Bradley and Crane, 1960), in this study and also other studies (Marth et al., 1956; Elfving et al., 2011) gibberellins appear to have stimulated axillary bud activation. Moreover, Ali and Fletcher, (2010) mentioned that there was a sequential role of auxin, cytokinin and gibberellin on apical dominance and the effectiveness of any one of these hormones in either inhibiting or releasing the bud from inhibition, depended upon the physiological stage of the bud, that is, whether it is undergoing mitosis or not. Further research is needed to elucidate the mode of action of GA-induced sylleptic axillary shoot formation by looking at the stages of bud development in relation to interactions and sequential actions of cytokinins and gibberellins.

Although, cytokinins are usually associated with activation of axillary buds and gibberellins with their continued growth, for kiwifruit gibberellins appeared to be more directly involved in activation, however further work is required. Elfving et al., (2011) stated that stimulation of axillary bud to produce lateral shoots (proleptic) by applied GA in cherry trees is likely the result of interaction with apical dominance control system. The initial expansion of winter dormant bud after receiving enough chilling units (endo-dormancy) in the spring occurs when they are exposed to favourable temperatures (i.e., loss of eco-dormancy). After breaking ecodormancy, their failure to continue to grow may reflect re-establishment of para-dormancy (apical dominance) manifested as a deficiency of gibberellins (Figure 7.3).

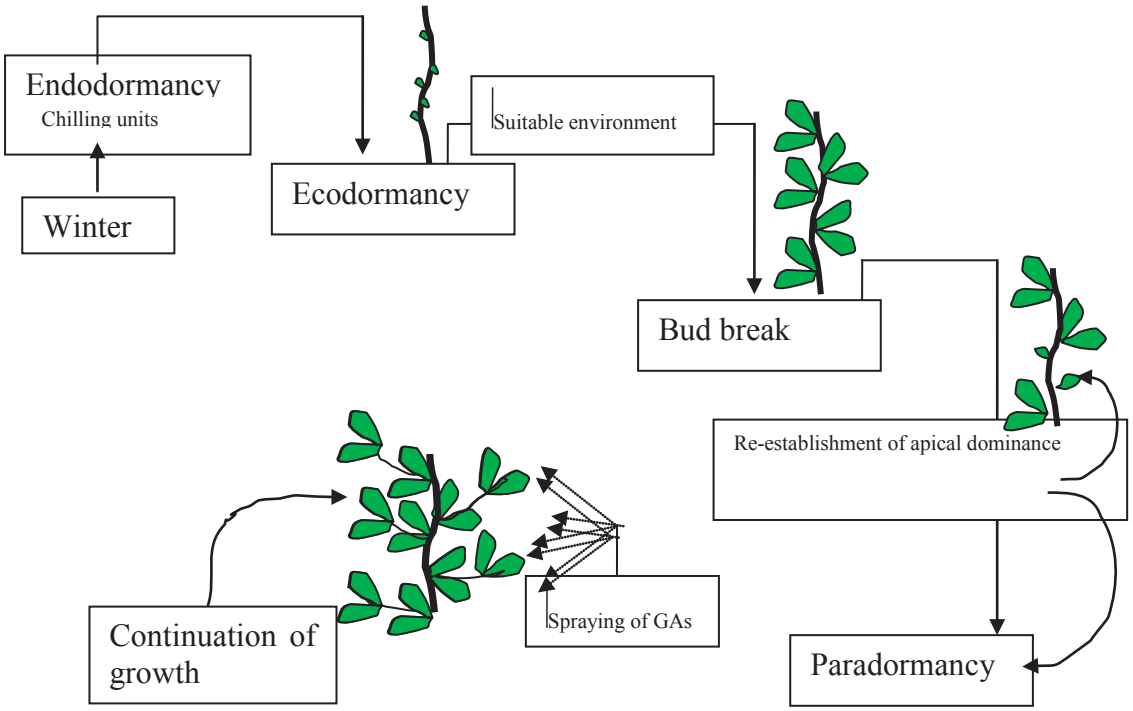

Figure 7.3 Diagrammatic representations of different stages in the shoot development passing through different stages of dormancy.

Moreover, anti-gibberellin Ca-Pro at 250 mg L^{-1} inhibited the release of lateral shoot axillary buds and promoted early termination of kiwifruit shoots. As Ca-Pro blocks the ultimate step of converting GA precursors (GA_{19} and GA_{20}) into bioactive GA_1 (Figure 6.1), the reduction in the growth with Ca-pro foliar sprays may be due to a lack of bioactive gibberellins. Thus, the vigorous growth of kiwifruit shoots may be due to bioactive gibberellins. Eighty percent of the shoots treated with Ca-Pro 250 mg L^{-1} terminated very early (12th Jan, 2010) while 100% of shoots treated with gibberellin foliar sprays (500 and 1000 mg L^{-1}) never showed any sign of termination by 8th February, 2010. Together these results with gibberellin and anti-gibberellin sprays clearly indicate that gibberellins promoted extension of active growth and anti-gibberellins early termination of a shoot, both involving apical and sub-apical meristems. However, future studies involving quantification of hormones in the SAM during normal shoot growth, during termination and after termination with or without gibberellins (GA_3 + GA_{4+7}) and anti-gibberellins (Ca-Pro at 250 mg L^{-1}), plus

quantification of hormones in xylem sap, would give confirmatory details of hormonal influence on vegetative growth in kiwifruit.

7.2.3 Flowering in apple and kiwifruit

Regarding flowering, GA_3+GA_{4+7} foliar sprays (800 mg L^{-1}) inhibited flowering for kiwifruit similar to composite apple trees in Chapter 2. Up until now, there are no studies for kiwifruit regarding levels of endogenous hormones. However, with the knowledge so far obtained for the vigorous growth of composite apple trees on vigorous rootstock, the vegetative vigour of kiwifruit vines may be related to the availability of sufficient levels of gibberellins. If composite apple tree 'RGRG' is compared to the vigorous kiwifruit vines, both may be having sufficient levels of gibberellins. The complete inhibition of flowering with exogenous gibberellin could be related to several months' persistence of the chemical in the treated organ, which may be similar to fruit-seed inhibition of flowering that supposedly led to biennial bearing in fruit trees (Hoad, 1978; Pharis and King, 1985). Further studies would be needed to compare a direct effect of gibberellins on inhibiting flowering to an indirect effect on shoot growth for kiwifruit vines. However, for kiwifruit, NPA foliar sprays increased flower formation significantly compared with the control (Table 5.4). This increase in number of flowers showed some similarity to 'M.9' rootstock. The most likely candidates in controlling the shift from vegetative to generative bud development in floral induction besides carbohydrates and florigen are pleiotropic plant hormones (Hegele et al., 2005). Feeding cytokinins into the xylem of apple spurs promoted flowering (Skogerbo, 1992). The flower promoting effect of single branch girdling in apple might also account for the buildup of cytokinin pool by formation and splitting cytokinin conjugates (Hegele et al., 2005) and builds up of carbohydrates above the girdling wound in the wood and bark. Therefore, the reduced primary shoot length and node number and early termination of growth with NPA treatment in the year applied for kiwifruit 'Hayward' may coincide with elevated cytokinins levels in the bud during next spring, which might be a favourable condition for floral induction [high ck/(auxin+GAs)] ratio.

7.2.4 Cytokinin - spring bud break for kiwifruit vines

Interestingly, during this study it was observed foliar sprays of cytokinins enhanced synchronised bud break i.e., 80% for 'Hort16A' in the first week itself (25th August, 2009) (Figure 5.6) and for 'Hayward' 50% and it was spread from 1st September to 6th October, 2009 (Figure 5.7). In New Zealand, for 'Hayward', the proportion of dormant buds that develop in spring is normally not more than 50% and can be as low as 30% (McPherson et al., 1994). The variation in bud break can cause a variation in the number of flowers/winter bud, which directly affects the commercial yield in kiwifruit (Richardson and McAneney, 1990; Cooper and Marshall, 1991). Therefore, hydrogen cyanamide (HC) is often used to increase bud break (Henzell and Briscoe, 1986). With HC, the increase in the bud break was greatest at the warmest sites (46% average increase at Kerikeri) but less at cooler sites (25% at TePuke). Even with HC, the effect of high winter temperature could not be negated (McPherson et al., 2001). In addition, HC will be banned from use in the near future. Therefore, the observations in this study on the effect of cytokinins is of considerable interest, the high and compact bud break for kiwifruit 'Hort16A' achieved with BAP foliar sprays was very impressive. After conducting further trials it may be possible to recommend BAP for obtaining uniform bud break when HC is eventually banned. Although, NPA also increased percentage bud break for kiwifruit 'Hayward' it cannot be used commercially because of toxicity, however, understanding the physiology and hormonal influence on bud break may be useful. As BAP is not toxic and registered for use on apple trees, trials with matured vine could be recommended to determine whether this response can be produced consistently from year to year on different kiwifruit cultivars.

7.3 Directions for future research-Summary

The use of dwarfing rootstocks for fruit trees is potentially useful; however, at this stage there are no size-controlling rootstocks to reduce kiwifruit vine vigour. As one way to understand the hormonal physiology behind vegetative vigour, the role of gibberellin in shoot extension in general for both apple trees and kiwifruit vines was

elucidated. A number of avenues exist to further investigate in order to be better able to control vegetative vigour:

1) Experimental work could be directed to study the relationship between root growth of different apple rootstocks under different environmental conditions and the growth rate of the shoot, in order to understand the reason for initial faster growth of apple scion on a dwarfing rootstock that occurs under the same environmental conditions.

2) In this study it was also found that stem part of the rootstock (shank) is responsible for hormonal alterations in the scion grafted to dwarfing rootstock. Therefore, it is suggested that further investigation on the nature of bark of different vigour controlling rootstocks would be worthwhile to add knowledge to the physiology of rootstock-induced dwarfing of scion. The mechanism by which the rootstock bark of 'M.9' may act to reduce the basipetal auxin transport to the root system still remains largely unknown, it would be worthy of investigating IAA efflux carriers at the plasma membrane (Estelle, 2001) and the rate of IAA metabolism of various rootstocks under different growing environments, which may also explain the plasticity of the growth of primary shoot of the scion on dwarfing rootstock in the first year from grafting (van Hooijdonk et al., 2009).

3) The results of this thesis indicate that gibberellins were involved in increasing both cell number and cell length in the elongation of matured internodes. But, further studies are suggested to elucidate the effect of gibberellins in the very young internode and also during the course of its elongation to elucidate the origins of differences in cell number and length with gibberellins during the period of elongation. That is, to what extent is an increase in cell number due to activity of apical meristem versus the sub-apical meristem? For the latter, an increase in cell number would be due to cell division in the elongating internode.

4) The increase in number of flower clusters for 'RGRG' composite apple tree is a very interesting result, which needs further evaluation as the result indicates that low vigour is not always associated with precocity. Therefore further work is

needed with a wider range of rootstocks in which assimilate supply and hormonal control is investigated.

5) Most importantly, as gibberellins are involved in the shoot termination, it is necessary to quantify levels of IAA and gibberellins (GA_1) in the shoot apical meristem periodically throughout the growing season, which may shed more light on the involvement of gibberellins in early shoot termination of apple shoots on dwarfing rootstocks and also kiwifruit vines treated with NAA, NPA and PBZ.

6) Unlike apples, for kiwifruit vines, restriction of auxin supply to root system by applying NPA to the base of the shoot did not affect growth, but, with foliar sprays of NPA and NAA, vegetative growth was reduced. To confirm these results, the experiment needed to be repeated with more replicates and an investigation into possible toxic side-effects of NPA sprays to the whole plant.

7) As there was high and very compact bud break i.e., 80% with BAP foliar sprays for the young vines in this study, it is recommended that further studies on trials using BAP foliar sprays to evaluate its effect on bud break in the orchard are needed as BAP is not toxic and registered for use. In addition, since gibberellin appears to stimulate bud release on proleptic shoot in cherry

8) By segregating seedlings of various crosses between kiwifruit, the stunted growth type of some seedlings could be sprayed with gibberellin at 500 and 800 mg L^{-1} to prolong the activity of SAM until the canopy is established and then the gibberellin withheld. Alternatively, these slow growing seedlings that are normally discarded by plant breeders may be of value as dwarfing rootstocks.

7.4 Final comment

The results of this thesis identified that gibberellins play an important role in stimulating apical and sub-apical meristem in order to increase shoot length both for composite apple trees on dwarfing rootstocks and also kiwifruit vines. The main role of gibberellin in vigour of apple and kiwifruit is in node formation and early termination of active shoot growth. The role of gibberellin in vigour of fruit trees does not lie in reducing internode length but in stimulating SAM by adding new metamers along the shoot. The results presented in Chapter 6, suggest that spraying of Ca-pro 250 mg L-1 to reduce vegetative vigour of may be worthwhile because of: 1) decrease in pruning costs and 2) effect on hormonal balance in the vine, which may increase resistance/tolerance to *Pseudomonas syringae pv. actinidiae* (Yamada, 1993; Chen et al., 2003; Wang et al, 2007) that is a severe threat to the NZ kiwifruit industry at the present time.

Appendix 1

Table 1 Appendix 1 Rootstock × scion interactions on the shoot croo-sectional area (SCA) of the primary shoots of ‘M.9’ and ‘RG’ scions on ‘M.9’ or ‘R.G’ rootstocks at the end of the first growing season (June, 2008) after tree grafting (August, 2007). Means are averaged over gibberellin treatment. Means sharing the same letter are not different at P=0.05 using Least squares means..... 296

Table 2 Rootstock × Gibberellin interactions on the shoot croo-sectional are (SCA)of the primary shoots of ‘M.9’ and ‘RG’ scions on ‘M.9’ or ‘R.G’ rootstocks at the end of the first growing season (June, 2008) after tree grafting (August, 2007). Means are averaged over scions. Means sharing the same letter are not different at P=0.05 using Least squares means..... 296

Table 3 Rootstock × Gibberellin interactions on the shoot croo-sectional are (SCA)of the primary shoots of ‘M.9’ and ‘RG’ scions on ‘M.9’ or ‘R.G’ rootstocks at the end of the first growing season (June,2008) after tree grafting (August, 2007). Means are averaged over rootstocks. Means sharing the same letter are not different at P=0.05 using Least squares means..... 297

Table 1 Appendix 1 Rootstock × scion interactions on the shoot croo-sectional are (SCA)of the primary shoots of 'M.9' and 'RG' scions on 'M.9' or 'R.G' rootstocks at the end of the first growing season (June, 2008) after tree grafting (August, 2007). Means are averaged over gibberellin treatment. Means sharing the same letter are not different at P=0.05 using Least squares means.

Rootstock	Scion	SCS (mm^2)
'M9'	RG	389.99 a
'M9'	M9	155.23 b
'RG'	RG	490.87 c
'RG'	M9	191.7 b

Table 2 Rootstock × Gibberellin interactions on the shoot croo-sectional are (SCA)of the primary shoots of 'M.9' and 'RG' scions on 'M.9' or 'R.G' rootstocks at the end of the first growing season (June, 2008) after tree grafting (August, 2007). Means are averaged over scions. Means sharing the same letter are not different at P=0.05 using Least squares means.

Rootstock	Gibberellin	SCS (mm^2)
'M9'	-GA	240.68 a
'M9'	GA (Nov)	250.16 a
'M9'	GA (Feb)	326.98 b
'RG'	-GA	356.86 b
'RG'	GA (Nov)	335.11 b
'RG'	GA (Feb)	331.91 b

Table 3 Rootstock × Gibberellin interactions on the shoot croo-sectional are (SCA)of the primary shoots of 'M.9' and 'RG' scions on 'M.9' or 'R.G' rootstocks at the end of the first growing season (June,2008) after tree grafting (August, 2007). Means are averaged over rootstocks. Means sharing the same letter are not different at P=0.05 using Least squares means.

Scion	Gibberellin	SCS (mm^2)
'M9'	-GA	164.42 a
'M9'	GA (Nov)	191.37 a
'M9'	GA (Feb)	164.61 a
'RG'	-GA	433.13 b
'RG'	GA (Nov)	393.89 b
'RG'	GA (Feb)	494.27 c

9. References

Achard, P., Herr, A., Baulcombe, D.C., Harberd, N.P. 2004. Modulation of floral development by a gibberellin-regulated microRNA. *Development* 131(14):3357-3365.

Akbas , F.A., Isikalan, C., Namli, S., Basaran, D. 2007. Micropropagation of Kiwifruit (*Actinidia deliciosa*). *International Journal of Agriculture & Biology* 9(3):489-493.

Akiyama, K., Hayashi, H. 2006. Strigolactones: chemical signals for fungal symbionts and parasitic weeds in plant roots. *Annals of Botany* 97(6):925-931.

Ali, A., Fletcher, R.A. 1970. Hormonal regulation of apical dominance in soybeans. *Canadian Journal of Botany* 48(11):1989-1994.

Alvarez, R., Nissen, S.J., Sutter, E.G. 1989. Relationship between indole-3-acetic acid levels in apple (*Malus pumila* Mill) rootstocks cultured *in vitro* and adventitious root formation in the presence of indole-3-butyric acid. *Plant Physiology* 89(2):439-443.

Arigita, L., Fernández, B., González, A., Tamés, R.S. 2005. Effect of the application of benzyladenine pulse on organogenesis, acclimatisation and endogenous phytohormone content in kiwi explants cultured under autotrophic conditions. *Plant Physiology and Biochemistry* 43(2):161-167.

Arney, S.E., Mancinelli, P. 1966. The basic action of gibberellic acid in elongation of 'Meteor' pea stems. *New Phytologist* 65(2):161-175.

Assar, P., Eshghi, S., Tafazoli, E., Rahemi, M., Khazaeipoul, Y.G., Monfared, A.S. 2009. Improving fruit quality in 'Hayward' kiwifruit using proper leaf to fruit ratios and girdling. *Horticulture, Environment and Biotechnology* 50(6):481-486.

Atkinson, C.J., Else, M. 2001. Understanding how rootstocks dwarf fruit trees. *The Compact Fruit Tree* 34(2):46-49.

Atkinson, C.J., Else, M., Taylor, L., Dover, C.J. 2003. Root and stem hydraulic conductivity as determinants of growth potential in grafted trees of apple (*Malus pumila* Mill.). *Journal of Experimental Botany* 54(385):1221-1229.

Atkinson, C.J., Webster, A.D., Vaughan, S.P., Lucas, A.S. 1997. Effects of root restriction on the physiology of apple tree growth. *Acta Horticulturae* 451:587-595.

Atkinson, C.J., Webster, A.D., Vaughan, S.P., Taylor, L., Kingswell, G. 2000. Interactions between root restriction, irrigation and rootstock treatments on 'Queen Cox' apple trees: Effects of soil and plant water relations. *Journal of Horticultural Science and Biotechnology* 75(4):376-382.

Atkinson, C.J., Else, M.A. 2003. Enhancing harvest index in temperate fruit tree crops through the use of dwarfing rootstocks. *International workshop on Cocoa breeding for improved production system*. Horticulture Research International, East Malling, United Kingdom. 118-131.

Avery, D.J. 1969. Comparisons of fruiting and deblossomed maiden apple trees, and of non-fruiting trees on a dwarfing and an invigorating rootstock. *New Phytologist* 68:323-336.

Avery, D.J. 1977. Maximum photosynthetic rate-a case study in apple. *New Phytologist* 78(1):55-63.

Awad, M.M., Kenworthy, A.L. 1963. Clonal rootstock, scion variety and time of sampling influences in apple leaf composition. *Proceedings of the American Society for Horticultural Science* 83:68-73.

Bainbridge, K., Sorefan, K., Ward, S., Leyser, O. 2005. Hormonally controlled expression of the *Arabidopsis MAX4* shoot branching regulatory gene. *The Plant Journal* 44(4):569-580.

Bangerth, F. 1992. Polar auxin transport as a signal in the regulation of tree and fruit development. *Acta Horticulturae* 329:70-76.

Bangerth, F. 1994. Response of cytokinin concentration in the xylem exudate of bean (*Phaseolus vulgaris* L.) plants to decapitation and auxin treatment, and relationship to apical dominance. *Planta* 194(3):439-442.

Bangerth, F., Li, C.J., Gruber, J. 2000. Mutual interaction of auxin and cytokinins in regulating correlative dominance. *Plant Growth Regulation* 32:205-217.

Bano, S., Scrimgeour, F. 2012. The Export Growth and Revealed Comparative Advantage of the New Zealand Kiwifruit Industry. *International Business Research* 5(2):p73.

Barden, J.A., Ferree, D.C. 1979. Rootstock does not affect net photosynthesis, dark respiration, specific leaf weight, and transpiration of apple leaves. *Journal of the American Society of Horticultural Science.;(United States)* 104(4):526-528.

Barratt, N.M., Davies, P.J. 1997. Developmental changes in the gibberellin-induced growth response in stem segments of light-grown pea genotypes. *Plant Growth Regulation* 21(2):127-134.

Barratt, N.M., Davies, P.J. 1996. Developmental and genotypic differences in the response of pea stem segments to auxin. *Plant Growth Regulation* 19(1):67-76.

Barthélémy, D., Caraglio, Y. 2007. Plant architecture: a dynamic, multilevel and comprehensive approach to plant form, structure and ontogeny. *Annals of Botany* 99(3):375-407.

Basler, E., McBride, R. 1977. Interaction of coumarin, gibberellic acid and abscisic acid in the translocation of auxin in bean seedlings. *Plant and Cell Physiology* 18(5):939-947.

Beakbane, A.B 1956. Possible mechanisms of rootstock effect. *Annals of Applied Biology* 44(3):517-521.

Beakbane, A.B., Thompson, E.C. 1947. Anatomical studies of stems and roots of hardy fruit trees IV. The root structure of some new clonal apple rootstocks budded with Cox's Orange Pippin. *Journal of Pomology and Horticultural Science* 23:206-211.

Beffa, R., Martin, H.V., Pilet, P.E. 1990. *In vitro* oxidation of indoleacetic acid by soluble auxin-oxidases and peroxidases from maize roots. *Plant Physiology* 94(2):485-491.

Ben-Tal, Y., Erner, Y. 1997. Flowering control by artificial gibberellins. *Acta Horticulturae 482*:21-26.

Beveridge, C.A. 2006. Axillary bud outgrowth: sending a message. *Current Opinion in Plant Biology* 9(1):35-40.

Beveridge, C.A., Kyozuka, J. 2010. New genes in the strigolactone-related shoot branching pathway. *Current Opinion in Plant Biology* 13(1):34-39.

Biasi, R., Costa, G., Manson, P.J. 1993. Light influence on kiwifruit (Actinidia deliciosa) quality. *Acta Horticulturae* 379:245-252.

Biasi, R., Costa, G., Succi, F. 1987. Effects of growth retardants on vegetation and fruiting of Hayward (*Actinidia deliciosa*). *Acta Horticulturae* 282:97-104.

Biasi, R., Marino, G., Costa, G. 1990. Propagation of Hayward (*Actinidia deliciosa*) from soft and semi-hardwood cuttings. *Acta Horticulturae* 282:243-250.

Bland, J. 1978. A Comparison of Certain Aspects of Ontogeny in the Long and Short Shoots of Mcintosh Apple During One Annual Growth Cycle. University of Minnesota, MN., PhD Thesis.

Bradford, K.J., Dilley, D.R. 1978. Effects of root anaerobiosis on ethylene production, epinasty, and growth of tomato plants *Plant Physiology* 61(4):506-509.

Bradley, M.V., Crane, J.C. 1957. Gibberellin-stimulated cambial activity in stems of apricot spur shoots. *Science* 126(3280):972-973.

Bradley, M.V., Crane, J.C. 1960. Gibberellin-induced inhibition of bud development in some species of Prunus. *Science* 131(3403):825-826.

Brewer, P.B., Dun, E.A., Ferguson, B.J., Rameau, C., Beveridge, C.A. 2009. Strigolactone acts downstream of auxin to regulate bud outgrowth in pea and *Arabidopsis*. *Plant Physiology* 150(1):482-493.

Brian, P.w., Hemming, H.G., Lowe, D. 1959. The effect of gibberellic acid on shoot growth of cupid sweet peas. *Physiologia Plantarum* 12(1):15-29.

Brian, P.W., Hemming, H.G. 1958. Complementary action of gibberellic acid and auxins in pea internode extension. *Annals of Botany* 22(1):1-17.

Brown, C.L., Sommer, H.E., Wetzstein, H. 1994. Morphological and histological differences in the development of dwarf mutants of sexual and somatic origin in diverse woody taxa. *Tree* 9(2):61-66.

Brown, C.L., Sommer, H.E. 1992. Shoot growth and histogenesis of trees possessing diverse patterns of shoot development. *American Journal of Botany* 79(3):335-346.

Brown, C.L., Sommer, H.E., Pienaar, L.V. 1995. The predominant role of the pith in the growth and development of internodes in *liquidambar styraciflua* (Hamamelidaceae). I. Histological basis of compressive and tensile stresses in developing primary tissues. *American Journal of Botany* 82(6):769-776.

Bulley, S.M., Wilson, F.M., Hedden, P., Phillips, A.L., Croker, S.J., James, D.J. 2005. Modification of gibberellin biosynthesis in the grafted apple scion allows control of tree height independent of the rootstock. *Plant Biotechnology Journal* 3(2):215-223.

Bus, V.G.M., Chagné, D., Bassett, H.C.M., Bowatte, D., Calenge, F., Celton, J.M., Durel, C.E., Malone, M.T., Patocchi, A., and Ranatunga, A.C. 2008. Genome mapping of three major resistance genes to woolly apple aphid (*Eriosoma lanigerum* Hausm.). *Tree Genetics & Genomes* 4(2):223-236.

Buttrose, M.S., Mullins, M.G. 1968. Proportional reduction in shoot growth of grapevines with root system maintained at constant relative volumes by repeated pruning. *Australian Journal of Biological Sciences* 21(6):1095-1102.

Buwalda, J.G., Meekings, J.S., Smith, G.S. 1991. Seasonal changes in photosynthetic capacity of leaves of kiwifruit (*Actinidia deliciosa*) vines. *Physiologia Plantarum* 83(1):93-98.

Carr, D.J., Reid, D.M., Skene, K.G.M. 1964. The supply of gibberellins from the root to the shoot. *Planta* 63(4):382-392.

Carraro, N., Peaucelle, A., Laufs, P., Traas, J. 2006. Cell differentiation and organ initiation at the shoot apical meristem. *Plant molecular biology* 60(6):811-826.

Carrera, M., Gómez-Aparisi, J. 1997. Behavior of 'Conference' pear, self-rooted or grafted on different rootstocks. *Acta Horticulturae* 475:143-148.

Catalano, M., Hill, T.A. 1969. Interaction between Gibberellic Acid and Kinetin in overcoming apical dominance, natural and induced by IAA, in tomato (*Lycopersicum esculenturn* Mill. cultivar potentate). *Nature* 222:985-986.

Celton, J.M., Tustin, D.S., Chagné, D., Gardiner, S.E. 2009. Construction of a dense genetic linkage map for apple rootstocks using SSRs developed from *Malus* ESTs and *Pyrus* genomic sequences. *Tree Genetics & Genomes* 5(1):93-107.

Centeno, M.L., Rodríguez, A., Feito, I., Fernández, B. 1996. Relationship between endogenous auxin and cytokinin levels and morphogenic responses in *Actinidia deliciosa* tissue cultures. *Plant Cell Reports* 16(1-2):58-62.

Cesoniene, L. 2008. Effect of 2-chlorethylphosphonic acid application on the growth and development of *Actinidia kolomikta*. *Scientific work of the Lithuanian Institute of Horticulture and Lithuanian University of Agriculture* 27(2):57-63.

Chalmers, D.J. 1986. Research and progress in cultural systems and management in temperate fruit orchards. *Acta Horticulturae* 175:215-226.

Champagnat, P. 1954. *The pruning of fruit trees.* Crosby-Lockwood, London.

Chan, B.G., Cain, J.C.1967.The effect of seed formation on subsequent flowering in apple. *Journal of the American Society for Horticultural Sciences* 91:63-68.

Chaplin, M.H., Westwood, M.N. 1980. Nutritional status of 'Bartlett' pear on cydonia and pyrus species rootstocks. *Journal of the American Society for Horticultural Science* 105(1):60-63.

Chen, C.M. 1981. Biosynthesis and enzymic regulation of the interconversion of cytokinin. *Proceedings in Life Sciences*:34-43.

Chen, C.M., Ertl, J.R., Leisner, S.M., Chang, C.C. 1985. Localization of cytokinin biosynthetic sites in pea plants and carrot roots. *Plant Physiology* 78(3):510-513.

Chesoniene, L. 1999. Comparison of some biological features and fruiting potential of *Actinidia kolomikta* cultivars. *Acta Horticulturae* 538:769-774.

Clearwater, M.J., Blattmann, P., Luo, Z., Lowe, R.G. 2007. Control of scion vigour by kiwifruit rootstocks is correlated with spring root pressure phenology. *Journal of Experimental Botany* 58(7):1741-1751.

Clearwater, M.J., Lowe, R.G., Hofstee, B.J., Barclay, C., Mandemaker, A.J., Blattmann, P. 2004. Hydraulic conductance and rootstock effects in grafted vines of kiwifruit. *Journal of Experimental Botany* 55(401):1371-1382.

Clearwater, M.J., Seleznyova, A.N., Thorp, T.G., Blattmann, P., Barnett, A.M., Lowe, R.G., Austin, P.T. 2006. Vigor-controlling rootstocks affect early shoot growth and leaf area development of kiwifruit. *Tree Physiolology* 26(4):505-515.

Cline, M.G. 1991. Apical dominance. *The Botanical Review* 57(4):318-358.

Cline, M.G. 2000. Execution of the auxin replacement apical dominance experiment in temperate woody species. *American Journal of Botany* 87(2):182-190.

Cline, M.G. 1994. The role of hormones in apical dominance. New approaches to an old problem in plant development. *Physiologia Plantarum* 90(1):230-237.

Cline, M.G. 1997. Concepts and terminology of apical dominance. *American Journal of Botany* 84(9):1064-1069.

Cline, M.G., Dong-II, K. 2002. A preliminary investigation of the role of auxin and cytokinin in sylleptic branching of three hybrid poplar clones exhibiting contrasting degrees of sylleptic branching. *Annals of Botany* 90(3):417-421.

Cockroft, B., Wallbrink, J.C. 1966. Soil properties and tree vigour in the Goulburn Valley. *Australian Journal of Experimental Agriculture and Animal Husbandary* 6(21):204-208.

Coenen, C., Lomax, T.L. 1997. Auxin--cytokinin interactions in higher plants: old problems and new tools. *Trends in plant science* 2:351-356.

Colby, H.L. 1935. Scion-stock chemistry and the fruiting relationship in apple tree. *Plant Physiology* 10(3):483-498.

Collett, C.E., Harberd, N.P., Leyser, O. 2000. Hormonal interactions in the control of *Arabidopsis* hypocotyl elongation. *Plant Physiology* 124(2):553-562.

Collins, T.J. (2007). ImageJ for microscopy. *Biotechniques* 43(1):25-30.

Cook, N.C., Bellstedt, D.U., Jacobs, G. 2001. Endogenous cytokinin distribution patterns at budburst in Granny Smith and Braeburn apple shoots in relation to bud growth. *Scientia Horticulturae* 87(1-2):53-63.

Cooke, J.E.K., Eriksson, M.E., Junttila, O. 2012. The dynamic nature of bud dormancy in trees: environmental control and molecular mechanisms. *Plant, Cell & Environment* 35(10):1707-1728.

Cooper, K.M., Marshall, R.R. 1991. Crop load and canopy management. *Acta Horticulturae* 297:501-508.

Costa, G., Biasi, R., Giuliani, R., Succi, F. 1991. Comparison of kiwifruit training systems. *Acta Horticulturae* 297:427-434.

Costa, G. 1999. Kiwifruit orchard management: new developments. *Acta Horticulturae* 498:111-126.

Costa, G., Sabatini, E., Spinelli, F., Andreotti, C., Spada, G., Mazzini, F. 2001. Prohexadione-Ca controls vegetative growth and cropping performance in pear. *Acta Horticulturae* 653:127-132.

Costes, E. García-Villanueva, E. 2007. Clarifying the effects of dwarfing rootstock on vegetative and reproductive growth during tree development: a study on apple trees. *Annals of Botany* 100(2):347-357.

Costes, E., Salles, J.C., Garcia, G. 2001. Growth and branching pattern along the main axis of two apple cultivars grafted on two different rootstocks. *Acta Horticulturae* 557:131-139.

Costes, E., Sinoquet, H., Kelner, J.J., Godin, C. 2003. Exploring within tree architectural development of two apple tree cultivars over 6 years. *Annals of Botany* 91(1):91-104.

Cowling, R.J., Harberd, N.P. 1999. Gibberellins control *Arabidopsis* hypocotyl growth via regulation of cellular elongation. *Journal of Experimental Botany* 50(337):1351-1357.

Crabbé, J.J. 1983. Vegetative vigor control over location and fate of flower buds, in fruit trees. *Acta Horticulturae 149*:55-64.

Cripps, J.E.L. 1970. A seasonal patern of apple root growth in western Australia. *Journal of Horticultural Science* 45:153-161.

Crochowska, M.J., Buta, G.J., Steffens, G.L., Faust, S.M. 1984. Endogenous auxin and gibberellin levels in low and high vigor apple seedlings. *Acta Horticulturae.* 146:125-134.

Crozier, A., Reid, D.M. 1971. Do roots synthesize gibberellins? *Canadian Journal of Botany* 49:967-975.

Cruz-Castillo, J.G., Lawes, G.S., Woolley, D.J., Ganesh, S. 1997. Evaluation of rootstock and 'Hayward' scion effects on field performance of kiwifruit vines using a multivariate analysis technique. *New Zealand Journal of Crop and Horticultural Science* 25(3):273-282.

Cummins, J.N., Aldwinckle, H.S. 1983. Breeding apple rootstocks. *Plant breeding Reviews.X*:294–394.

Currie, M.B. 1997. Source-sink relationship in kiwifruit: Carbohydrate and hormone effects on fruit growth at the cell, organ and whole plant level. *PhD thesis:* Massey University, Palmerston North, New Zealand.

Currie, M.B., Barnett, A.M., Boyd, L.M., Max, S. 2005. Trunk girdling: Risks and opportunities. *New Zealand Kiwifruit Journal Jan/Feb*:12-16.

Cutter, E.G. 1965. Recent experimental studies of the shoot apex and shoot morphogenesis. *The Botanical Review* 31(1):7-113.

Cutting, J.G.M., Lyne, M.C. 1993. Girdling and the reduction in shoot xylem sap concentrations of cytokinins and gibberellins in peach. *Journal of Horticultural Science* 68(4):619-626.

Cutting, J.G.M., Strydom, D.K., Jacob, G., Bellstedt, D.U., Van Der Merwe, K.J., Weiler, E.W. 1991. Changes in xylem constituents in response to rest breaking agents applied to apple before bud break. *Journal of the American Society for Horticultural Science* 116(4):680-683.

Dalziel, J., Lawrence, D.K. 1984. Biochemical and biological effects of kaurene oxidase inhibitors, such as paclobutrazol, p. 43-57. In: Menhcnett, R., Lawrence , K. (eds.). *British Plant Growth Regulator Group Mongraph (No. 11): Biochemical*

Aspects of Synthetic and Naturally Occurring Plant Growth Regulators. British Plant Growth Regulator Group, Wantage.

Dann, I.R., Wildes, R.A., Chalmers, D.J. 1984. Effects of limb girdling on growth and devolopment of competing fruit and vegetative tissues of peach trees *Australian Journal of Plant Physiology* 11(2):49-58.

Davies, P.J. 1995. Plant hormones: Physiology, Biochemistry and Molecular Biology. Kluwer Academic Publishers, Dordrecht, The Netherlands; Norwell, MA, USA.

Davis, L.D. 1957. Flowering and alternate bearing. *Proceedings of the American Society of Horticultural Science* 70:545-556.

Davison, R.M. 1990. The physiology of the kiwifruit. In: Kiwifruit-Science and management, pp. 127-154, Warrington, I.J., Weston, G.C., eds. Ray Richards Publisher in association with the New Zealand Society of Horticultural Science, Auckland, New Zealand.

Daykin, A., Scott, I.M., Francis, D., Causton, D.R. 1997. Effects of gibberellin on the cellular dynamics of dwarf pea internode development. *Planta* 203(4):526-535.

Dennis, F. G. Jr., Edgerton, L.J. 1966. Effects of gibberellins and ringing upon apple fruit development and flower bud formation. *Proceedings of the American Society of Horticultural Science* 88:14-24.

Digby, J., Wareing, P.F. 1966. The effect of applied growth hormones on cambial division and the differentiation of the cambial derivatives. *Annals of botany* 30(3):539-548.

Dry, P.R., Loveys, B.R. 1998. Factors influencing grapevine vigour and the potential for control with partial rootzone drying. *Australian Journal of Grape and Wine Research* 4(3):140-148.

Dun, E.A., Brewer, P.B., Beveridge, C.A. 2009. Strigolactones: discovery of the elusive shoot branching hormone. *Trends in plant science* 14(7):364-372.

Durbak, A., Yao, H., McSteen, P. 2012. Hormone signalling in plant development. *Current Opinion in Plant Biology* 15:92-96.

Early, J.D., Martin, G.C. 1988. Translocation and breakdown of 14C-labeled paclobutrazol in 'Nemaguard' peach seedlings. *HortScience* 23:196-200.

East Malling (2005). Report to Department for Environment, food and rural affairs, United Kingdom. Identifying the long-distance hormonal signals in the xylem

sap from dwarfing rootstocks. Report No.HH3730STF. http://www.defra.gov.uk/.

Elfving, D.C. 1984. Factors affecting apple-tree response to chemical branch-induction treatments [Plant growth regulators, 6-benzylamino purine, gibberellic acid, growth habit, New York, Ontario]. *Journal of the American Society for Horticultural Science* 109(4):476-481.

Elfving, D.C., Schecter, I., Hutchinson, A. 1993. The history of the vineland (V.) apple rootstocks. *Fruit Varieties Journal* 47(1):52-58.

Elfving, D.C., Visser, D.B., Henry, J.L. 2006. The use of bioregulators in the production of deciduous fruit trees. *Acta Horticulturae* 727:57-66.

Elfving, D.C., Visser, D.B., Henry, J.L. 2011. Gibberellins stimulate lateral branch development in young sweet cherry Trees in the orchard. *International Journal of Fruit Science* 11(1):41-54.

Epstein, E., Ludwig Müller, J. 1993. Indole-3 butyric acid in plants: occurrence, synthesis, metabolism and transport. *Physiologia Plantarum* 88(2):382-389.

Ercisli, S., Esitken, A., Cangi, R., Sahin, F. 2003. Adventitious root formation of kiwifruit in relation to sampling date, IBA and Agrobacterium rubi inoculation. *Plant Growth Regulation* 41(2):133-137.

Eriksson, M.E., Israelsson, M., Olsson, O., Moritz, T. 2000. Increased gibberellin biosynthesis in transgenic trees promotes growth, biomass production and xylem fiber length. *Nature Biotechnology* 18:784-788.

Eriksson, M.E., Moritz, T. 2002. Daylength and spatial expression of a gibberellin 20-oxidase isolated from hybrid aspen (Populus tremula L. x P. tremuloides Michx.). *Planta* 214(6):920-930.

Ernani, P.R., Rogeri, D.A., Proença, M.M., Dias, J. 2008. Addition of nitrogen had no effect on yield and quality of apples in an high density orchard carrying a dwarf rootstock. *Revista Brasileira de Fruticultura* 30(4):1113-1118.

Esau, K. 1965. *Plant Anatomy.* John Wiley and Sons, New York.

Estabrooks, E.N. 1993. Paclobutrazol sprays reduce vegetative growth and increase fruit production in young McIntosh apple trees. *Canadian Journal of Plant Science* 73(4):1127-1135.

Estelle, M. 2001. Plant hormones: Transporters on the move. *Nature* 413:374-375.

Evans, J.R., Evans, R.R., Regusci, C.L., Rademacher, W. 1999. Mode of action, metabolism, and uptake of BAS 125W, prohexadione-calcium. *HortScience* 34(7):1200-1201.

Evans, R.R., Evans, J.R., Rademacher, W. 1996. Prohexadione calcium for suppression of vegetative growth in eastern apples. *Acta Horticulturae* 451:663-666.

Evert, R.F. 2006. *Esau's Plant anatomy*, 3rd edition. John Wiley and sons, Incorporated, New Jersey.

Fagoaga, C., Tadeo, F.R., Iglesias, D.J., Huerta, L., Lliso, I., Vidal, A.M., Talon, M., Navarro, L., Garcia-Martinez, J.L., Pena, L. 2007. Engineering of gibberellin levels in citrus by sense and antisense overexpression of a *GA 20-oxidase* gene modifies plant architecture. *Journal of Experimental Botany* 58(6):1407-1420.

Fallahi, E., Colt, W.M., Fallahi, B., Chun, I.J. 2002. The importance of apple rootstocks on tree growth, yield, fruit quality, leaf nutrition, and photosynthesis with an emphasis on 'Fuji'. *HortTechnology* 12(1):38-44.

Faust, M., Erez, A., Rowland, L.J., Wang, S.Y., Norman, H.A. 1997. Bud dormancy in perennial fruit trees: physiological basis for dormancy induction, maintenance, and release. *HortScience* 32(4):623-629.

Fazio, G., Aldwinekle, H.S., Robinson, T.L., Cummins, J. 2005. Geneva®935 A new fire blight resistant, semidwarfing apple rootstock. *HortScience* 7:1027.

Fazio, G., Robinson, T. 2008. Modification of nursery tree architecture with apple rootstocks. A Breeding Perspective. *New York Fruit Quaterly*, 16(1):13-16.

Ferguson, A.R. 1984. Kiwifruit: A botanical review. *Horticultural Reviews* 6 (ed J. Janick) John wiley & sons, Inc., Hoboken, NJ, USA.

Ferguson, A.R. 2007. The need for characterisation and evaluation of germplasm: kiwifruit as an example. *Euphytica* 154(3):371-382.

Ferguson, A.R., McNeilage, M.A. 1999. *Actinidia* germplasm for kiwifruit breeding. *Acta Horticulturae* 498:37-42.

Ferguson, A.R., Seal, A.G. 2008. Kiwifruit. *Temperate Fruit Crop Breeding.* Pp 235-264.

Ferguson, A.R., Huang, H. 2007. Genetic resources of kiwifruit: domestication and breeding. *Plant Breeding Reviews* 29:1-121.

Ferguson, B.J., Beveridge, C.A. 2009. Roles for auxin, cytokinin, and strigolactone in regulating shoot branching. *Plant Physiology* 149(4):1929-1944.

Ferrando, M., Spiess, W.E.L. 2000. Review: Confocal scanning laser microscopy. A powerful tool in food science. *Food Science and Technology International* 6(4):267-284.

Ferree, D.C., Myers, S.C., Schupp, J.R. 1991. Root pruning and root restriction of fruit trees-current review. *Acta Horticulturae* 322:153-166

Ferree, D.C., Carlson, R.F. 1987 Apple rootstocks. *In: Rootstocks for fruit crops,* Eds: Rom, R. C. and Carlson, R. F. New York, USA.

Fleet, C.M., Sun, T.P. 2005. A *DELLA*cate balance: the role of gibberellin in plant morphogenesis. *Current opinion in plant biology* 8(1):77-85.

Fletcher, J.C., Brand, U., Running, M.P., Simon, R., Meyerowitz, E.M. 1999. Signalling of cell fate decisions by *CLAVATA3* in *Arabidopsis* shoot meristems. *Science* 283(5409):1911-1914.

Flore, J.A., Lakso, A.N. 1989. Environmental and physiological regulations of photosynthesis in fruit crops. *Horticultural Reviews* 11:111-157.

Foo, E., Bullier, E., Goussot, M., Foucher, F., Rameau, C., Beveridge, C.A. 2005. The branching gene *RAMOSUS1* mediates interactions among two novel signals and auxin in pea. *The Plant Cell* 17(2):464-474.

Foo, E., Morris, S.E., Parmenter, K., Young, N., Wang, H., Jones, A., Rameau, C., Turnbull, C.G.N., Beveridge, C.A. 2007. Feedback regulation of xylem cytokinin content is conserved in pea and *Arabidopsis*. *Plant Physiology* 143(3):1418-1428.

Forshey, C.G., Elfving, D.C. 1989. The relationship between the vegetative growth and fruiting in apple trees. *Horticultural reviews* 11:229-287.

Foster, T., Kirk, C., Jones, W.T., Allan, A.C., Espley, R., Karunairetnam, S., Rakonjac, J. 2007a. Characterisation of the *DELLA* subfamily in apple (*Malus* x *domestica* Borkh.). *Tree Genetics & Genomes* 3(3):187-197.

Foster, T.M., Seleznyova, A.N., Barnett, A.M. 2007b. Independent control of organogenesis and shoot tip abortion are key factors to developmental plasticity in kiwifruit (*Actinidia*). *Annals of botany* 100(3):471-481.

Fu, X., Harberd, N.P. 2003. Auxin promotes *Arabidopsis* root growth by modulating gibberellin response. *Nature* 421:740-743.

Fujii, J.A., Kennedy, R.A. 1985. Seasonal changes in the photosynthetic rate in apple trees: A comparison between fruiting and nonfruiting trees. *Plant Physiology* 78(3):519-524.

Fujii, T., Nito, N. 1972. Studies on the compatibility of grafting of fruit trees. I. Callus fusion between rootstock and scion. *Journal of Japan Society of Horticultural. Sci*ence 41:1-10.

Galston, A.W., Purves, W.K. 1960. The mechanism of action of auxin. *Annual Review of Plant Physiology* 11:239-276.

Gaspar, T., Kevers, C., Faivre-Rampant, O., Crevecoeur, M., Penel, C.L., Greppin, H., Dommes, J. 2003. Changing concepts in plant hormone action. *In Vitro Cellular & Developmental Biology-Plant* 39(2):85-106.

Geisler, D., Ferree, D.C. 1984. Response of plants to root pruning. *Horticultural Reviews* 6:155-188.

Gendreau, E., Traas, J., Desnos, T., Grandjean, O., Caboche, M., Hofte, H. 1997. Cellular basis of hypocotyl growth in *Arabidopsis thaliana*. *Plant Physiology* 114(1):295-305.

Gerasopoulos, D., Drogoudi, P.D. 2005. Summer-pruning and preharvest calcium chloride sprays affect storability and low temperature breakdown incidence in kiwifruit. *Postharvest Biology and Technology* 36(3):303-308.

Goldschmidt, E.E., Monselise, S.P. 1970. Hormonal control of flowering in citrus and some other woody perennials, p. 758-766. In: Carr, D. J. (ed.) *Plant Growth Substances*. Springer Verlag, Berlin/Heidelberg/New York.

Goldschmidt, E.E., Tamim, M., Goren, R. 1997. Gibberellins and flowering in citrus and other fruit trees: A critical analysis. *Acta Horticulturae* 463:201-208.

Goldsmith, M.H.M., Cataldo, D.A., Karn, J., Brenneman, T., Trip, P. 1974. The rapid non-polar transport of auxin in the phloem of intact *Coleus* plants. *Planta* 116(4):301-317.

Gomez-Roldan, V., Fermas, S., Brewer, P.B., Puech-Pagès, V., Dun, E.A., Pillot, J.P., Letisse, F., Matusova, R., Danoun, S., Portais, J. 2008. Strigolactone inhibition of shoot branching. *Nature* 455:189-194.

Goren, R., Huberman, M., and Goldschmidt, E. 2003. Girdling: physiological and horticultural aspects. *Horticultural Reviews* 30:1-36.

Graebe, J.E. 1987. Gibberellin biosynthesis and control. *Annual Review of Plant Physiology* 38:419-465.

Granier, C., Inze, D., Tardieu, F. 2000. Spatial distribution of cell division rate can be deduced from that of $p34^{cdc2}$ kinase activity in maize leaves grown at contrasting temperatures and soil water conditions. *Plant Physiology* 124(3):1393-1402.

Grant, J.A., Ryugo, K. 1982. Influence of developing shoots on flowering potential of dormant buds of *Actinidia chinensis*. *HortScience* 17(6):977-978.

Grauslund, J. 1972. Gibberellins in diffusates from shoots of apple trees. *Physiologia Plantarum* 27:65-70.

Green, P.B. 1969. Cell morphogenesis. *Annual Review of Plant Physiology* 20:365-394.

Greene, D. 1989. Gibberellins A_{4+7} influence fruit set, fruit quality, and return bloom of apples. *Journal of the American Society for Horticultural Science* 114(4):619-625.

Greenhalgh, W. and Edgerton, L. 1967. Interaction of alar and gibberellin on growth and flowering of the apple. *Proceedings of American Society for Horticultural Science.* 91:9-17.

Greulach, V.A., Haesloop, J.G. 1958. The Influence of gibberellic acid on cell acid on cell division and cell elongation in *Phaseolus vulgaris*. *American Journal of Botany* 45(7):566-570.

Grochowska, M. 1967. Occurrence of free phloretic acid (p-hydroxy-dihydro cinnamic acid) in xylem sap of the apple tree. *Bulletin de l'Académie Polonaise des Sciences* 15:455-459.

Grochowska, M.J., Karaszewska, A., Jankowska, B., Maksymiuk, J. 1984. Dormant pruning influence on auxin, gibberellin, and cytokinin levels in apple-trees. *Journal of the American Society for Horticultural Science* 109:312-318.

Gunckel, J.E. and Wetmore, R.H. 1946. Studies of development in long shoots and short shoots of *Ginkgo biloba* L. II. Phyllotaxis and the organization of the primary vascular system; primary phloem and primary xylem. *American Journal of Botany* 33(6):532-543.

Gurr, A., Samish, R.M 1968. The role of auxins and auxin destruction in the vigor effect induced by various apple root stocks. *Beitrage Biology Pflanzen* 45:91-111.

Gutiérrez-Pesce, P., Rugini, E. 2009. Kiwifruit, p. 185-212. In: Kole C., Hall TC (eds.) *Compendium of Transgenic Crop Plants (Vol. 5): Transgenic Tropical and Subtropical Fruits and Nuts*. Blackwell, Oxford, UK.

Guttridge, C.G. 1962. Inhibition of fruit-bud formation in apple with gibberellic acid. *Nature* 196:1008.

Guttridge, C.G., Thompson, P.A. 1959. Effect of gibberellic acid on length and number of epidermal cells in petioles of strawberry. *Nature* 183:197-198.

Hall, S.M., Hillman, J.R. 1975. Correlative inhibition of lateral bud growth in *Phaseolus vulgaris* L. timing of bud growth following decapitation. *Planta* 123(2):137-143.

Hallé, F., Oldeman, R.A.A., Tomlinson, P.B. 1978. *Tropical trees and forests: An architectural analysis*. Berlin:Springer-Verlag. Pp 441.

Hammerton, J.L. 1975. Effects of growth regulators on pigeon pea (*Cajanus cajan*) *Experimental Agriculture* 11(4):241-245.

Hansen, P. 1971. The effect of fruiting upon transpiration rate and stomatal opening in apple leaves. *Physiologia Plantarum* 25(2):181-183.

Hanson, J.B., Trewavas, A.J. 1982. Regulation of plant cell growth: the changing perspective. *New Phytologist* 90(1):1-18.

Harbage, J.F., Stimart, D.P. 1996. Ethylene does not promote adventitious root initiation on apple microcuttings. *Journal of the American Society for Horticultural Science* 121(5):880-885.

Hartmann, H.T., Kester, D.E., Davies, F.T., Geneve, R.L. 1975. *Plant Propagation: Principles and Practices*. Prentice-Hall, Englewood Cliffs, NJ.

Hay, A., Kaur, H., Phillips, A., Hedden, P., Hake, S., Tsiantis, M. 2002. The gibberellin pathway mediates *KNOTTED1*-type homeobox function in plants with different body plans. *Current Biology* 12:1557-1565.

Hayward, A., Stirnberg, P., Beveridge, C., Leyser, O. 2009. Interactions between auxin and strigolactone in shoot branching control. *Plant Physiology* 151(1):400-412.

Hedden, P., Thomas, S. G. 2007. Gibberelin metabolism and signal transduction, p. 147-184. In: Heddon, P., Thomas, S. G.(eds.) *Annual Plant Reviews (Vol. 24): Plant Hormone Signalling.* Blackwell, Oxford, UK.

Hedden, P., Graebe, J.E. 1985. Inhibition of gibberellin biosynthesis by paclobutrazol in cell-free homogenates of *Cucurbita maxima* endosperm and *Malus pumila* embryos. *Journal of Plant Growth Regulation* 4(1-4):111-122.

Hegele, M., Bangerth, F., Naphrom, D., Sruamsiri, P., Manochai, P. 2005. Control of flower induction in tropical/subtropical fruit trees by phytohormones using the example of longan and mango. *Acta Horticulturae* 727:217-276.

Henzell, R., Briscoe, M.R. 1985. An observation on shoot promotion on chemically pruned kiwifruit vines. *Acta Horticulturae* 172:281-284.

Henzell, R.F., Briscoe, M.R. 1986a. Hydrogen cyanamide: a tool for consistently high kiwifruit production. *New Zealand kiwifruit special publication* 1:8-11.

Henzell, R.F., Briscoe, M.R., Lauren, D.R. 1986b. Evaluation of two plant growth regulators as chemical pruning agents for kiwifruit vines in summer. *New Zealand Journal of Experimental Agriculture* 14(2):199-203.

Hetherington, S., Jones, K.M., Koen, T.B. 1992. Stimulation of bud production in *Eucalyptus globulus* by paclobutrazol application. *South African Forestry Journal* 160(1):39-41.

Hibbs, A.R. 2000. *Confocal Microscopy for Biologists: An Intensive Introductory Course*. Kluwer Academic/Plenum Publishers, New York.

Hirst, P.M., Ferree, D.C. 1995. Rootstock effects on shoot morphology and spur quality of 'Delicious' apple and relationships with precocity and productivity. *Journal of the American Society for Horticultural Science* 120(4):622-634.

Hoad, G.V. 1978. The role of seed derived hormones in the control of flowering in apple. *Acta Horticuturae 80*:93-104.

Hoffman, D.E. 2011. Changes in the transcriptome and metabolome during the initiation of growth cessation in hybrid aspens. PhD thesis. Swedish University of Agricultural Science., Umea

Holmsen, T.W. 1960. Pith development in normal and short internode seedlings of *Prunus persica* var. Lovell. *American Journal of Botany* 47(3):173-175.

Howell, S. 1998. *Molecular Genetics of Plant Development.* Cambridge University Press, Cambridge.

Huang, H.W., Ferguson, A.R. 2006. *Actinidia* in China: natural diversity, phylogeographical evolution, interspecific gene flow and kiwifruit cultivar improvement. *Acta Horticulturae* 753:31-40.

Ibrahim, I., Dana, M.N. 1971. Gibberellin-like activity in apple rootstocks. *HortScience* 6:541-542.

Igbedioh, S. 1991. Effects of agricultural pesticides on humans, animals, and higher plants in developing countries. *Archives of environmental health: An International Journal* 46(4):218-224.

Ingram, T.J., Reid, J.B., Murfet, C., Potts, W.C. 1984. Internode length *Pisum*. IV. The effect of the *Le* gene on gibberellin metabolism *Physiologia Planarum* 59(4):607-616.

Irving, D.E., Drost, J.H. 1987. Effects of water deficit on vegetative growth, fruit growth and fruit quality in 'Cox's Orange Pippin' apple. *Journal of Horticultural Science* 62:427-432.

Jackson, J.E. 1988. World-wide development of high density planting in research and practice. *Acta Horticulturae* 243:17-28.

Jackson, J.E. 2003. *Biology of Apples and Pears*. Cambridge University Press, United Kingdom.

Jackson, S.D. 2004. *Plant Architecture*. John Wiley & Sons, Ltd.

James, D.J. 1983. Adventitious root formation '*in vitro*' in apple rootstocks (*Malus pumila*) I. Factors affecting the length of the auxin sensitive phase in M.9. *Physiologia Plantarum* 57(1):149-153.

James, D.J., Thurbon, I.J. 1981. Phenolic compounds and other factors controlling rhizogenesis *in vitro* in the apple rootstocks M.9 and M.26. *Zeitschrift fuer Pflanzenphysiologie (Germany, FR)* 105(1):11-20.

Jasinski, S., Piazza, P., Craft, J., Hay, A., Woolley, L., Rieu, I., Phillips, A., Hedden, P., Tsiantis, M. 2005. *KNOX* action in *Arabidopsis* is mediated by coordinate regulation of cytokinin and gibberellin activities. *Current Biology* 15(17):1560-1565.

Jasinski, S., Riou-Khamlichi, C., Roche, O., Perennes, C., Bergounioux, C., Glab, N. 2002. The *CDK* inhibitor *NtKIS1a* is involved in plant development, endoreduplication and restores normal development of *cyclin D3*; 1-overexpressing plants. *Journal of cell science* 115:973-982.

Jaumien, F., Czarnecki, B., Poniedzialek, W., Mitrut, T. 1992. Very similar effects of a mixture of GA_3 and BA (6-benzylaminopurine) and of GA_{4+7} and BA on branching of some apple cultivars in nursery. *Acta Horticulturae* 329:35-42.

Johnson, R., Lakso, A.N. 1985. Relationships between stem length, leaf area, stem weight, and accumulated growing degree-days in apple shoots. *Journal of the American Society for Horticultural Science* 110(4):586-590.

Johnson, X., Brcich, T., Dun, E.A., Goussot, D.M., Haurogne, K., Beveridge, C.A., Rameau, C. 2006. Branching genes are conserved across species. Genes controlling a novel signal in pea are coregulated by other long-distance signals. *Plant Physiology* 142(3):1014-1026.

Jones, O.P. 1973. Effects of cytokinins in xylem sap from apple trees on apple shoot growth. *Journal of Horticultural Science* 48:181-188.

Jones, O.P. 1974. Xylem sap composition in apple trees effect of the graft union. *Annals of botany* 38(2):463-467.

Jones, O.P. 1986. Endogenous growth regulators and rootstock/scion interactions in apple and cherry trees. *Acta Horticulturae* 179:177-184.

Jones, O.P., Lacey, H.J. 1968. Gibberellin-like substances in the transpiration stream of apple and pear trees. *Journal of Experimental Botany* 19(3):526-531.

Jones, O.P. 1967. Effect of benzyl adenine on isolated apple shoots. *Nature* 215:1514-1515.

Jupe, S.C., Causton, D.R., Scott, I.M. 1988. Cellular basis of the effects of gibberellin and the *pro* gene on stem growth in tomato. *Planta* 174(1):106-111.

Kamboj, J.S., Blake P.S., Quinlan, J.D., Baker, D.A. 1999a. Identification and quantitation by GC-MS of zeatin and zeatin riboside in xylem sap from rootstock and scion of grafted apple trees. *Plant Growth Regulation* 28(3):199-205.

Kamboj, J.S., Browning, G., Quinlan, J.D., Blake, P.S., Blaker, D.A. 1996. Polar transport of [^{3}H]-IAA in apical shoot segments of different apple rootstocks. *Journal of Horticultural Science* 72(5):773-780.

Kamboj, J.S., Blake, P.S., Quinlan, J.D., Webster, A.D., Baker, D.A. 1997. Recent advances in studies on the dwarfing mechanism of apple rootstocks. *Acta Horticulturae* 451:75-82.

Kamboj, J.S., Browning, G., Blake, P.S., Quinlan, J.D., Baker, D.A. 1999b. GC-MS-SIM analysis of abscisic acid and indole-3-acetic acid in shoot bark of apple rootstocks. *Plant Growth Regulation* 28(1):21-27.

Kamijima, O. 1981. Consideration on the mechanism of expression of dwarf genes in rice plants. II: The actions of dwarf genes on cell division and cell elongation in parenchyma of internode. *Japanese Journal of Breeding* 31(3):302-315.

Kaminek, M., Armstrong, D.J. 1990. Genotypic variation in cytokinin oxidase from *Phaseolus* callus cultures. *Plant Physiology* 93(4):1530-1538.

Kapulnik, Y., Resnick, N., Mayzlish-Gati, E., Kaplan, Y., Wininger, S., Hershenhorn, J., Koltai, H. 2011. Strigolactones interact with ethylene and auxin in regulating root-hair elongation in *Arabidopsis*. *Journal of Experimental Botany* 62(8):2915-2924.

Kato, T., Ito, H. 1962. Physiological factors associated with the shoot growth of apple trees. *Tohuku Journal of Agricultural Research* 13:1-21.

Kayal, W.E.L., Allen, C.C.G., Ju, C.J.T., Adams, E., King-Jones, S., Zaharia, L.I., Abrahams, S.R., Cooke, J.E.K. 2011. Molecular events of apical bud formation in white spruce, *Picea glauca*. *Plant Cell & Environment* 34(3):480-500.

Kefeli, V.I., Kadyrov, C.S. 1971. Natural growth inhibitors, their chemical and physiological properties. *Annual Review of Plant Physiology* 22:185-196.

Kende, H., Sitton, D. 1967. The physiological significance of kinetin and gibberellin-like root hormones. *Annals of the New York Academy of Sciences* 144:235-243.

Kende, H., Zeevert, J. 1997. The five 'classical' plant hormones. *Plant Cell* 9(7):1197-1210.

Ketchie, D.O. 1984. Flowering, spur formation and limb angles of Delicious apple strains. *Fruit Varieties Journal* 38(4):150-152.

Knight, R. 1925. Water relations of apples. *East Malling Annual Report* 13:55-56.

Kobayashi, M., Sakurai, A., Saka, H., Takahashi, N. 1989. Quantitative analysis of endogenous gibberellins in normal and dwarf cultivars of rice. *Plant Cell Physiology* 30(7):963-969.

Koltai, H., LekKala, S.P., Bhattacharya, C., Mayzlish-Gati, E., Resnick, N., Wininger, S., Dor, E., Yoneyama, K., Hershenhorn, J. 2010. A tomato strigolactone-impaired mutant displays aberrant shoot morphology and plant interactions. *Journal of Experimental Botany* 61(6):1739-1749.

Koshioka, M., Taylor, J.S., Edwards, G.R., Looney, N.E., and Pharis, R.P. 1985. Identification of gibberellins A_{19} and A_{20} in vegetative tissue of apple. *Agricultural and Biological Chemistry* 49(4):1223-1226.

Kosina, J. 2010. Effect of dwarfing and semi-dwarfing apple rootstocks on the growth and productivity of selected apple cultivars. *Horticultural science* 37(4):121-126.

Kozlowski, T.T. 1964. Shoot growth in woody plants. *The Botanical Review* 30(3):335-392.

Krikorian, A., Kelly, K., Smith, D. 1995. Hormones in tissue culture and micropropagation, p. 774-796. In: Davies, P. J. (ed.) *Plant Hormones: Physiology, Biochemistry and Molecular Biology.* Kluwer Academic Publishers, Dordrecht.

Kuraishi, S., Muir, R.M. 1962. Increase in diffusible auxin after treatment with gibberellin. *Science* 137(3532):760-761.

Labonte, R.N. 1989. Pesticides and healthy public policy. *Canadian Journal of Public Health. Revue canadienne de santé publique* 80(4):238-242.

Lakso, A.N. 1980. Aspects of canopy photosynthesis and productivity in the apple tree. *Acta Horticulturae* 114:100-109.

Lakso, A.N., Wünsche, J.N., Palmer, J.W., Corelli Grappadelli, L. 1999. Measurement and modeling of carbon balance of the apple tree. *HortScience* 34:1040-1047.

Lang, G.A. 2005. Underlying principles of high density sweet cherry production. *Acta Horticulturae* 667:325-336.

Lavee, S. 1986. Usefulness of growth regulators for controlling vine growth and improving grape quality in intensive vineyards. *Acta Horticulturae* 206:89-108.

Law, D.M. 1987. Gibberellin-enhanced indole-3-acetic acid biosynthesis: D-Tryptophan as the precursor of indole-3-acetic acid. *Physiologia Plantarum* 70(4):626-632.

Lawes, G.S. 1984. Winter temperatures and kiwifruit bud development. *Orchardist of New Zealand* 57(3):110.

Lawes, G.S. 1990. Propagation of kiwifruit. In: Warrington, I. G. and Weston, G. C. (eds.), Kiwifruit: Science and management. Ray Rcards Publisher/ New Zealand society for Horticutural science, Auckland, New Zealand.

Leather, G.R., Forrence, L.E., Abeles, F.B. 1972. Increased ethylene production during clinostat experiments may cause leaf epinasty. *Plant Physiology* 49(2):183-186.

Ledger, S.E., Janssen, B.J., Karunairetnam, S., Wang, T., Snowden, K.C. 2010. Modified *carotenoid cleavage dioxygenase8* expression correlates with altered branching in kiwifruit (*Actinidia chinensis). New Phytologist* 188(3):803-813.

Lenhard, M., Jurgens, G., Laux, T. 2002. The *WUSCHEL* and *SHOOTMERISTEMLESS* genes fulfil complementary roles in *Arabidopsis* shoot meristem regulation. *Development* 129:3195-3206.

Leopold, A.C., Kriedemann, P.E. 1975. *Plant Growth and Development.* 2nd edn. McGraw-Hill, New York.

Letham, D.S. 1994. Cytokinins as phytohormones: sites of biosynthesis, translocation, and function of translocated cytokinin, p.57–80. In: David, W. S. M., Machteld, C. M. (eds.) *Cytokinins: Chemistry, Activity and Function*. CRC Press, Boca Raton, FL.

Letham, D.S. 1969. Cytokinins and their relation to other phytohormones. *Bioscience* 19(4):309-316.

Lever, B.G. 1985. 'Cultar'-a technical overview. *Acta Horticulturae* 179:459-466.

Li, C.J., Guevara, E., Herrera, J., Bangerth, F. 1995. Effect of apex excision and replacement by 1-naphtylacetic acid on cytokinin concentration and apical dominance in pea plants. *Physiologia Plantarum* 94(3):465-469.

Li, J., Wang, X., Mo, L., Li, R., Liang, M. 2003. A New Kiwifruit Cultivar 'Shimei' from *Actinidia deliciosa*. *Acta Horticulturae* 610:95-100.

Linsley-Noakes, G.C. 1989. Improving flowering of kiwifruit in climatically marginal areas using hydrogen cyanamide. *Scientia Horticulturae* 38(3):247-259.

Little, C.H.A., MacDonald, J.E. 2003. Effects of exogenous gibberellin and auxin on shoot elongation and vegetative bud development in seedlings of *Pinus sylvestris* and *Picea glauca*. *Tree physiology* 23(2):73-83.

Little, C.H.A., Savidge, R.A. 1987. The role of plant growth regulators in forest tree cambial growth. *Plant Growth Regulation* 6:137-169.

Liu, Z.H., Wang, W.C., Yan, S.Y. 1997. Effect of hormone treatment on callus formation and endogenous indole-acetic acid and polyamine contents of soybean hypocotyl cultivated *in vitro*. *Botanical Bulletin of Academia Sinica* 38:171-176.

Ljung, K., Bhalerao, R.P., Sandberg, G. 2001. Sites and homeostatic control of auxin biosynthesis in *Arabidopsis* during vegetative growth. *The Plant Journal* 28(4):465-474.

Loach, K. 1987. Hormone applications and adventitious root formation in cuttings-a critical review. *Acta Horticulturae* 227:126-133.

Lockard, R.G., Schneider, G.W., Kemp, T.R. 1982. Phenolic compounds in two size-controlling apple rootstocks. *Journal of the American Society for Horticultural Science* 107(2):183-186.

Lockard, R.G., Schneider, G.W. 1981. Stock and scion growth relationship and the dwarfing mechanism in apple. *Horticultural Reviews* 3:315-375.

Lomax, T.L., Muday, G.K., Rubery, P.H. 1995. Auxin transport. In: Davies PJ, ed. *Plant Hormones:* Dordrecht: Kluwer, 509-530.

Long, J.A., Barton, M.K. 1998. The development of apical embryonic pattern in *Arabidopsis*. *Development* 125:3027-3035.

Looney, N.E., Kamienska, A., Legge, R.L., Pharis, R.P. 1977. Metabolism of ^{3}H gibberellin A_4 in relation to flower initiation in apple. *Acta Horticulturae* 80:105-114.

Looney, N.E., Lane, W.D. 1983. Spur-type growth mutants of McIntosh apple: a review of their genetics, physiology and field performance. *Acta Horticulturae* 146:31-46.

Looney, N.E., Pharis, R.P., Noma, M. 1985. Promotion of flowering in apple trees with gibberellin A and C-3 epi-gibberellin A. *Planta* 165(2):292-294.

Looney, N.E., Taylor, J.S., Pharis, R.P. 1988. Relationship of endogenous gibberellin and cytokinin levels in shoot tips to apical form in four strains of McIntosh apple. *Journal of the American Society for Horticultural Science* 113:395-398.

Loreti, F., Piccotino, D., Baroni, G., Xiloyannis, C. 1991. Effect of propagation technique on vegetative growth and fruiting in kiwifruit. *Acta Horticulturae* 297:183-192.

Love, J., Bjorklund, S., Vahala, J., Hertzberg, M., Kangasjarvi, J., Sundberg, B. 2009. Ethylene is an endogenous stimulator of cell division in the cambial meristem of *Populus*. *Proceedings of the National Academy of Sciences of the United States of America* 106(14):5984-5989.

Lowe, R. 2005 Kiwi plant named '51-1785'. (US Patent No. 20060253946 P1) Google Patent: http://www.google.com/search?tbm=pts&hl=en&q= 20060253946.

Lowe, R., Cheng, C. 2008. Kiwifruit variety named 'HORT22D'. (US Patent No. 20080289070 P1) Google Patent: http://www.google.com/search?tbm=pts&hl=en&q=20080289070.

Lowe, R., Marsh, H. 1999. Kiwi plant named Tomua. (US Patent No. PP11065 P) Google Patent: http://www.google.com/search?tbm=pts&hl=en&q=USPP11065.

Lowe, R.G., Wang, Z.Y., Patterson, K.J., Gould, K.S. 1992. Rootstocks-enhancing the vine's potential. *New Zealand kiwifruit, special publication* 4:33-36.

Lowe, R.G., Hofstee, B. 2011. Kiwi plant named 'Bruce.(US Patent No.PP21613P3) Google Patent:http://www.google.com/search?tbm=pts&hl=en&q=USPP21613.

Luckwill, L. 1974. A new look at the process of fruit bud formation in apple. *XIX International Horticultural Congress. Warsaw* 3:237-245.

Luckwill, L.C., Silva, J.M. 1979. The effects of daminozide and gibberellic acid on flower initiation, growth and fruiting of apple cv. Golden Delicious. *Journal of Horticultra Science* 54:217-223.

Luckwill, L.C., Whyte, P. 1968. Hormones in the xylem sap of apple trees. *Society of Chemical Industry, London* 31:87-101.

Luckwill, L.C. 1970. The control of fruitfulness of apple trees, p. 237-254. In: Luckwill, L. C., Cutting, C. V. (eds.) *The Physiology of Tree Crops.* Academic Press, London.

Lyndon, R.F. 1998. *The Shoot Apical Meristem: Its Growth and Development*. Cambridge University Press, Cambridge, UK.

Marcelle, R., Sironval, C. 1963. Effect of gibberellic acid on flowering of apple trees. *Nature* 197:pp. 405.

Marini, R.P., Anderson, J.L., Barritt, B.H., Brown, G.R., J. Cline, W.P.C.J., .Domoto, P.A., Ferree, D.C., Garner, J., Greene, G.M., Hampson, C., Hirst, P., . Kushad, M.M., Mielke, E., Mullins, C.A., Parker, M., .Perry, R.L., Prive, J.P., Robinson, T., Rom, C.R., Roper, T., Schupp, J.R., Stover, E., R., U. 2000. Performance of 'Gala' apple on four semi-dwarf rootstocks; A five year summary of the 1994 NC-140 Semi-dwarf rootstock trial. *Journal of the American Pomological Society* 54:84-91.

Marth, P.C., Audia, W.V., Mitchell, J.W. 1956. Effects of gibberellic acid on growth and development of plants of various genera and species. *Botanical Gazette* 118(2):106-111.

Martin, G. 1987. Apical dominance. *HortScience* 22:824-833.

Martin, G.C., Stahly, E.A. 1967. Endogenous growth regulating factors in bark of M.9 and M.16 apple trees. *Proceedings of the Amererican Society for Horticultural Science.* 91:31-38.

Massai, R., Piccotino, D., Raffaelli, M., Xiloyannis, C., Baroni, G. 1997. Growth and yield of kiwifruit vines planted at different distances along the row. *Acta Horticuturae.* 444:169-174.

McPherson, H.G., Hall, A.J., Stanley, C.J. 1994. Seasonal and regional variation in budbreak and flowering of kiwifruit vines in New Zealand. *New Zealand Journal of Crop and Horticultural Science* 22(3):263-276.

McPherson, H.G., Richardson, A.C., Snelgar, W.P., Currie, M.B. 2001. Effects of hydrogen cyanamide on budbreak and flowering in kiwifruit (*Actinidia deliciosa* ‘Hayward'). *New Zealand Journal of Crop and Horticultural Science* 29(4):277-285.

Medford, J.I. 1992. Vegetative apical meristems. *The Plant Cell* 4(9):1029-1039.

Medjdoub, R., Val, J., Blanco, A. 2004. Prohexadione-Ca inhibits vegetative growth of ‘Smoothee Golden Delicious' apple trees. *Scientia Horticulturae* 101(3):243-253.

Michalczuk, L. 2002. Indole-3-acetic acid level in wood, bark and cambial sap of apple rootstocks differing in growth vigour. *Acta Physiologiae Plantarum* 24(2):131-136.

Miller, S.R. 1965. Growth inhibition produced by leaf extracts from size-controlling apple rootstocks. *Canadian Journal of Plant Science* 45:519-524.

Miller, S.A., Broom, F.D., Thorp, T.G., Barnett, A.M. 2001. Effects of leader pruning on vine architecture, productivity and fruit quality in kiwifruit (*Actinidia deliciosa* cv. Hayward). *Scientia Horticulturae* 91(3-4):189-199.

Miller, S.S., Tworkoski, T. 2003. Regulating vegetative growth in deciduous fruit trees. *Plant Growth Regulation Societ of America Quarterly* 31(1):8-46.

Miller, S.R., Nelson, S.H., Heeney, H.B. 1961. Studies on apple rootstock selections relating respiration rates to an anatomical method of predicting dwarfness. *Canadial Journal of Plant Sciences* 41(2):221-226.

Miller, S.S. 2002. Prohexadione-calcium controls vegetative shoot growth in apple. *Journal of Tree Fruit Production* 3(1):11-28.

Miller, S.S., Eldridge, B.J. 1986. Use of 6-benzylamino purine and promalin for improved canopy development in selected apple cultivars. *Scientia Horticulturae* 28(4):355-368.

Mok, D.W.S., Mok, M.C. 2001. Cytokinin metabolism and action. *Annual Review of Plant Physiology and Plant Molecular Biology* 52:89-118.

Moncaleán, P., Canal, M.J., Feito, I., Rodríguez, A., Fernández, B. 1999. Cytokinins and mineral nutrition in *Actinidia deliciosa* (Kiwi) shoots cultured *in vitro*. *Journal of Plant Physiology* 155(4-5):606-612.

Moncalean, P., Cañal, M.J., Fernandez, H., Fernández, B., Rodríguez, A. 2003. Nutritional and gibberellic acid requirements in kiwifruit vitroponic cultures. *In Vitro Cellular & Developmental Biology-Plant* 39(1):49-55.

Monteith, J.L., Moss, C.J. 1977. Climate and the efficiency of crop production in Britain. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 281(980):277-294.

Moore, T.C. 1979. *Biochemistry and Physiology of Plant Hormones*. Springer-Verlag New York.

Morgan, D.G., Mees, G.C. 1958. Gibberellic acid and the growth of crop plants. *The Journal of Agricultural Science* 50(1):49-59.

Moriguchi, K., Yano, T., Shinkai, S., Sagawa, M., Inoue, H., Ochi, M. 2002. Effects of girdling kiwifruit trees during the growth period on vegetative growth, fruit growth and quality. *Bulletin of Ehime Fruit Tree Experiment Station* 15:55-65.

Morris, D.A., Thomas, A.G. 1978. A microautoradiographic study of auxin transport in the stem of intact pea seedlings (*Pisum sativum* L.). *Journal of Experimental Botany* 29(1):147-157.

Morris, S.E., Cox, M.C.H., Ross, J.J., Krisantini, S., Beveridge, C.A. 2005. Auxin dynamics after decapitation are not correlated with the initial growth of axillary buds. *Plant Physiology* 138(3):1665-1672.

Mostafa, E.A.M., Saleh, M.M.S. 2006. Influence of spraying with gibberellic acid on behaviour of Anna apple trees. *Journal of Applied Sciences Research* 2(8):477-483.

Motosugi, H., Nishijima, T., Hiehata, N., Koshioka, M., Suguira, A. 1996. Endogenous gibberellins in the xylem exudate from apple trees. *Bioscience, Biotechnology, and Biochemistry* 60:1500-1502.

Müller, D., Leyser, O. 2011. Auxin, cytokinin and the control of shoot branching. *Annals of Botany* 107(7):1203-1212.

Multani, D.S., Briggs, S.P., Chamberlin, M.A., Blakeslee, J.J., Murphy, A.S., Johal, G.S. 2003. Loss of an MDR transporter in compact stalks of maize *br2* and sorghum *dw3* mutants. *Science* 302(5642):81-84.

Naito, R., Fujimoto, J., Yamamura, H. 1983. Effects of shoot vigor and SADH application to clusters before anthesis on berry set in Kyoho grapes. *American Journal of Enology and Viticulture* 34(3):142-147.

Nishimura, A., Tamaoki, M., Sakamoto, T., Matsuoka, M. 2000. Over-expression of tobacco knotted 1-type class1 homeobox genes alters various leaf morphology. *Plant and Cell Physiology* 41(5):583-590.

Nitsch, J.P. 1957. Growth responses of woody plants to photoperiodic stimuli. *Proceedings of the American Society for Horticultural Science* 70:512-525.

Nitsch, J.P., Nitsch, C. 1959. Photoperiodic effects in woody plants: Evidence for the interplay of growth-regulating substances. In: *Photoperiodism and Related Phenomena in Plants and Animals American association for the advancement of science. Washington, D.C.*

Nordstrom, A., Tarkowski, P., Tarkowska, D., Norbaek, R., Astot, C., Dolezal, K., Sandberg, G. 2004. Auxin regulation of cytokinin biosynthesis in *Arabidopsis thaliana*: A factor of potential importance for auxin-cytokinin-regulated development. *Proceedings of the National Academy of Sciences of the United States of America* 101(21):8039-8044.

Norelli, J.L., Jones, A.L., Aldwinckle, H.S. 2003. Fire blight management in the twenty-first century: Using new technologies that enhance host resistance in apple. *Plant Disease* 87(7):756-765.

Ockerse, R. 1970. The dependence of auxin-induced pea stem growth on gibberellin. *Botanical Gazette* 131(1):95-97.

Olien, W.C., Lakso, A.N. 1986. Effect of rootstock on apple (*Malus domestica*) tree water relations. *Physiologia Plantarum* 67(3):421-430.

Oliveira, C.M., Browning, G. 1993. Gibberellin structure-activity effects on flower initiation in mature trees and on shoot growth in mature and juvenile *Prunus avium*. *Plant Growth Regulation* 13(1):55-63.

Oliveira, M.M., Fraser, L.G. 2005. Actinidiaceae: *Actinidia* spp. Kiwifruit, p 2-29. In: Litz, R. E. (ed.) *Biotechnology of Fruit and Nut Crops*. CABI, Wallingford, UK.

Olsen, J.E. 2010. Light and temperature sensing and signalling in induction of bud dormancy in woody plants. *Plant Molecular Biology* 73(1-2):37-47.

Owens, C.L., Stover, E. 1999. Vegetative growth and flowering of young apple trees in response to prohexadione-calcium. *HortScience* 34:1194-1196.

Paderson, M.K., Burton, J.D., Coble, H.D., Collins, J.R., Fritz, D. 1997. Efficacy of Finish and its mechanism of action. *Proceedings Beltwide Cotton Conference* 2:1363-1365

Palmer, J.W. 1999. High density orchards: An option for New Zealand? *Compact Fruit Tree* 32:115-118.

Palmer, J.W. 2007. Apples and kiwifruit, can we learn from each other? *Acta Horticulturae* 753:359-368.

Palmer, J.W., Adams, H.M. 1997. Early results with intensive systems of apples on virus-free M.9 rootstock in New Zealand. *Acta Horticulturae* 451:487-494.

Palni, L.M.S., Burch, L., Horgan, R. 1988. The effect of auxin concentration on cytokinin stability and metabolism. *Planta* 174(2):231-234.

Parkinson, M., Yeoman, M.M. 1982. Graft formation in cultured, explanted internodes. *New Phytologist* 91(4):711-719.

Patterson, K.J., Currie, M.B., Black, M.Z., Martin, P.J., Nicholls, P., Jagielo, C. 2009. Kiwifruit root manipulations - potential for fruit dry matter improvements. *New Zealand Kiwifruit Journal* 194:29-32.

Pharis, R.P., King, R.W. 1985. Gibberellins and reproductive development in seed plants. *Annual Review of Plant Physiology* 36:517-568.

Phillips, I., Vlitos, A., Cutler, H. 1959. The influence of gibberellic acid upon the endogenous growth substances of the Alaska pea. *Contributions from Boyce Thompson Institute* 20:111-120.

Pilet, P.E., Galston, A.W. 1955. Auxin destruction, peroxidase activity, and peroxide genesis in the roots of *Lens culinaris*. *Physiologia Plantarum* 8(4):888-898.

Pilet, P.E. 1977. Growth inhibitors in growing and geostimulated maize roots. *Plant Growth Regulation*: pp 115-128.

Plich, H., Jankiewicz, L.S. 1970. Stimulation of apple internode elongation-synergistic effect of applied cytokinin, auxin and gibberellin. *Bull. Acad. Polon. Sci. Cl. V* 18:233-236.

Polito, V.S., Grant, J.A. 1984. Initiation and development of pistillate flowers in *Actinidia chinensis*. *Scientia Horticulturae* 22(4):365-371.

Potter, I., Fry, S.C. 1993. Xyloglucan endotransglycosylase activity in pea internodes [Effects of applied gibberellic acid]. *Plant Physiology* 103(1):235-241.

Preston, A. 1959. Apple rootstock studies. *Journal of Horticultural Science* 33:194-201.

Priestley, J.H. 1929. Cell growth and cell division in the shoot of the flowering plant. *New Phytologist* 28:54-81.

Prochazka, S., Jacobs, W.P. 1984. Transport of benzyladenine and gibberellin A_1 from roots in relation to the dominance between the axillary buds of pea (*Pisum sativum* L.) cotyledons *Plant Physiology* 76(1):224-227.

Proctor, J.T.A., Watson, R.L., Landsberg, J.J. 1976. The carbon budget of a young apple tree. *Journal of the American Society for Horticultural Science* 101:579-582.

Psarras, G., Merwin, I.A., Lakso, A.N., Ray, J.A. 2000. Root growth phenology, root longevity, and rhizosphere respiration of field grown ‘Mutsu' apple trees on ‘Malling 9' rootstock. *Journal of the American Society for Horticultural Science* 125:596-602.

Quinlan, J.D., Richardson, P.J. 1983. Effect of paclobutrazol (PP333) on apple shoot growth. *Acta Horticulturae*146:105-112.

Rademacher, W. 1993. PGRs-present situation and outlook. *Acta Horticulturae* 329:296-296.

Rademacher, W. 2000. Growth retardants: effects on gibberellin biosynthesis and other metabolic pathways. *Annual Reviews of Plant Physiology and Plant Molecular Biology* 51:501-531.

Rademacher, W., Bucci, T. 2002. New plant growth regulators: High risk investment? *HortTechnology* 12:64-67.

Rademacher, W., Spinelli, F., Costa, G. 2005. Prohexadione-Ca: Modes of action of a multifunctional plant bioregulator for fruit trees, *Acta Horticulturae* 727:95-106.

Ramirez, H. 1998. Identification of endogenous gibberellins in Red Delicious apple seeds. *Acta Horticulturae* 463: 231-233.

Ramirez, H., Hoad, G.V. 1981. Effects of growth substances on fruit-bud initiation in apple. *Acta Horticulturae* 120:131-136.

Rao, Y.V., Berry, W.E. 1941. The Carbohydrate relations of a single scion variety grafted on Malling rootsocks IX and XIII. A contribution of the physiology of dwarfing. *The Journal of pomology* 18:193-225.

Reed, R.C., Brady, S.R., Muday, G.K. 1998. Inhibition of auxin movement from the shoot into the root inhibits lateral root development in *Arabidopsis*. *Plant Physiology* 118(4):1369-1378.

Reid, J.B., Murfet, I.C., Potts, W.C. 1983. Internode length in *Pisum* II. Additional information on the relationship and the action of loci *Le, La, Cry, Na,* and *Lm*. *Journal of Experimental Botany* 34(3):349-364.

Reinecke, D.M., Bandurski, R.S. 1987. Auxin biosynthesis and metabolism. *Plant Hormones and Their Role in Plant Growth and Development. pp.*24-42.

Reinhardt, D., Mandel, T., and Kuhlemeier, C. 2000. Auxin regulates the initiation and radial position of plant lateral organs. *The Plant Cell* 12(4):507-518.

Reinhardt, D., Pesce, E.R., Stieger, P., Mandel, T., Baltensperger, K., Bennett, M., Traas, J., Friml, J., Kuhlemeier, C. 2003. Regulation of phyllotaxis by polar auxin transport. *Nature* 426:255-260.

Reiser, V., Raitt, D.C., Saito, H. 2003. Yeast osmosensor Sln1 and plant cytokinin receptor Cre1 respond to changes in turgor pressure. *The Journal of Cell Biology* 161(6):1035-1040.

Rezai, N. 2003. Taking the confusion out of confocal microscopy. *BioTeach Journal* 1:75–80.

Richards, D., Rowe, R.N. 1977. Effects of root restriction, root pruning and 6-benzylaminopurine on the growth of peach seedlings. *Annals of Botany* 41(4):729-740.

Richards, D.E., King, K.E., Ait-ali, T., and Harberd, N.P. 2001. How gibberellin regulates plant growth and development: a molecular genetic analysis of gibberellin signalling. *Annual Review of Plant Physiology and Plant Molecular Biology* 52:67-88.

Richards, F.J. 1951. Phyllotaxis: its quantitative expression and relation to growth in the apex. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 235(629):509-564.

Richardson, A.C., McAneney, K.J. 1990. Influence of fruit number on fruit weight and yield of kiwifruit. *Scientia Horticulturae* 42(3):233-241.

Richardson, P.J., Quinlan, J.D. 1986. Uptake and translocation of paclobutrazol by shoots of M.26 apple rootstock. *Plant Growth Regulation* 4(4):347-356.

Rinne, P.L.H., Welling, A., Vahala, J., Ripel, L., Ruonala, R., Kangasjarvi, J., van der Schoot, C. 2011. Chilling of dormant buds hyperinduces *FLOWERING LOCUS T* and recruits GA-inducible 1, 3-beta-glucanases to reopen signal conduits and release dormancy in *Populus*. *The Plant Cell* 23(1):130-146.

Riou-Khamlichi, C., Huntley, R., Jacqmard, A., Murray, J.A.H. 1999. Cytokinin activation of *Arabidopsis* cell division through a *D-type cyclin*. *Science* 283(5407):1541-1544.

Ripetti, V., Escoute, J., Verdeil, J.L., Costes, E. 2008. Shaping the shoot: the relative contribution of cell number and cell shape to variations in internode length between parent and hybrid apple trees. *Journal of Experimental Botany* 59(6):1399-1407.

Roberts, A.N., Blaney, L.T. 1967. Qualitative, quantitative, and positional aspects of interstock influence on growth and flowering of the apple. *Proceedings of the American Society for Horticultural Science*. 91:39-50.

Robinson, T.L. 1992. Physiological basis for orchard system perfomance. *Compact Fruit Tree* 25:24-28.

Robinson, T.L., Anderson, L., Azarenko, A., Barritt, B.H., Brown, G., Cline, J., Crassweller, R., Domoto, P., Embree, C., Fennell, A. 2004. Performance of cornell-geneva rootstocks across North America in multi location NC-140 rootstock Trials. *Acta Horticulturae* 658:241-245.

Robinson, T.L., Fazio, G., Aldwinckle, H.S., Hoying, S.A., Russo, N. 2006. Field performance of Geneva® apple rootstocks in the Eastern USA. *Sodininkyst ir daržininkyst* 25(3):181-191.

Robitaille H.A., Carlson, R.F. 1971. Response of dwarfed apple trees to stem injections of gibberellic and absiscic acid. *HortScience* 6:539-540.

Robitaille, H.A., Carlson, R.F. 1976. Gibberellic and abscisic acid-like substances and the regulation of apple shoot extension. *Journal of the American Society for Horticultural Science* 101:388-392.

Rogers, W.S. 1939. Root studies VIII. apple root growth in relation to rootstock, soil, seasonal and climatic factors. *Journal of Pomology and Horticultural Science* 17:99-130.

Rogers, W.S., Beakbane, A.B. 1957. Stock and scion relations. *Annual Reviews of Plant Phisology* 8:217-236.

Rom, R.C. and Carlson, R.F. 1987. *Rootstocks for Fruit Crops*. Wiley, New York.

Rosati, P., Gaggioli, D. 1989. Orchard response of micropropagated sour cherry and apple cultivars. *Scientia Horticulturae* 39(3):201-209.

Ross, J.J. 1998. Effects of auxin transport inhibitors on gibberellins in pea. *Journal of Plant growth regulation* 17(3):141-146.

Ross, J.J., Davidson, S.E., Wolbang, C.M., Bayly-Stark, E., Smith, J.J., Reid, J.B. 2002. Developmental regulation of the gibberellin pathway in pea shoots. *Functional Plant Biology* 30(1):83-89.

Ross, J.J., Murfet, I.C., Reid, J.B. 1997. Gibberellin mutants. *Physiologia Plantarum* 100(3):550-560.

Ross, J.J., O'Neil, D.P., Smith, J.J., Hubb, L., Kerckhoffs, J., Elliot, R.C. 2000. Evidence that auxin promotes gibberellin A1 biosythesis in pea. *The plant Journal* 21(6):547-552.

Ross, J.J., O'Neill, D.P., Wolbang, C.M., Symons, G.M., Reid, J.B. 2001. Auxin-gibberellin interactions and their role in plant growth. *Journal of Plant Growth Regulation* 20:346-353.

Ross, J.J., Reid, J.B., Dungey, H.S. 1992. Ontogenetic variation in levels of gibberellin A in *Pisum*. *Planta* 186(2):166-171.

Ross, J.J., Weston, D.E., Davidson, S.E., Reid, J.B. 2011. Plant hormone interactions: how complex are they? *Physiologia Plantarum* 141(4):299-309.

Rouffa, A.S., Gunckel, J.E. 1951. Leaf initiation, origin, and pattern of pith development in the Rosaceae. *American Journal of Botany* 38(4):301-307.

Rupp, H.M., Frank, M., Werner, T., Strnad, M., Schmulling, T. 1999. Increased steady state mRNA levels of the *STM* and *KNAT1* homeobox genes in cytokinin overproducing *Arabidopsis thaliana* indicate a role for cytokinins in the shoot apical meristem. *The Plant Journal* 18(5):557-563.

Rusholme Pilcher, R.L., Celton, J.M., Gardiner, S.E., Tustin, D.S. 2008. Genetic markers linked to the dwarfing trait of apple rootstock 'Malling 9'. *Journal of the American Society for Horticultural Science* 133(1):100-106.

Ryugo, K. 1985. Promotion and inhibition of flower initiation and fruit set by plant manipulation and hormones, a review. *Acta Horticulturae* 179:301-308.

Ryugo, K., Breen, P.J. 1974. Indoleacetic acid metabolism in cuttings of plum (*Prunus cerasifera* x *P. munsoniana* cv. Marianna 2624). *Proceedings of the American Society for Horticultural Science.* 99:247-251.

Sachs, R.M., Bretz, C.F., Lang, A. 1959. Shoot histogenesis: the early effects of gibberellin upon stem elongation in two rosette plants. *American Journal of Botany* 46(5):376-384.

Sachs, R.M. 1965. Stem elongation. *Annual Review of Plant Physiology* 16:73-96.

Sachs, R.M., Lang, A., Bretz, C.F., Roach, J. 1960. Shoot histogenesis: subapical meristematic activity in a caulescent plant and the action of gibberellic acid and Amo-1618. *American Journal of Botany* 47(4):260-266.

Sachs, T., Thimann, K.V. 1967. The role of auxins and cytokinins in the release of buds from dominance. *American Journal of Botany* 54(1):136-144.

Sachs, T. 2005. Auxin's role as an example of the mechanisms of shoot/root relations. *Plant and Soil* 268(1):13-19.

Sakamoto, T., Kamiya, N., Ueguchi-Tanaka, M., Iwahori, S., Matsuoka, M. 2001. *KNOX* homeodomain protein directly suppresses the expression of a gibberellin biosynthetic gene in the tobacco shoot apical meristem. *Genes & development* 15:581-590.

Sakamoto, T., Morinaka, Y., Ishiyama, K., Kobayashi, M., Itoh, H., Kayano, T., Iwahori, S., Matsuoka, M., Tanaka, H. 2003. Genetic manipulation of gibberellin metabolism in transgenic rice. *Nature Biotechnology* 21:909-913.

Salamini, F. 2003. Hormones and the Green Revolution. *Science Signalling* 302(5642):71-72.

Sale, P., Lyford, P. 1990. Cultural, management and harvesting practices for kiwifruit in New Zealand. *In* IJ Warington, GC Weston, eds, Kiwifruit: Science and Management. New Zealand Society for Horticultural Science, Wellindton, pp 247-296.

Salinero, M.C., Vela, P., Sainz, M.J. 2009. Phenological growth stages of kiwifruit (*Actinidia deliciosa* 'Hayward'). *Scientia Horticulturae* 121(1):27-31.

Sansavini, S., Bassi, D., Giunchi, L. 1980. Tree efficiency and fruit quality in high-density apple orchards. *Acta Horticulturae* 114:114-136.

Sansavini, S., Bonomo, R., Finotti, A., Palara, U. 1986a. Foliar and soil application of paclobutrazol on Gloster apple. *Acta Horticulturae* 179:489-496.

Sansavini, S., Marangoni, B., Buscaroli, C., Corelli, L., Tazzari, G. 1986b. The relationship between spacing and rootstock effects in an intensive planting trial of two apple cultivars. *Acta Horticulturae*160:23-37.

Saure, M.C. 1987. Summer pruning effects in apple-a review. *Scientia Horticulturae* 30:253-282.

Saure, M.C. 1992. Interference of pruning with endogenous growth control. *Acta Horticulturae* 322:241-248.

Schaeffer, G.W., Sharpe Jr, F.T. 1969. Release of axillary bud inhibition with benzyladenine in tobacco. *Botanical Gazette* 130(2):107-110.

Schechter, I., Elfving, D.C., Proctor, J. 1991. Canopy development, photosynthesis, and vegetative growth as affected by apple rootstocks. *Fruit Varieties Journal* 45:229-237.

Schechter, I., Proctor, J.T.A., Elfving, D.C. 1994. Carbon exchange rate and accumulation in limbs of fruiting and nonfruiting apple trees. *Journal of the American Society for Horticultural Science* 119(2):150-156.

Schomburg, F.M., Bizzell, C.M., Lee, D., Zeevaart, J.A.D., Amasino, R.M. 2003. Overexpression of a novel class of gibberellin 2-oxidases decreases gibberellin levels and creates dwarf plants. *The Plant Cell* 15(1):151-163.

Schoof, H., Lenhard, M., Haecker, A., Mayer, K.F.X., Jurgens, G., Laux, T. 2000. The stem cell population of *Arabidopsis* shoot meristems is maintained by a regulatory loop between the *CLAVATA* and *WUSCHEL* genes. *Cell* 100(6):635-644.

Seal, A.G 2003. The plant breeding challenges to making kiwifruit a worldwide mainstream fresh fruit. *Acta. Horticulturae* 610:75-80.

Sebanek, J. 1965. The interaction of endogenous gibberellins in correlation with cotyledons and axillary buds in the pea (*Pisum sativum* L.). *Biologia Plantarum* 7(3):194-198.

Seleznyova, A., Thorp, T.G., Barnett, A.M., Costes, E. 2002. Quantitative analysis of shoot development and branching patterns in *Actinidia*. *Annals of Botany* 89(4):471-482.

Seleznyova, A.N., Thorp, T., White, M., Tustin, S., Costes, E. 2003. Application of architectural analysis and AMAPmod methodology to study dwarfing phenomenon: the branch structure of 'Royal Gala' apple grafted on dwarfing and non-dwarfing rootstock/interstock combinations. *Annals of Botany* 91(6):665-672.

Seleznyova, A.N., Tustin, D.S., Thorp, T.G. 2008. Apple dwarfing rootstocks and interstocks affect the type of growth units produced during the annual growth cycle: precocious transition to flowering affects the composition and vigour of annual shoots. *Annals of Botany* 101(5):679-687.

Seleznyova, A.N., Tustin, D.S., White, M.D., Costes, E. 2005. Analysis of the earliest observed expression of dwarfing rootstock effects on young apple trees, using markovian models. *Acta Horticulturae 732*:79-84.

Shani, E., Yanai, O., Ori, N. 2006. The role of hormones in shoot apical meristem function. *Current Opinion in Plant Biology* 9:484-489.

Sheriff, L.J., McKay, M.J., Reid, J.B., Willis, C.L. 1994. Decapitation reduces the metablism of gibberellinA_{20} to A_1 in *Pisum sativum* L. decreasing the *Le/le* difference. *Plant Physiology* 104(1):277-280.

Shim, K.K., Ha, Y.M. 1999. Kiwifruit production and research in Korea. *Acta Horticulturae* 498:127-132.

Shimizu-Sato, S., Tanaka, M., Mori, H. 2009. Auxin–cytokinin interactions in the control of shoot branching. *Plant Molecular Biology* 69(4):429-435.

Sim, B.L., Lawes, G.S. 1981. Propagation of kiwifruit from stem cuttings. *Gartenbauwissenschaft* (Germany, F.R) 46(2):65-68.

Simons, R.K. 1986. Graft-union characteristics as related to dwarfing in apple (*Malus domestica* Borkh.). *Acta Horticulturae* 160:57-66.

Simons, R.K. 1987. Compatibility and stock-scion interactions as related to dwarfing. In: R.C., R. and Carlson, R. F. (eds.), Rootstocks for fruit crops. John Wiley & sons Inc, New York.

Skene, K.G.M. 1967. Gibberellin-like substances in root exudate of *Vitis vinifera*. *Planta* 74(3):250-262.

Skene, K.G.M. 1972. Cytokinins in the xylem sap of grape vine canes: Changes in activity during cold-storage. *Planta* 104(1):89-92.

Skogerbo, G. 1992. Effects of root pruning and trunk girdling on xylem cytokinin content of apple (*Malus* x *domestica* Borkh.). *Norwegian Journal of Agricultural Sciences* 6:499-499.

Smit, M., Meintjes, J.J., Jacobs, G., Stassen, P.J.C., Theron, K.I. 2005. Shoot growth control of pear trees (*Pyrus communis* L.) with prohexadione-calcium. *Scientia Horticulturae* 106(4):515-529.

Smith, G.C., Clark, C.J., Boldingh, H.L. 1992a. Seasonal accumulation of starch by components of the kiwifruit vine. *Annals of Botany* 70(1):19-25.

Smith, V.A., Knatt, C.J., Gaskin, P., Reid, J.B. 1992b. The distribution of gibberellins in vegetative tissues of *Pisum sativum* L.: I. Biological and biochemical consequences of the *le* mutation. *Plant Physiology* 99(2):368-371.

Smulders, M.J.M., Croes, A.F., Wullems, G.J. 1988. Polar transport of 1-naphthaleneacetic acid determines the distribution of flower buds on explants of tobacco. *Plant Physiology* 88(3):752-756.

Snelgar, W.P., Hopkirk, G. 1988. Effect of overhead shading on yield and fruit quality of kiwifruit (*Actinidia deliciosa*). *Journal of Horticultural Science* 63(4):731-742.

Snelgar, W.P., Hopkirk, G., Seelye, R.J., Martin, P.J., Manson, P.J. 1998. Relationship between canopy density and fruit quality of kiwifruit. *New Zealand Journal of Crop and Horticultural Science* 26(3):223-232.

Snelgar, W.P., Manson, P.J. 1992. Determination of the time of flower evocation in kiwifruit vines. *New Zealand Journal of Crop and Horticultural Science* 20(4):439-447.

Snelgar, W.P., Thorp, T.G. 1988. Leaf area, final fruit weight and productivity in kiwifruit. *Scientia Horticulturae* 36:241-249.

Snelgar, W.P., Manson, P.J., Martin, P.J. 1992. Influence of time of shading on flowering and yield of kiwifruit vines. *Journal of Horticultural Science* 67:481-487.

Snyder, W.E. 1949. Some responses of plants to 2, 3, 5-triiodobenzoic acid. *Plant Physiology* 24(2):195-206.

Soejima, J., Bessho, H., Tsuchiya, S., Komori, S., Abe, K., Kotoda, N. 1998. Breeding of Fuji apples and performance on JM rootstocks. *Compact Fruit Tree* 31(1):22-24.

Soejima, J., Yoshida, Y., Haniuda, T., Bessho, H., Tsuchiya, S., Masuda, T., Komori, S., Sanada, T., Ito, Y., Sadamori, S., Kashimura, Y. 2010. New aple rootstocks 'JM 1', 'JM 7' and 'JM 8'. *Bulletin of the National Institute of fruit Sciene* 11:1-16.

Solereder, H. 1908. *Systematic Anatomy of the Dicotyledons: A Handbook for Laboratories of Pure and Applied Botany* (English ed., translated by Boudle, L. A, Fritsch, F. E.). Clarendon Press, Oxford.

Sorce, C., Massai, R., Picciarelli, P., Lorenzi, R. 2002. Hormonal relationships in xylem sap of grafted and ungrafted *Prunus* rootstocks. *Scientia Horticulturae* 93(3-4):333-342.

Soumelidou, K., Morris, D.A., Battey, N.H., Barnett, J.R., John, P. 1994a. Auxin transport capacity in relation to the dwarfing effect of apple rootstocks. *Journal of Horticultural Science* 69:719-725.

Spray, C., Phinney, B.O., Gaskin, P., Gilmour, S.J., MacMillan, J. 1984. Internode length in *Zea mays* L. *Planta* 160(5):464-468.

Srinivasan, C., Mullins, M.G. 1981. Physiology of flowering in the grapevine a review. *American Journal of Enology and Viticulture* 32(1):47-63.

Stanica, F., Peticila, A.G., Davidescu, V.E., Dumitrascu, M., Madjar, R.M. 2002. Use of composed rooting substrates for kiwifruit (*Actinidia* sp.) hardwood cuttings propagation. *Acta Horticulturae* 608:249-251.

Stant, M.Y. 1961. The effect of gibberellic acid on fibre-cell length. *Annals of Botany* 25(4):453-462.

Steffens, G.L., Byun, J.K., Wang, S.Y. 1985. Controlling plant growth via the gibberellin biosynthesis system–I. Growth parameter alterations in apple seedlings. *Physiologia Plantarum* 63(2):163-168.

Steffens, G.L., Hedden, P. 1992. Effect of temperature regimes on gibberellin levels in thermosensitive dwarf apple trees. *Physiologia Plantarum* 86(4):539-543.

Stuart, N.W., Cathey, H.M. 1961. Applied aspects of the gibberellins. *Annual Review of Plant Physiology* 12:369-394.

Sussex, I.M., Kerk, N.M. 2001. The evolution of plant architecture. *Current Opinion in Plant Biology* 4(1):33-37.

Suezawa, K., Matsuta, N., Omura, M., Yamaki, S. 1988. Plantlet formation from cell suspensions of kiwifruit (*Actinidia chinensis* Planch. var. chinensis). *Scientia Horticulturae* 37(1-2):123-128.

Swarbrick, T. 1929. Factors governing flower bud formation. VII. The seasonal elongation of apple varieties on some vegetative rootstocks and its possible relation to fruit bud formation. *Journal of Pomology Horticultural Science* 7:100-129.

Tafazoli, E., Beyl, C. 1993. Changes in endogenous abscisic acid and cold hardiness in *Actinidia* treated with triazole growth retardants. *Journal of Plant Growth Regulation* 12(2):79-83.

Takahashi, N., Kobayashi, M. 1991. Organ-specific gibberellins in rice: roles and biosynthesis. In: Takahashi N. Phinney BO, MacMillan J (eds) Gibberellins. Springer, Berlin Heidelberg New York, pp 9-21.

Tamas, I.A., Schlossberg-Jacobs, J.L., Lim, R., Friedman, L.B., Barone, C.C. 1989. Effect of plant growth substances on the growth of axillary buds in cultured stem segments of *Phaseolus vulgaris* L. *Plant Growth Regulation* 8(2):165-183.

Tanaka-Ueguchi, M., Itoh, H., Oyama, N., Koshioka, M., Matsuoka, M. 1998. Over-expression of a tobacco homeobox gene, *NTH15*, decreases the expression of a gibberellin biosynthetic gene encoding GA_{20}-oxidase. *The Plant Journal* 15(3):391-400.

Tandonnet, J.P., Cookson, S.J., Vivin, P., Ollat, N. 2010. Scion genotype controls biomass allocation and root development in grafted grapevine. *Australian Journal of Grape and Wine Research* 16(2):290-300.

Tanimoto, E., Yanagishima, N., Masuda, Y. 1967. Effect of gibberellic acid on dwarf and normal pea plants. *Physiologia Plantarum* 20(2):291-298.

Tersoglio, E., Naranjo, G. 2009. Identification of the beginning of the ecodormancy in " Bing" sweet cherry variety. *ITEA* 105:272-281.

Testolin, R. 1990. Kiwifruit yield efficiency, plant density, and bud number per surface unit. *Journal of the American Society for Horticultural Science* 115(5):704-707.

Theron, K.I., Steyn, W.J. 2007. Girdling: Science behind the age-old technique. *Acta Horticulturae* 800:51-60.

Thimann, K.V. 1937. On the nature of inhibitions caused by auxin. *American Journal of Botany* 24(7):407-412.

Thomidis, T. 2007. Field Susceptibility of Four Peach Rootstocks to *Phytophtora citrophthora* and *P. syringae*. *Phytopathologia Mediterranea* 39:404-409.

Thorp, G., Barnett. A, Lowe. R, Palmer. J, Clearwater. M, Duffy. A, Hofstee. B, Paterson. T, M, B., Hoyt. S, Manning. M, McKenna. C, and S, D. 2012. Rootstock Guide.

Titova, J.S., Shishkanov, G.V. 1977. Photosynthesis and pigment content in apple on different rootstocks. *Horticultural Abstracts* 47:4283.

Tombesi, A., Antognozzi, E., Palliotti, A. 1993. Influence of light exposure on characteristics and storage life of kiwifruit. *New Zealand Journal of Crop and Horticultural Science* 21(1):85-90.

Tooke, F., Battey, N. 2003. Models of shoot apical meristem function. *New Phytologist* 159(1):37-52.

Torr, T. 2009. December canopy management and dry matter in kiwifruit. Technical Tips (December 2009), East Pack Ltd., Te Puke, NZ. http://www.eastpack.co.nz/vdb/document/85

Traas, J., Vernoux, T. 2002. The shoot apical meristem: the dynamics of a stable structure. *Philosophical Transactions of the Royal Society: Biological Sciences* 357:737-747.

Tromp, J. 1973. The interactiont of growth regulating substances and tree orientation on growth and flower bud formation in apple. *Acta Horticulturae* 34:185-188.

Tromp, J. 1982. Flower-bud formation in apple as affected by various gibberellins. *Journal of Horicultural Science* 57:277-282.

Tromp, J. 1996. Sylleptic shoot formation in young apple trees exposed to various soil temperature and air humidity regimes in three successive periods of the growing season. *Annals of botany* 77(1):63-70.

Tromp, J., Ovaa, J. 1990. Seasonal changes in the cytokinin composition of xylem sap of apple. *Journal of Plant Physiology* 136(5):606-610.

Tsukamoto, Y., Sano, Y. 1958. Studies on the dormancy of potato tuber. I. The relation between gibberellin treatment and change of growth substance. *Bulletin Research Institute. Food Science. Kyoto University* 21:39-50.

Tsukaya, H. 2006. Mechanism of leaf-shape determination. *Annual Review of Plant Biology* 57:477-496.

Tubbs, F.R. 1951. East Malling Research Station. *Proceedings of the Royal Society of London. Series B, Biological Sciences* 139(894):1-18.

Tubbs, F.R. 1967. Tree size control through dwarfing rootstocks. *Proceedings of XVII International Horticultural Congress* 3:43-56.

Tubbs, F.R. 1973. Research field in the interaction of rootstock and scion in woody perenials-Part 2. *Horticutural Abstracts* 43:325-335.

Tukey, H.B. 1964. Dwarfed Fruit Trees. Macmillan, New York.

Tukey, L.D. 1982. Vegetative control and fruiting on mature apple trees treated with PP333. *Acta Horticulturae 137*:103-110.

Turnbull, C.G.N., Raymond, M.A.A., Dodd, R.I.C., Morris, S.E. 1997. Rapid increases in cytokinin concentration in lateral buds of chickpea (*Cicer arietinum* L.) during release of apical dominance. *Planta* 202(3):271-276.

Tustin, D.S., Cashmore, W.M., Bensley, R. 2001. Pomological and physiological characteristics of Slender Pyramid central leader apple (*Malus domestica*) planting systems grown on intermediate vigour, semi-dwarfing, and dwarfing rootstocks. *New Zealand Journal of Crop and Horticultural Science* 29(3):195-208.

Tustin, D.S., Palmer, J.W. 2008. Adoption and implementation of intensive planting system for apple - the New Zealand experience *Acta Horticulturae* 772:475-479.

Tworkoski, T.T., Miller, S. 2007. Rootstock effect on growth of apple scions with different growth habits. *Scientia Horticulturae* 111(4):335-343.

Ucler, A., Parlak, S., and Yucesan, Z. 2004. Effects of IBA and cutting dates on the rooting ability of semi-hardwood kiwifruit (*Actinidia deliciosa* A. Chev.) Cuttings. *Turkish Journal of Agriculture & Forestry* 28:195-201.

Umehara, M., Hanada, A., Yoshida, S., Akiyama, K., Arite, T., Takeda-Kamiya, N., Magome, H., Kamiya, Y., Shirasu, K., Yoneyama, K. 2008. Inhibition of shoot branching by new terpenoid plant hormones. *Nature* 455:195-200.

Ushiyama, K., Kita, N., Suyama, K., Aono, N. 1992. Bacterial canker disease of wild *Actinidia* plants as the infection source of outbreak of bacterial canker of

kiwifruit caused by *Pseudomonas syringae* pv. actinidiae. *Annals of Phytopathological Society of Japan* 58(3):426-426.

Vaadia, Y., Itai, C. 1968. Interrelationships of growth with reference to the distribution of growth substances, p. 65-79. In: Whittington, W. J. (ed.), Root growth . Butterworth, London.

van Hooijdonk, B.M. 2009. The physiological basis of vigour control by apple rootstocks-an unresolved paradigm: 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.

van Hooijdonk, B.M. 2012. Restricting basipetal transport of auxin in the rootstock stem of composite 'Royal Gala' apple trees reduces root growth and cytokinins in the xylem sap. In: (Poster), E. S. P. (ed.).

van Hooijdonk, B.M., Woolley, D.J., Warrington, I.J., Tustin, D.S. 2010. Initial alteration of scion architecture by dwarfing apple rootstocks may involve shoot-root-shoot signalling by auxin, gibberellin, and cytokinin. *Journal of Horticultural Science & Biotechnology* 85:59-65.

van Hooijdonk, B.M., Woolley, D.J., Warrington, I.J., Tustin, D.S. 2011. Rootstock modify scion architecture, endogenous hormones, and root growth of newly grafted 'Royal Gala' apple trees. *Jounal of the American Society for Horticultural Science* 136(2):93-102.

Van Onckelen, H., Palme, K., Verbelen, J.P., Van Der Straeten, D. 2003. The *Arabidopsis* mutant *alh1* illustrates a cross talk between ethylene and auxin. *Plant Physiology* 131(3):1228-1238.

Van Overbeek, J. 1935. The growth hormone and the dwarf type of growth in corn. *Proceedings of the National Academy of Sciences of the United States of America* 21(5):292-299.

Vanneste, J., Yu, J., Cornish, D., Max, S., Clark, G. 2011. Presence of *Pseudomonas syringae* pv. actinidiae, the causal agent of bacterial canker of kiwifruit, on symptomatic and asymptomatic tissues of kiwifruit. *New Zealand Plant Protection* 64:241-245.

Vernoux, T., Kronenberger, J., Grandjean, O., Laufs, P., Traas, J. 2000. *PIN-FORMED* 1 regulates cell fate at the periphery of the shoot apical meristem. *Development* 127:5157-5165.

Vogel, J.T., Walter, M.H., Giavalisco, P., Lytovchenko, A., Kohlen, W., Charnikhova, T., Simkin, A.J., Goulet, C., Strack, D., Bouwmeester, H.J. 2010. *SlCCD7* controls strigolactone biosynthesis, shoot branching and mycorrhiza induced apocarotenoid formation in tomato. *The Plant Journal* 61(2):300-311.

Vogler, H., Kuhlemeier, C. 2003. Simple hormones but complex signalling: Growth and development. *Current Opinion in Plant Biology* 6:51-56.

Volz, R.K., Gibbs, H.M., Popenoe, J. 1994. Branch induction on apple nursery trees: effects of growth regulators and defoliation. *New Zealand Journal of Crop and Horticultural Science* 22(3):277-283.

Wagenmakers, P.S., Wertheim, S.J. 1991. Planting systems for fruit trees in temperate climates. *Critical Reviews in Plant Sciences* 10(4):369-385.

Wang, Z.Y., Gould, K.S., Patterson, K.J. 1994. Comparitive root anatomy of five *Actinidia* species in relation to rootstock effects on kiwifruit flowering. *Annals of Botany* 73(4):403-413.

Wang, D., Pajerowska-Mukhtar, K., Culler, A.H., Dong, X. 2007. Salicylic acid inhibits pathogen growth in plants through repression of the auxin signalling pathway. *Current Biology* 17(20):1784-1790.

Ward, B.G., Henzell, R.F., Holland, P.T., Spiers, A.G. 1999. Non-spray methods to control invasive weeds in urban areas. *Urban weeds and pests* :1-5.

Ward, B.G., Henzell, R.F. 2004. Use of herbicidal gels on woody weeds. Document Science Internal Series. 162. Department of Conservation, Wellington. 21p.

Wardlaw, C.W. 1957. On the organization and reactivity of the shoot apex in vascular plants. *American Journal of Botany* 44(2):176-185.

Wareing, P.F. 1977. Growth substances and intigration in the whole plant. *Symposia of the Society for Experimental Biology* 31:337-366.

Warrington, I.J. 2000. New Zealand Horticulture–Success of an Export-led Industry. Annual IDFTA Conference report, Napier, New Zealand. http://www.virtualorchard.net/idfta/cft/2000/july/cftjuly2000p66.pdf.

Warrington, I.J., Ferree, D.C., Schupp, J., Frank, G., Dennis Jr, F.G., Baugher, T.A. 1990. Strain and rootstock effects on spur characteristics and yield of 'Delicious' apple strains. *Journal of the American Society for Horticultural Science* 115(3):348-356.

Weaver, R.J. 1956. Plant regulators in grape production. 26 p. California Agricultural Experiment Station Bulletin. 752.

Webster, A.D. 1997. A review of fruit tree rootstock research and development. *Acta Horticulturae* 451:53-74.

Webster, A.D. 2000. Rootstocks for temperate fruit crops: Current uses, future potential and alternative strategies. *Acta Horticulturae* 557:25-34.

Webster, A.D., Smith, R., Watkins, R. 1986. Apple rootstock studies. I: Preliminary evaluations of several MM.106 × M.27 hybrids. *Journal of Horticultural Science* 61:429-437.

Webster, A.D., Wertheim, S.J. 1993. Comparisons of species and hybrid rootstocks for European plum cultivars. *Journal of Horticultural Science* 68:861-869.

Webster, A.D. 1995. Rootstock and interstock effects on deciduous fruit tree vigour, precocity, and yield productivity. *New Zealand Journal of Crop and Horticultural Science* 23(4):373-382.

Webster, A.D. 2004. Vigour mechanisms in dwarfing rootstocks for temperate fruit trees. *Acta Horticulturae* 658:29-41.

Webster, T. 2002. Dwarfing rootstocks: past, present and future. *Compact Fruit Tree* 35:67-72.

Wertheim, S.J., Estabrooks, E.N. 1994. Effect of repeated sprays of 6-benzyladenine on the formation of sylleptic shoots in apple in the fruit-tree nursery. *Scientia Horticulturae* 60(1-2):31-39.

Wheeler, A.W. 1960. Changes in a leaf-growth substance in cotyledons and primary leaves during the growth of dwarf bean seedlings. *Journal of Experimental Botany* 11(2):217-226.

White, J. 1979. The plant as a metapopulation. *Annual Review of Ecology and Systematics* 10:109-145.

White, J.C., Medlow, G.C., Hillman, J.R., Wilkins, M.B. 1975. Correlative inhibition of lateral bud growth in *Phaseolus vulgaris* L. Isolation of indoleacetic acid from the inhibitory region. *Journal of Experimental Botany* 26(3):419-424.

White, M., Tustin, S. 2002. New apple rootstock alternatives for the southern hemisphere. *Compact Fruit Tree* 33:112-115.

Wickson, M., Thimann, K.V. 1958. The antagonism of auxin and kinetin in apical dominance. *Physiologia Plantarum* 11(1):62-74.

Williams, A.H. 1960. The distribution of phenolic compounds in apple and pear trees. *Phenolics in plant health and disease. Pergamon Press, New York*:3-7.

Williams, M.W., Edgerton, L.J. 1982. Vegetative growth control of apple and pear trees with ICI PP333 (Paclobutrazol) a chemical analog of Bayleton. *Acta Horticulturae* 137:111-116.

Williams, M.W., Stahly, E.A. 1968. Effect of cytokinins on apple shoot development from axillary buds. *HortScience* 3:68-69.

Williams, M.W. 1984. Use of bioregulators to control vegetative growth of fruit trees and improve fruiting efficiency. *Acta Horticulturae* 146:97-104.

Wolbang, C.M., Ross, J.J. 2001. Auxin promotes gibberellin biosynthesis in decapitated tobacco plants. *Planta* 214(1):153-157.

Wolbang, C.M., Chandler, P.M., Smith, J.J., Ross, J.J. 2004. Auxin from the developing inflorescence is required for the biosynthesis of active gibberellins in barley stems. *Plant Physiology* 134(2):769-776.

Wollenweber, B., Porter, J.R., Lübberstedt, T. 2005. Need for multidisciplinary research towards a second green revolution. *Current Opinion in Plant Biology* 8(3):337-341.

Woodward, A.W., Bartel, B. 2005. Auxin: regulation, action, and interaction. *Annals of Botany* 95(5):707-735.

Wünsche, J.N., Lakso, A.N. 2000. Apple tree physiology-implications for orchard and tree management. *Compact Fruit Tree* 33:82-88.

Xin, M., Qin, Z., Wang, L., Wu, T., Zhou, X. 2012. Genetic identification of a dwarf mutant in cucumber (*Cucumis sativus* L.). *African Journal of Biotechnology* 11(20):4493-4498.

Xu, Y.L., Li, L., Wu, K., Peeters, A.J.., Gage, D.A., Zeevaart, J.A. 1995. The *GA5* locus of *Arabidopsis thaliana* encodes a multifunctional gibberellin 20-oxidase: molecular cloning and functional expression. *Proceedings of the National Academy of Sciences of the United States of America* 92(14):6640-6644.

Yadava, U.L., Lockard, R.G. 1977. Abscisic acid and gibberellin-in three ungrafted apple (*Malus sylvestris*) rootstock clones. *Physiologia Plantarum* 40(4):225-229.

Yamada, T. 1993. The role of auxin in plant-disease development. *Annual review of phytopathology* 31:253-273.

Yamaguchi, S. 2008. Gibberellin metabolism and its regulation. *Annual review of plant biology* 59:225-251.

Yang, T., Davies, P.J., Reid, J.B. 1996. Genetic dissection of the relative roles of auxin and gibberellin in the regulation of stem elongation in intact light-grown peas. *Plant Physiology* 110(3):1029-1034.

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(2):297-300.

Yuri, J.A., Talice, J.G., Verdugo, J., del Pozo, A. 2010. Responses of fruit growth, quality, and productivity to crop load in apple cv. Ultra Red Gala/MM111. *Scientia Horticulturae* 127(3):305-312.

Zhu, L.H., Li, X.Y., Welander, M. 2008. Overexpression of the *Arabidopsis gai* gene in apple significantly reduces plant size. *Plant Cell Reports* 27(2):289-296.

Ziv, M., Melamud, H., Bernstein, Z., Lavee, S. 1981. Necrosis in grapevine buds (*Vitis vinifera* cv. Queen of Vineyard). II. Effect of gibberellic acid (GA_3) application. *Vitis* 20:105-114.