Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

The physiology and control of crown bud formation and development in gentians

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.



Uttara Chandani Samarakoon

Abstract

The spread in time to flower harvest maturity and an inability to produce an adequate number of crown buds during crop establishment, were perceived as problems by commercial growers of gentians for cut flowers. By identifying and investigating plant and environment related factors that influence growth and development, the current thesis aimed to investigate both the causes of these problems and identify potential solutions.

Crown buds which appeared during the previous growth cycle were the source of floral shoot production and, in both true seedlings and clonally propagated plants, these buds typically originated on the transition zone between shoots and roots. When formed on the transition zone the first and, as a consequence, apical bud of the crown bud cluster, originated adventitiously. The individual crown buds which subsequently initiated within the cluster developed as axillary buds from that apical bud. As evident from the appearance of two to five times more crown buds and shoots, and crown bud clusters, the naturally occurring short photoperiodic regime promoted the crown bud formation process, compared to a long photoperiodic regime. With the application of Ethephon, or Ethephon followed by either Thidiazuron or Paclobutrazol, it was possible to achieve a similar increase in the number of crown buds under the long photoperiodic regime. In contrast to the strong photoperiodic response, exposure to the cooler temperatures experienced outside did not influence the crown bud formation process.

Once formed, crown buds were under different types and degrees of dormancy (i.e. endo, para or eco). Dependent on both the developmental stage of plants and the hierarchical position of buds within a crown bud cluster, shoot emergence and subsequent development to flowering varied in the quantity, quality of shoots, and timing in response to dormancy breaking treatments (chilling, gibberellic acid (GA₃), or clipping), Hence when applied prior to shoot emergence, application of chilling to plants of the cultivar 'Diva' resulted in an eight-fold increase in the number of floral shoots, along with a 83 day reduction in the spread in time to harvest maturity. The requirement of chilling could be substituted by GA₃. In contrast, when applied following the start of shoot emergence, only application of GA₃ was effective in increasing shoot emergence and narrowing the spread in time to harvest maturity.

negative relationship with floral shoot length was evident with hierarchical position of the crown bud from proximal to the distal end. This influence of hierarchical position was most evident following application of chilling or GA₃, potentially explaining up to 28 days spread in duration to harvest maturity in 'Diva'. While the aforementioned factors related to shoot emergence, development and hierarchical position contribute to the sources of variability within a plant or an individual cluster, both the sources and extent of variation in time to harvest maturity, varied within cultivars, with wider spread in time to harvest maturity in 'Starlet' (41 days) than 'Diva' (35 days) and 'Spotlight' (29 days).

Within a bud cluster, a positive relationship between duration to harvest maturity and a

The present study has increased the understanding of factors contributing to growth and development of gentians as an ornamental crop. Treatments have been developed to allow manipulation of the formation of crown buds, subsequent emergence and compactness of flowering duration.

Acknowledgements

First and foremost, I would like to express my sincere gratitude to Dr. Keith Funnell, my primary supervisor for his constructive critique and suggestions as well as the encouragement from the stage of planning the experiments to the submission of the thesis. Your persistence in developing the writing style and patience in editing chapters multiple times is phenomenal. Without your excellent supervision, guidance and understanding this thesis would not have been possible. Your time and effort into this thesis is therefore, greatly appreciated.

I would like to convey my gratitude to Dr. David Woolley, my co-supervisor for sharing his passion in plant physiology, which led me to evaluate the results in different ways. Also I would like to appreciate your valuable and constructive critique of the chapters and the long discussions which enabled me to develop my thinking further.

I am highly grateful to Mr. Ed Morgan my co-supervisor for his valuable suggestions in the thesis and the encouragement throughout the research program. Also I would like to express my special thanks for providing me the opportunity to present results, locally and internationally.

I also wish to thank Mr. Garry Burge (late) my co-supervisor for his kind words and encouragement during the initial stage of the study.

I would like to acknowledge Dr. Barbara Ambrose for teaching me wax embedding protocols for light microscopy and Prof. Michael McManus for letting me use the facilities at IMBS. I am also thankful for the support extended for microscopy work from Dr. Dimitry Sokolov, Mr. Doug Hopcroft and Dr. Jianyu Chen at the Manawatu Microscopy and Imaging Centre, and Dr. Matthew Perrot at IVABS.

Technical staff at Institute of Natural Resources, as well as at Plant Growth Units is greatly acknowledged for the support extended in carrying out experiments, especially Mr. James Slater and Mrs. Kay Sinclair for spending many hours with me in heated greenhouses during data collection.

I would like to thank for the Statistical help I received from Mr. Duncan Hedderley and also grateful for being so understandable about my work.

Dr. Jocelyn Eason of the New Zealand Institute for Plant & Food Research Limited and AgroFresh, Philadelphia, USA is greatly appreciated for the provision of 1-MCP for experiments.

Financial support received for the research and publications from the New Zealand Institute for Plant & Food Research Limited, via New Zealand Foundation for Research, Science and Technology (contract C02X0702) and George Mason Charitable Trust is gratefully acknowledged.

It is due to the Massey University Doctoral Scholarship, Massey University Doctoral Completion Bursary and numerous other scholarships that I have managed to carry out my PhD without much of a financial constraint and, therefore, I would like to acknowledge my appreciation of the contribution they made.

I am highly grateful to Dilantha and Amal for supporting me in many ways

My friend Noveline must be remembered for all the happy times and talks we shared.

I owe my gratitude to my family especially my husband Suranga who had to convert his obsession with insects to electronics to support the family while I am studying, and for tolerating me when I was mentally strained. A big thank goes to my daughter Oneli, who kept me amused with her lively charisma. Watching you grow into the wonderful person you are now was the best form of relaxation I had during this period.

My sincere thanks also goes to my parents for empowering me to be who I am today by providing me with the required genetic composition, nurturing environment and continuous blessings. Ultimately, I have to agree most of the genes required for the PhD, came from my dad.

Finally I would pay gratitude to Buddhist philosophy, which enabled me to enhance the focus and concentration to my research and face the stressful situations with the right understanding.

Table of Contents

Abstract		i
Acknow	ledgements	iii
Table of	Contents	v
List of F	igures	xvii
List of T	ables	xxxv
List of A	Abbreviations	xxxvii
List of P	bublications	xxxviii
Chapter	1 General introduction and literature review	1
1.1	Introduction	1
1.2	Morphology, ecology and growth cycle	2
1.2.1	Morphology	2
1.2.2	Natural habitat of parental lines of recently introduced hybrids	3
1.2.3	Growth cycle of cultivars used in cut flower production	4
1.3	Problematic situation	6
1.3.1	Spread in time to flower harvest maturity	6
1.3.2	Vegetative propagation – crown buds	8
1.3.3	Potential commercial implications of the thesis	8
1.4	The research strategy for the thesis	9
1.5	Plant form and the developmental process	10
1.5.1	Types of buds	10
1.5.1.1	Apical, axillary and adventitious buds	10

1.5.2	Methods for morphological and anatomical investigations	12
1.5.2.1	Macroscopy	12
1.5.2.2	Microscopy	12
1.5.2.3	Staining of the tissues for microscopy	13
1.6	Influence of plant and environment related factors during the developmental process.	14
1.6.1	Initiation and development of buds	14
1.6.2	Bud dormancy	15
1.6.2.1	Paradormancy	17
1.6.2.2	Endodormancy	18
1.6.2.3	Ecodormancy	19
1.6.2.4	Involvement of growth regulators in initiation and development of	
	buds and bud dormancy	19
1.6.3	Shoot emergence, development and flowering	22
1.6.3.1	Vernalization and breaking endodormancy	22
1.6.3.2	Development of floral shoots	23
1.6.4	Concept of chill units	24
1.6.5	Concept of thermal time	25
1.7	Overview of the thesis	26
Chapter	2 Sources of variation in time to harvest maturity of flowering	
	shoots in three cultivars of gentian	29
2.1	Abstract	29
2.2	Introduction	29
2.3	Materials and methods	31
2.4	Results	33

2.4.1	Spread in time to harvest maturity among cultivars	33
2.4.1.1	Distribution of time to harvest maturity	33
2.4.1.2	Duration to shoot emergence	39
2.4.1.3	Correlations between key variables	39
2.4.1.4	Outliers in duration to harvest maturity	40
2.4.2	Spread in time to harvest maturity within cultivars	41
2.5	Discussion	43
2.6	Conclusion	46
Chapter	3 Seasonal Changes in Crown Buds and Flowering of Gentian	
	'Spotlight'	47
3.1	Abstract	47
3.2	Introduction	48
3.3	Materials and methods	50
3.3.1	General materials and methods	50
3.3.2	Procedure for tracking buds	50
3.3.3	Treatments and experimental design	51
3.3.4	Variables recorded	52
3.3.5	Dissection of crown buds	52
3.3.6	Data analysis	53
3.4	Results	53
3.4.1	Shoot emergence – first growth cycle	53
3.4.2	Shoot growth and development – first growth cycle	54
3.4.3	Timing of flowering – first growth cycle	56
3.4.4	Timing and quality of crown buds initiated – first growth cycle	57

3.4.5	Sequence of shoot and crown bud development	58
3.4.6	Crown bud morphology – beginning of growth second growth cycle	60
3.4.7	Shoot emergence – second growth cycle	60
3.4.8	Timing of bud appearance and shoot emergence	60
3.4.9	Influence of exposure of the crown	61
3.5	Discussion	62
3.5.1	Preformed crown buds	62
3.5.2	Appearance of new crown buds	63
3.5.3	Timing of bud appearance and shoot emergence	64
3.5.4	Influence of environment on shoot emergence and development	65
3.5.5	Conclusions	67
Chanter	4 Crown buds; changes in paradormancy during the growing	
Спарист		
Спарист	seasonseason	69
4.1		
_	season	69
4.1	Season	69
4.1	Abstract Introduction	69 69
4.1 4.2 4.3	Abstract	69 69 71
4.1 4.2 4.3 4.3.1	Abstract	697171
4.1 4.2 4.3 4.3.1 4.3.2	Abstract	69717171
4.1 4.2 4.3 4.3.1 4.3.2 4.3.3	Abstract	6971717171
4.1 4.2 4.3 4.3.1 4.3.2 4.3.3 4.3.4	Season Abstract Introduction Materials and Methods Plant materials and production Treatments Macroscopic examination Histological examination	697171717273
4.1 4.2 4.3 4.3.1 4.3.2 4.3.3 4.3.4 4.3.5	Season Abstract Introduction Materials and Methods Plant materials and production Treatments Macroscopic examination Histological examination Statistical design and data analysis	697171727374

4.5	Discussion	79
4.5.1	Conclusion	83
Chapter	5 Anatomy, ontogeny and origin of crown bud clusters of gentians	85
5.1	Abstract	85
5.2	Introduction	85
5.3	Materials and methods	89
5.3.1	Management of planting material and sampling	89
5.3.1.1	Experiment 1; Basic anatomical features of shoot/stem, root and	
	transition zone	89
5.3.1.2	Experiment 2; Crown bud clusters - Origin and ontogeny	90
5.3.1.3	Experiment 3; Hierarchical arrangement of crown buds	90
5.3.2	Histology - Light Microscope	91
5.3.3	Histology - Confocal Microscope	91
5.3.4	Macroscopic observations	92
5.4	Results and discussion	93
5.4.1	Experiment 1; Basic anatomical and morphological features of shoot, root, axillary buds and transition zone	
5.4.1.1	Histology of floral shoots and storage roots	93
5.4.1.2	Histology of axillary buds on floral shoots	93
5.4.1.3	Transition zone/crown tissue in seedlings	94
5.4.1.4	Development of crown buds in the transition zone of seedlings	99
5.4.2	Experiment 2; Crown bud clusters - Origin and ontogeny	105
5.4.2.1	Macroscopic features of the transition zone in plants from tissue	
	culture	105
5.4.2.2	Histological features of transition zone in plants from tissue culture	105

5.4.2.3	Origin for bud clusters; adventitious or axillary?	108
5.4.2.4	Contrast between a seedling and a plant from tissue culture, in	
	development of the transition zone and bud initials	110
5.4.3	Experiment 2; Crown buds within a cluster; origin and ontogeny	119
5.4.3.1	Macroscopic features of the arrangement of buds within a cluster	119
5.4.3.2	Histological studies of ontogeny of buds within the cluster	119
5.4.3.3	Buds within the cluster - axillary or adventitious?	121
5.4.4	Experiment 3; Hierarchical arrangement of crown buds within the cluster	126
5.4.4.1	Macroscopic features of the crown bud cluster \pm GA ₃	126
5.4.4.2	Confocal microscopy of shoot, storage root and cluster stem \pm GA ₃	126
5.4.4.3	Cluster stem	127
5.4.4.4	Cluster stem and the hierarchy of buds in the cluster	128
5.5	Overall Conclusion	134
Chapter	6 Crown bud formation process: preliminary screening for	
	influence of photoperiod, temperature and growth regulators	137
6.1	Abstract	137
6.2	Introduction	137
6.3	Materials and methods	142
6.3.1	General management of plants	142
6.3.2	Experiment One: Effect of photoperiod, temperature and GA ₃	144
6.3.3	Experiment Two: Effect of GA ₃ , TDZ, NAA, ethephon and NPA under long days	145
6.3.4	Experimental design, data collection and analysis	146
6.4	Results	147

6.4.1	Overview	147
6.4.2	Experiment One: Effect of photoperiod, temperature and GA ₃	147
6.4.3	Experiment Two: Effect of GA ₃ , TDZ, NAA, ethephon and NPA under long days	150
6.5	Discussion	153
6.5.1	Influence of environmental factors; photoperiod and temperature	153
6.5.2	Influence of exogenous growth regulators	155
6.5.2.1	Application of GA ₃ and its interaction with photoperiod	155
6.5.2.2	Influence of exogenous growth regulators (TDZ, ethephon and NPA)	
	under inhibitory long days	157
6.5.3	Conclusions	159
Chapter	7 Crown bud formation process: Influence of photoperiod, exogenous growth regulators & their interactions	161
7.1	Abstract	161
7.2	Introduction	161
7.3	Materials and methods	166
7.3.1	General management of plants	166
7.3.2	Experiment One: Photoperiod, TDZ, PBZ, and ethephon	167
7.3.2.1	Treatment application	167
7.3.2.2	Experimental design	168
7.3.2.3	Variables recorded	171
7.3.3	Experiment Two: Photoperiod and temperature	171
7.3.4	Experiment Three: Photoperiod, GA ₃ , PBZ, ethephon, 1-MCP alone or in combination	172
7.3.4.1	Treatment application	172

7.3.4.2	Experimental design	173
7.3.4.3	Variables recorded	173
7.3.5	Data Analysis	173
7.4	Results	174
7.4.1	Experiment One: Photoperiod, TDZ, PBZ, and ethephon	174
7.4.1.1	Overview	174
7.4.1.2	Quantitative changes in crown buds and crown shoots	175
7.4.1.3	Qualitative characteristics of crown buds and crown shoots	181
7.4.1.4	Buds and shoots from roots	184
7.4.1.5	Diameter of the transition zone	186
7.4.1.6	Growth of existing primary and secondary shoots and storage roots	187
7.4.1.7	Second growth cycle - survival and development	188
7.4.2	Experiment Two: Photoperiod and temperature	190
7.4.3	Experiment Three: Photoperiod, GA ₃ , PBZ, ethephon, 1-MCP alone or in combination	191
7.5	Discussion	192
7.5.1	Influence of photoperiod	192
7.5.1.1	Quantity and quality of crown buds and resulting shoots	192
7.5.1.2	Photoperiod induced photo-assimilate distribution	193
7.5.1.3	Cold vs photoperiod	194
7.5.2	Influence of gibberellins	196
7.5.3	Influence of TDZ	198
7.5.4	Influence of ethephon	199
7.5.5	Interactions between the plant growth regulators	203

7.5.6	Interaction between the photoperiodic regime and plant growth regulators	205
7.5.7	Practical horticultural implications	208
7.6	Conclusions	210
Chapter	8 Shoot emergence and development; Effect of chilling, GA ₃ , PBZ and developmental stage	213
8.1	Abstract	213
8.2	Introduction	213
8.3	Materials and methods	218
8.3.1	General management of plants	218
8.3.2	Treatment application	219
8.3.3	Experiment One; chilling or GA ₃ when crown buds unemerged	221
8.3.4	Experiment Two; chilling, GA ₃ , or PBZ when shoots recently emerged	221
8.3.5	Experiment Three; chilling, GA ₃ or PBZ when shoots emerged and developed	222
8.3.6	Variables recorded	222
8.3.7	Variables calculated	223
8.3.8	Experimental design and statistical analysis	224
8.4	Results	225
8.4.1	Overview	225
8.4.2	Experiment One; chilling or GA ₃ when crown buds unemerged	225
8.4.2.1	Influence in the third growth cycle	228
8.4.3	Experiment Two; chilling, GA ₃ , or PBZ when shoots recently emerged	230

8.4.3.1	Influence in the third growth cycle	233
8.4.4	Experiment Three; chilling, GA ₃ or PBZ when shoots emerged and developed	234
8.4.4.1	Influence in the third growth cycle	237
8.5	Discussion.	239
8.5.1	Possible roles of chilling and gibberellin in dormancy and flowering	239
8.5.1.1	Endodormancy	239
8.5.1.2	Paradormancy	241
8.5.1.3	Flowering and shoot development.	242
8.5.2	Crown bud formation process	245
8.5.3	Potential commercial applications	246
8.6	Conclusion	249
Chapter	9 Shoot emergence and development; Differential response of	
	buds within the cluster	251
9.1	Abstract	251
9.2	Introduction	251
9.3	Materials and methods	253
9.3.1	General management of plants	253
9.3.2	Treatment application	253
9.3.3	Procedure for tracking buds	255
9.3.4	Variables recorded and calculated	255
9.3.5	Experimental design and statistical analysis	256
9.4		
	Results	257
9.4.1	Results	

9.4.3	Shoot emergence - second growth cycle.	264
9.4.4	Morphological and developmental changes over two growth cycles	265
9.5	Discussion	266
9.5.1	Developmental potential and bud hierarchy	266
9.5.2	Presence of paradormancy and differential responses within the cluster	268
9.5.3	Future directions	271
9.5.4	Position of new crown bud clusters	272
9.5.5	Conclusion	273
Chapter	· 10 General discussion	275
10.1	Introduction	275
10.2	Crown bud formation process	277
10.2.1	Origin and ontogeny	277
10.2.2	Factors controlling the crown bud formation process	278
10.3	Shoot emergence and development through to harvest maturity	280
10.3.1	Types of dormancy and factors influencing shoot emergence	280
10.3.2	Shoot development post emergence	284
10.4	Potential commercial implications	286
10.4.1	An inadequate number of crown buds	286
10.4.2	The variation in spread of flower harvest maturity	287
10.4.3	Overall floral productivity	290
10.5	Conclusion	291
Appendi	ces	293

Appendix I; Stages of flower bud development and macroscopic evidence for	
flower initiation in 'Spotlight'	293
Appendix II; Plant to plant variability in number of crown buds	297
Appendix III; Morphological features of seedlings of parental lines	298
Appendix IV; Shoot emergence and development as influenced by clipping, chilling and GA ₃ in genotype '03/04-114'	299
Appendix V; Fresh weight of crown buds in response to photoperiodic regime, growth regulators and their interactions	301
Appendix VI; Article accepted for publication in Proceedings of the International Symposium of Ornamental Crops, IHC 2010	302
Bibliography	311

List of Figures

Figure 1.1 Recently developed cultivars of gentians; (A) 'Showtime Spotlight', (B) 'Showtime Diva' and, (C) 'Starlet'	2
Figure 1.2 Typical features of the crown present in cultivars of gentian derived primarily from the parental line of <i>Gentiana triflora</i> . Morphological features include; rhizome, crown buds, crown shoots, storage roots and feeder roots	3
Figure 1.3 Diagram depicting typical seasons corresponding to the stages of growth and development of mature gentian plants during an annual growth cycle, i.e. dormant crown buds in winter, shoot emergence in spring, and flowering in summer through autumn.	5
Figure 1.4 Number of floral shoots harvested per week from 3 cultivars in a commercial field in New Zealand (Feb-May 2007).	7
Figure 1.5 Diagram depicting signals and typical seasons corresponding to the different types of dormancy that is associated with dormancy of herbaceous geophytes and woody plants. Within the parts of the annual growth cycle, details are presented of the three types of bud dormancy (red text), the general seasons in which they might predominantly occur (green text) and relevant signals	16
Figure 1.6 Diagram presenting the developmental stages of apical dominance before and after release of apical dominance of the shoot apex.	
Figure 1.7 Schematic presentation of chapters within this PhD thesis according to their relevant objectives along a developmental pathway from bud through to anthesis for gentian plants.	28
Figure 2.1 Daily average temperature encountered in the open field during September 2007 to July 2008.	32
Figure 2.2 Variables recorded and calculated related to shoots reaching emergence and harvest maturity during the current study	32
Figure 2.3 Duration to harvest maturity among gentian cultivars ('Spotlight' ($n = 123$), 'Diva' ($n = 97$) and 'Starlet' ($n = 97$)) in either (A), calendar days; or (B), growing degree-days (GDD); (C), duration to shoot emergence in calendar days.	

Solid and dashed vertical lines in the centre of each box indicate the median and mean, respectively. Boundaries of box indicate 25^{th} and 75^{th} percentiles, whiskers indicate 10^{th} and 90^{th} percentiles and, solid dots indicate one or more individuals as outliers beyond these percentile limits. Figure 2.4 (A), Correlation between duration to shoot emergence and duration to harvest ('Diva' (); $R^2 = 0.33$, 'Spotlight'(); $R^2 = 0.35$, 'Starlet' (-); $R^2 = 0.51$); (B), GDD to shoot emergence and duration to harvest ('Diva'; $R^2 = 0.33$; 'Spotlight'; $R^2 = 0.35$, 'Starlet'; $R^2 = 0.70$); (C), Duration to shoot emergence	38
and duration to harvest from shoot emergence (Diva; $R^2 = 0.70$, 'Spotlight'; $R^2 = 0.43$, 'Starlet'; $R^2 = 0.45$); (D), GDD to shoot emergence and from shoot emergence to harvest ('Diva'; $R^2 = 0.54$, 'Spotlight'; $R^2 = 0.25$, 'Starlet'; $R^2 = 0.35$). Regression correlations were significant at $P < 0.0001$	40
Figure 2.5 Distribution of time to harvest maturity for floral shoots in individual plants of each gentian cultivar. Solid and dashed vertical lines in the centre of each box indicate the median and mean, respectively. Boundaries of box indicate 25 th and 75 th percentiles, whiskers indicate 10 th and 90 th percentiles and, solid dots indicate one or more individual shoots as outliers beyond these percentile	
limits.	42
Figure 3.1 Mean monthly temperature encountered in two treatment environments used during the first (September 2007 to August 2008) and second (September 2008 to August 2009) growth cycle	52
Figure 3.2 (A) and (B), respectively, photograph of a representative plant of 'Spotlight', and diagrammatic illustration of the same, illustrating key organs at the start of experiment, (B-H); Developmental sequence and physical location of buds and shoots on the crown over a period of 2 years, on one representative plant; size of buds ("primary-crown buds" were thick (> 1 mm diameter) and purple-coloured buds; "secondary-crown buds" were thin (< 1 mm diameter) and pale-coloured; "root buds" developed directly from storage roots). Shoots in the diagram are relative to the sizes observed during the experiment, where the smallest coloured dot is representative of less than 1 mm diameter in size; red open circle indicates a single bud cluster.	55
open enere maleaces a single oud cluster))

rigure 3.3 Cumulative total of emerged shoots, floral shoots, and new buds per	
plant for 'Spotlight' grown outside during the first growth cycle (September 2007 to Avgust 2008). The nettern of change within the protected environment.	
2007 to August 2008). The pattern of change within the protected environment	5.6
(data not presented) was the same as for those grown outside	30
Figure 3.4 Box and whisker plot for number of shoots harvested at each date for	
tracked or control within two growing environments, during the first (September	
2007 to August 2008) growth cycle for 'Spotlight'. Solid and dashed vertical	
lines in the centre of the box indicates median and mean respectively.	
Boundaries of box indicates 25 th and 75 th percentiles, whiskers show 10 th and	
90^{th} percentiles and solid dots show the outliers these percentile limits. (n =	
number of shoots harvested from all five replicates).	57
Figure 3.5 Pattern of change in; (A), shoot length until harvesting commenced	
and; (B), cumulative crown bud number per plant, in two different environments	
during first growth cycle for 'Spotlight'. Vertical bars = standard error.	
Regression correlations were significant at $P < 0.0001$.	59
Figure 3.6 During the first growth cycle, distribution of date of appearance of;	
(A), all buds on the crown; (B), date of appearance of crown of buds that	
emerged into shoots; (C), the date of appearance of un-emerged buds for	
'Spotlight'. Solid and dashed vertical lines in the centre of the box indicates	
median and mean respectively. Boundaries of box indicates 25 th and 75 th	
percentiles, whiskers show 10 th and 90 th percentiles and solid dots show the	
outliers.	61
Figure 4.1 Stages of shoot and bud development, and period of harvesting during	
the first growth cycle of plants of 'Spotlight', with reference to when plants were	
clipped (Clip 1 - 12 th December 2007, Clip 2 - 20 th February 2008, or control -	
15 th June 2008) and samples taken for microscopy and visual inspection.	
(Adapted from Figure 3.3).	73
Figure 4.2 Emerged Shoots of plants of 'Spotlight' clipped on 12 th December	
2007, as observed on 9 th January 2008; (A) greenhouse and, (B) outside. (C)	
Shoot emergence from the crown area of clipped plants from the outside and	
greenhouse environments. (D) Exposed crown showing no remaining crown	
buds on an outside grown plant	76

Figure 4.3 Two types of shoots which emerged following clipping of plants of	
'Spotlight'; (a) axillary shoot from current season's axils and, (b) rosette from	
crown bud.	76
Figure 4.4 Shoot among the flowing aliming in plants of 'Smotlight'. (A)	
Figure 4.4 Shoot emergence following clipping in plants of 'Spotlight'; (A)	
shoots directly from the crown as a rosette and as elongated shoots from the	
axillary buds of current season's floral shoot, (B) flower buds in shoots which	
emerged from an axillary bud (flower buds visible in March). cs - crown shoot,	
ab - axillary shoot.	77
Figure 4.5 Longitudinal sections of the crown of 'Spotlight' prior to applying	
clipping treatments; (A) Emergence of buds from different positions ('x' and	
'y') on a segment of crown; (B) Region 'x' of image (A) at higher	
magnification, presenting three crown buds, with two covered with a single scale	
leaf; (C) Region 'y' at higher magnification, presenting buds arising as a cluster.	
cb - crown bud, sl - scale leaf. Scale bar = 1 mm	79
co - crown odd, si - scare rear. Scare oar – 1 mm.	/ 0
Figure 4.6 (A) Macroscopic appearance of crown region of 'Spotlight' prior to	
applying clipping treatments; (B) transverse section at position I in image (A),	
immediately above the visible crown bud, (C) transverse section at position II, at	
the level of the bud where attachment to the main stem is visible, (D) transverse	
section at position III, below the crown bud. c - cortex, cb - crown bud, cs -	
shoot developed from the crown, sl - scale leaf, vc - vascular connection. Scale	
bars = 1 mm	79
Figure 5.1 Transverse section of (A-C) floral shoot and (D-F) storage root of	
'Spotlight' at increasing magnification; (A) full section indicating vascular ring,	
(B) magnified view of the vascular ring (red coloured square in Figure 5.1A),	
(C) arrangement of xylem vessels and phloem fibers, (D) full section of	
secondary storage root, (E) magnified view of secondary storage root indicating	
xylem of vascular tissues in the center, (F) full section of a primary storage root.	
(G) Radial arrangement of xylem vessels. As indicated by staining with	
Toluidine Blue, tissue stained light blue are xylem vessels: c - cortex, e -	
epidermis, p – pith, ph – phloem, vc - vascular cambium, vr - vascular ring, vy –	
vascular rays, x – xylem.	95

Figure 5.4 Acropetal sequence of transverse sections within the transition zone of a seedling of *Gentiana straminea* in its first growth cycle from germination; (A) intact plant illustrating transition zone, (B) full section closer to root with vascular bundles concentrated towards the centre, red square indicating position of magnified image of (C) and, within (C) indicating with the red square the magnified position shown in (D) illustrating xylem vessels. (E) Full section with vascular bundles diverging to form separate groups with red square magnified in (F), and four gaps indicated by "*" with extensions of vascular strands magnified in (G), (H), (I) and (J) as indicated by white arrows. (K) Development of a bud from one gap indicated by red box (L - Q) sectioned acropetally indicating base of the bud (white arrows) and, (R) primordia. (S) Shoot closer to rosette showing, (T) pith in the centre and, (U) separation of

vascular bundles. As indicated by staining with Toluidine Blue, tissue stained	
light blue are xylem vessels: lp - leaf primordia, p - pith, vb - vascular bundles,	
vs - vascular strands, x - xylem	2
Figure 5.5 Transverse sections of transition gone of a seedling of Centiana	
Figure 5.5 Transverse sections of transition zone of a seedling of <i>Gentiana</i>	
straminea in first growth cycle following germination; (A) intact plant	
illustrating transition zone, (B) section indicating development of two bud	
initials (white arrows) from one corner of the gaps of vascular grouping within	
the transition zone, (C, D) two well-developed buds with primordia at another	
corner of the transition zone. (E) Intact plant of a seedling of Gentiana	
straminea illustrating transition zone and crown buds, (F) longitudinal section	
through transitional zone illustrated in (E), indicating bud with primordia and,	
(G) vascular traces connecting bud and transition zone: lp - leaf primordia, tz -	
transition zone. 103	3
Figure 5.6 Longitudinal sections of transitional zone of a seedling of <i>G. Lutea</i> in	
first growth cycle following germination; (A) intact plant illustrating transition	
zone, (B) full section at transition zone indicating positions where magnified	
images of (C) and (F) were taken. (C) Positions of bud initials further magnified	
in (D) and (E), indicating bud initials at different stages of development.	
Positions indicated in (F) further magnified in (G), indicating bud initials and	
(H), buds with primordia. (I) Section taken on a different plane of axis,	
indicating the position magnified in (J), a potential site for bud initiation. bi -	
bud initial, tz - transition zone	4
Figure 5.7 Development of the transition zone and bud clusters from different	
positions on plants of 'Diva' produced from tissue culture, at increasing periods	
following deflasking; (A) transition zone of a plant before visible evidence of a	
bud cluster after 6 months, (B) bud cluster developed at a node after 9 months,	
•	
(C) thickening of the region below the lowest node after one year, (D) magnified	
view within red coloured square of (C), illustrating crown buds (white arrows).	
(E) Bud development at different positions on a single plant after one year (June	
2009; white arrow indicates a cluster arising from proximal end of storage	
roots), and (F) subsequent shoot emergence from the bud cluster on the	
transition zone of the same plant after a further 6 months. cbc - crown bud	
cluster, cb – crown bud, cs - crown shoot, n – node	2

derived from tissue culture following deflasking; (A) intact plant indicating transition zone, (B) full section at the base of the shoot indicating positions of magnified images of where (C), (D), and (E) were taken, illustrating vascular tissues and root initials. As indicated by staining with Toluidine Blue, tissue stained light blue are xylem vessels: ri - root initials, tz - transition zone, x - xylem.	113
Figure 5.9 Acropetal sequence of transverse sections of the eventual transition zone of a plant of 'Diva' derived from tissue culture six months following deflasking; (A) intact plant indicating transition zone, (B) partial section at the root end indicating unorganized tissue, (C) partial section indicating position from which magnified image of (D) was taken, indicating the vascular groupings at the base of the shoot. (E-F) progressive development of vascular ring and presence of xylem as two layers (black arrows). As indicated by staining with Toluidine Blue, tissue stained light blue are xylem vessels: c – cortex, tz - transition zone, x – xylem.	114
Figure 5.10 Acropetal sequence of transverse sections through the eventual transition zone (i.e. shoot) of a plant of 'Diva' derived from tissue culture at six months from deflasking; (A) a crown bud (red circle) and root initials at root end and, (B) vascular tissues converging at base of the shoot with (C-E), a bud cluster at stage 1, and the eventual transition zone with secondary thickening. (F-J) magnified images of acropetal sequence of transverse sections below, and through, the bud cluster indicating; (F-H) non-differentiated tissue at attachment to main shoot and, (I-J) association between buds within the cluster. As indicated by staining with Toluidine Blue, tissue stained light blue are xylem vessels; cb - crown bud, cbc - crown bud cluster, ps - primary shoot, x - xylem vessels.	115
Figure 5.11 Acropetal sequence of transverse sections through the eventual transition zone (i.e. shoot) of a plant of 'Diva' derived from tissue culture at six months from deflasking; (A) intact plant indicating transition zone and crown bud cluster, (B-F) full sections indicating changes in vascular ring at the lowest node and the initiation of a crown bud cluster (white arrow) at the node, (G-J) full sections below and through the bud cluster indicating changes along the bud	

Figure 5.8 Transverse sections of an eventual transition zone of a plant of 'Diva'

cluster at Stage 3, (G-H) at the base of the cluster and, (I-J) within the buds of	
the cluster. As indicated by staining with Toluidine Blue, tissue stained light	
blue are xylem vessels. ab – apical bud, c - cortex, cb – crown bud, cbc - crown	
bud cluster, ps - primary shoot, vg - gap in vascular ring, vr - vascular ring, x -	
xylem vessels.	116
Figure 5.12 Acropetal sequence of transverse sections through the eventual	
transition zone (i.e. shoot) of a plant of 'Diva' derived from tissue culture at	
eight months from deflasking; (A) intact plant indicating transition zone and	
crown bud cluster, (B) red object indicating segment magnified in (C)	
illustrating vascular grouping at the root end. (D) full section at the base of shoot	
illustrating enlarged cortex and, (E, F) development of the bud cluster on the	
shoot (white arrow), and the gap in the vascular ring on the other side (black	
arrow). As indicated by staining with Toluidine Blue, tissue stained light blue	
are xylem vessels. c - cortex, cbc - crown bud cluster, tz -transition zone, x -	
xylem vessels, vr - vascular ring.	117
Figure 5.13 Sections through crown bud clusters initiated at different positions	
on plants of 'Diva' during the first growth cycle from deflasking; (A, B)	
transverse sections indicating crown buds at proximal end of roots (red circle),	
(C) a crown bud cluster on the base of the primary shoot with visible vascular	
connection to vascular ring, (D) a bud cluster developing in between two shoots	
at the transition zone, (E) an intact plant with a new shoot emerging from the	
cluster located at the base of a shoot, (F) transverse section through the eventual	
transition zone of that plant indicating remaining buds within the cluster still	
enveloped within scale leaves (red circle), (G) bud clusters at the root end of	
eventual transition zone from two positions, one at a distance from the main	
shoot (red circle) and other with connection to primary shoot, indicating position	

Figure 5.14 Sequential dissections of a crown bud cluster on a plant of 'Diva' derived from tissue culture during its second growth cycle, i.e. 15 months from deflasking; (A - D) progressive removal of individual crown buds and scale

cb - crown bud, cbc - crown bud cluster, ps - primary shoot, vr - vascular ring, x

- xylem strands. 118

leaves from oldest (1) to most recent (6). Numbering and age based on distance from apex and location of scale leaves.	122
Figure 5.15 Longitudinal sections through crown bud clusters of 'Diva' during the first growth cycle following deflasking. The same bud cluster viewed along different planes of axis are presented in A-B, and another cluster in C-F, so as to show the association between buds and scale leaves, from plants with crown bud clusters at; (A-B) 'Stage 1', i.e. two crown buds in cluster, (C-F) 'Stage 2', i.e. three crown buds in cluster. cb - crown bud, sl - scale leaves	123
Figure 5.16 Transverse section through the transition zone of 'Diva' six months following deflasking; (A) indicating position of a crown bud cluster at <i>Stage 3</i> of development and magnified in, (B-D) a sequence of longitudinal sections indicating vascular connections between the buds and arrangement of scale leaves at different planes of axis. As indicated by staining with Toluidine Blue, tissue stained light blue are xylem vessels. ab - apical bud, cb - crown bud, ps - primary shoot, sl - scale leaves, tz - transition zone, vs - vascular strands	124
Figure 5.17 (A-E) Acropetal sequence of transverse sections of the crown bud cluster of the plant in Figure 5.12A, illustrating the arrangement of buds within a cluster at <i>Stage 4</i> of cluster development, with early initiated crown buds numbered as; cb1, cb2, cb3 and axillary buds of these buds as cb1.1 and cb2.1. Positions indicated in (C) and (E) are magnified in (F) & (G) to indicate axillary buds and illustrate the greater number of primordia within the earliest initiated bud (cb1) of the cluster and, (H) to indicate latest bud initiated from the cluster stem. cb - crown bud, cs - cluster stem, lp – leaf primordium, sl - scale leaf, vr - vascular ring	125
Figure 5.18 Plants of '03/04-114' following treatment application of either, (A) control or (B) foliar GA ₃ , after seven months. (C) control and treated plants after 10 months (7/12/09). (D) Compact bud clusters (red circle) in control treated plant. (E-I) Elongated stem with separated bud cluster, (E & F) at the distal end (red circle and white arrows), (F & G) with scale leaves (white arrows) and, (H & I) buds/shoots arising at the distal end (white arrows).	
Figure 5.19 Progressive dissection of a bud cluster on an elongated stem of '03/04-114' following foliar application of GA ₃ ; (A) non-dissected with most	

distal-positioned crown bud/shoot (1) emerging, (B) crown buds 2-4 revealed,	
(C-E) crown buds 4-6 revealed. (F) Diagrammatic illustration of arrangement of	
buds within the cluster (cross-sectional view); earliest formed axillary bud (1) to	
the last (5). More buds, including the apical bud of the stem cluster, were present	
within crown bud 5.	131
Figure 5.20 Transverse section of (A. & D) flored shoot and (C. & D) stores a root	
Figure 5.20 Transverse section of (A & B) floral shoot and (C & D) storage root	
of 'Diva' at increasing magnification; (A) section at the base of the shoot	
illustrating vascular ring, (B) magnified image illustrating lignified cells, (C) full	
section of the storage root indicating xylem vessels in the centre, and magnified	
in (D). Orange and red fluorescence indicates the xylem vessels. c - cortex, p -	
pith, vr - vascular ring, x – xylem.	132
Figure 5.21 Transverse sections of the elongated cluster stem following	
treatment with GA ₃ and control 'Diva'; (A) proximal end of the elongated stem	
with the centre magnified in (B) to illustrate the vascular ring similar to stem,	
indicating position of further magnification presented in (C), indicating xylem	
vessels. (D) Distal end of the elongated stem indicating position of magnified	
image presented within (E), indicating the arrangement of xylem vessels and, (F)	
possible initiation of a bud. (G) Arrangement of xylem vessels at base of the	
cluster in control treatment. Orange and red fluorescence indicates the xylem	
vessels. cb - crown bud, p - pith, vr - vascular ring, x – xylem	133
Figure 5.22 Schematic diagram illustrating the arrangement of crown bud	
clusters on the transition zone, and hierarchical arrangement of buds within the	
cluster, as apparent following elongation of the compressed cluster stem (i.e.	
when GA treated).	134
Figure 6.1 Schematic representation illustrating the composition of treatments	
applied within Experiments One and Two, commencing from the transfer to	
greenhouses at 0 wk following deflasking and growth in fog tent	144
greenhouses at 6 wk following deflusking and growth in fog tent	,,,177
Figure 6.2 Number of crown buds, crown shoots, and their combined total per	
plant for 'Diva' 61/2 months from the start of treatment application (Experiment	
One). Treatment abbreviations as detailed in Figure 6.1. Means, with the same	
letter are not significant at $P \le 0.05$ (LSD) for each variable located in the order	

from the base of the stack bar; i.e. lower case letters for number of crown buds	
or crown shoots, and upper case letters for the combined number	148
Figure 6.3 Dry weight of primary, secondary and tertiary shoots (combined) per	
plant for 'Diva' 61/2 months from the start of treatment application (Experiment	
One). Treatment abbreviations as detailed in Figure 6.1. Means with the same	
letter are not significant at $P \le 0.05$ (LSD) for each variable located in the order	
from the base of the stack bar; i.e. lower case letters for dry weight of primary or	
secondary and tertiary shoots, and upper case letters for the combined weight of	
all shoots.	149
Figure 6.4 (A) Typical levels of growth for plants of 'Diva' 6½ months from	
treatment with (GA) or without (Control) GA3, under long days. (B & C) Level	
of development of secondary shoots from the axillary buds on the primary shoot,	
as apparent from two positions along the primary shoot, 4 weeks following	
application of GA 3 (Experiment One).	150
Figure 6.5 Number of crown buds, crown shoots, and their combined total per	
plant for 'Diva' 5 months following application of plant growth regulators under	
long days (Experiment Two). Treatment abbreviations as detailed in Figure 6.1.	
Means, with the same letter are not significant at $P \leq 0.05$ (LSD), for each	
variable located in the order from the base of the stack bar; i.e. lower case letters	
for number of crown buds or crown shoots, and upper case letters for the	
combined number.	151
Figure 6.6 Dry weight of primary and secondary shoots (combined) per plant for	
'Diva' 5 months following application of plant growth regulators under long	
days (Experiment Two). Treatment abbreviations as detailed in Figure 6.1.	
Means with the same letter are not significant at $P \leq 0.05$ (LSD) for each	
variable located in the order from the base of the stack bar; i.e. lower case letters	
for dry weight of primary or secondary and tertiary shoots, and upper case letters	
for the combined weight of all shoots.	152
Figure 6.7 Representative samples of crown buds in the transition zone of 'Diva'	
6½ months from the start of treatment application in Experiment One and Two;	
(A) Outside, (B) LD→SD, (C) TDZ and, (D) ethephon.	153

Figure 7.1 A representative plant of 'Diva' at the start of treatment application	
within each of three experiments (First growth cycle; 31st March 2009). The	
primary shoot and secondary shoots carried opened/opening flowers	168
Figure 7.2 Schematic representation illustrating the composition of treatments	
Figure 7.2 Schematic representation illustrating the composition of treatments	
applied within Experiments One, Two and Three during the first growth cycle.	
Left to right presents the time line for both treatment application and sampling	
dates commencing 31 st March through to 9 th September 2009. As indicated by	
the open arrows, the timing of sampling refers to the duration from the last	
treatment application (0 to 20 weeks)	170
Figure 7.3 Combined number of visible crown buds and crown shoots per plant	
of 'Diva' (Experiment One) for the; (A) main effect of duration from last	
treatment application ^x , (B) main effect of treatment with growth regulators 4	
weeks following the last treatment application, (C & D) combined number of	
buds and shoots developed from the transition zone or storage roots 20 weeks	
following the last treatment application within two photoperiodic regimes (C,	
LD; D, LD→ SD). Abbreviations for treatments as described in Table 7.1.	
Vertical lines represent standard error values. Means followed by different	
letters were significantly different at $P \leq 0.05$; upper case letters apply to	
variables representing combined value within stack bar in Figure 7.3A. n.s. =	
not significantly different (Number of root bud and shoots at LD and number of	
crown buds and shoots at LD→ SD).	179
Figure 7.4 Number of crown bud clusters per plant of 'Diva' at 20 weeks	
following the last treatment application (Experiment One) under; (A) Long day	
or, (B) short day photoperiodic regimes and, (C) combined number of visible	
crown buds and crown shoots within a cluster, for the main effect of application	
of growth regulators. Abbreviations for treatments as described in Table 7.1.	
Means followed by different letters were significantly different at $P \le 0.05$; for	
data that were sqroot transformed, transformed means and mean separation letter	
are presented at the top of each column.	180
Figure 7.5 Diameter of crown buds on plants of gentian cultivar 'Diva' at 20	
weeks following the last treatment application within two photoperiodic regimes	
(Experiment One); (A) LD, (B) LD→SD. Abbreviations for treatments as	

followed by different letters were significantly different at $P \le 0.05$.	181
Figure 7.6 Number of crown shoots per plant of 'Diva' (Experiment One); (A &	
B) during the 20 weeks following the last treatment application within two	
photoperiodic regimes (A, LD; B, LD→SD) and, (C) for the main effect of	
treatment with application of growth regulators at 20 weeks following the last	
treatment application. (D) Percentage of crown shoots emerged as a proportion	
to the combined number of crown buds and crown shoots for the main effect of	
treatment with application of growth regulators 20 weeks following the last	
treatment application. Abbreviations for treatments as described in Table 7.1.	
Means followed by different letters were significantly different at $P \le 0.05$. n.s.	
= not significantly different.	182
Figure 7.7 (A) Shoot length (B), number of nodes and (C), diameter of new	
shoots developed from crown, at 12 weeks following last treatment application	
for the main effect of treatment with application of growth regulators to plants of	
'Diva' Experiment One). Abbreviations for treatments as described in Table 7.1.	
For each variable, means followed by different letters were significantly	
different at $P \le 0.05$.	183
Figure 7.8 (A-B) Combined number of visible root buds and shoots per plant	
during the 20 week period following the last treatment application within two	
photoperiodic regimes (A, LD; B, LD→ SD) for plants of 'Diva' (Experiment	
One). Abbreviations for treatments as detailed in Table 7.1. Means followed by	
different letters were significantly different at $P \le 0.05$, n.s. = not significantly	
different.	184
Figure 7.9 Macroscopic features near the transition zone of representative	
samples of plants of 'Diva' from treatments under the long photoperiodic regime	
20 weeks following the last treatment application (Experiment One); (A) Control	
with no crown buds, (B) ethephon with clusters comprising both crown buds and	
crown shoots, (C) PBZ with clusters comprising crown buds, (D) PBZETH with	
clusters comprising both buds and shoots, (E) TDZ with small and thin crown	
buds and crown shoots, (F) TDZETH with clusters comprising both crown buds	
and grown shoots. Abbreviations for treatments as detailed in Table 7.1	185

Figure 7.10 Macroscopic features near the transition zone of representative
samples of plants of 'Diva' from treatments under the naturally occurring short
photoperiodic regime 20 weeks following the last treatment application
(Experiment One); (A) Control with clusters of crown buds, (B) ethephon with
clusters comprising both crown buds and crown shoots, (C) PBZ with clusters of
crown buds, (D) PBZETH, (E) TDZ, (F) TDZETH with clusters comprising
buds and shoots from roots, which were small and thin. Abbreviations for
treatments as detailed in Table 7.1.
Figure 7.11 (A-B) Dry weight of primary and secondary shoots per plant as at
12th week following the last treatment application within two photoperiodic
regimes (A, LD; B, LD→ SD) of 'Diva' (Experiment One). Abbreviations for
treatments as described in Table 7.1. Means followed by different letters were
significantly different at $P \le 0.05$; data were log transformed for analysis, hence
both transformed means and mean separation letter are presented above each
column
Figure 7.12 Combined number of visible crown buds and crown shoots and total
above-ground dry weight per plant of 'Diva'15 weeks following the last
treatment application (Experiment Two). Abbreviations for treatments as
described in Table 7.1. Vertical lines represent \pm standard error values. Means
followed by different letters were significantly different at $P \le 0.05$; number of
buds and shoots data were sqroot transformed for analysis, hence both
transformed mean and mean separation letter are presented above each column191
Figure 7.13 Combined number of visible crown buds and crown shoots per plant
for the main effect of application with growth regulators at the 12 th week
following the last treatment application of 'Diva' (Experiment Three).
Abbreviations for treatments as described in Table 7.1. Means followed by
different letters were significantly different at $P \le 0.05$; for data that were sqroot
transformed, transformed means were presented along with mean separation
letter
Figure 7.14 Schematic diagram based on Chapters 6 and 7, illustrating potential
origin of buds (shoot, transition zone or root) and potential strategies for
promotion using photoperiod of the growing environment and/or application of
growth regulators. Treatment application abbreviations as per Table 7.1206
5.5 " all 125 allators. Treatment approached a dore vitations as per ratio /.1200

stages used for: (A) Experiment One, plants with buds unemerged, (B) Experiment Two, plants with buds recently emerged, (C) Experiment Three, plants with emerged and developed shoots.	219
Figure 8.2. Arrangement of treatment combinations within Experiments One, Two and Three	220
Figure 8.3. (A) Proportion of shoots emerged and reaching harvest maturity as compared with unemerged crown buds present at the time of first treatment application, (B) duration to harvest maturity for each shoot and, (C) shoot length at harvest, following increasing duration of chilling at 5 °C or application of GA ₃ to plants of 'Diva' with unemerged buds (Experiment One). For each variable, means with different letters were significantly different. Within (A) mean separation is based on the log transformed means. Vertical lines represent	
\pm SEM. Mean separation by DNMRT at $P < 0.05$.	227
Figure 8.4. Number of nodes and number of nodes with floral axillaries at harvest following increasing duration of chilling at 5 °C or application of GA_3 to plants of 'Diva' with unemerged buds (Experiment One). For each variable means indicated by different letters were significantly different. Vertical lines represent SEM. Mean separation by DNMRT at $P < 0.05$.	228
Figure 8.5. Spread in time to harvest maturity of shoots following treatment application to plants of 'Diva' at three developmental stages comprising; (A) unemerged buds (Experiment One), (B) shoots recently emerged (Experiment Two), (C) elongated shoots (Experiment Three). The number of shoots contributing to the distribution is indicated to the right of each treatment's box plot. Solid lines in the centre of each box indicate the median. Boundaries of the box indicate the 25 th and 75 th percentiles, whiskers indicate 10 th and 90 th percentiles and, solid dots indicate one or more individuals as outliers beyond these percentile limits.	2229
Figure 8.6. (A) Proportion of shoots reaching harvest maturity as compared with the combined number of unemerged crown buds and crown shoots present at the time of first treatment application, and (B) shoot length at harvest of shoots developed following increasing duration of chilling at 5 °C and/or application of	

GA_3 or PBZ to plants of Diva' with shoots recently emerged (Experiment Two). For each variable means indicated by different letters were significantly different. Vertical lines represent \pm SEM. Mean separation by DNMRT at $P < 0.05$.
Figure 8.7. Number of nodes and axillaries with flowers, at harvest of shoots developed following increasing duration of chilling at 5 °C and/or application of GA_3 or PBZ to plants of 'Diva' with shoots recently emerged (Experiment Two). For each variable means indicated by different letters were significantly different. Vertical lines represent SEM. Mean separation by DNMRT at $P < 0.05$
Figure 8.8. During the third growth cycle, following increasing duration of chilling at 5 °C and/or application of GA ₃ or PBZ to plants of 'Diva' with shoots recently emerged (Experiment Two); (A) combined number of crown buds and shoots per cluster and, (B) the proportion of shoots emerged as compared to the combined number of crown buds and crown shoots at the beginning of third growth cycle. For each variable means indicated by different letters were significantly different. Vertical lines represent \pm SEM. Mean separation by
Figure 8.9. (A) Proportion of shoots reaching harvest maturity as compared with combined number of unemerged crown buds and crown shoots present at the time of first treatment application and, (B) shoot length at harvest of shoots (present at the beginning of the treatment application; adjusted means are presented) following increasing duration of chilling at 5 °C and/or application of GA_3 to plants of 'Diva' with elongated shoots (Experiment Three). Within (A) mean separation is based on the log transformed means. For each variable means indicated by different letters were significantly different. Vertical lines represent \pm SEM. Mean separation by DNMRT at $P < 0.05$
Figure 8.10. The combined number of crown buds and shoots per plant, during the third growth cycle, following increasing duration of chilling at 5 °C and/or application of GA_3 or PBZ to plants of 'Diva' with elongated shoots (Experiment Three). For each variable means indicated by different letters were significantly different. Vertical lines represent \pm SEM. Mean separation by DNMRT at $P < 0.05$

Figure 9.1 Schematic representation illustrating the sequence and composition of	
treatments applied.	254
Figure 9.2 A representative plant of 'Diva' with a single bud cluster at differing stages of development during the first growth cycle within the Clipped + GA treatment, illustrating the methodology of identifying the hierarchical position of crown buds; (A), prior to treatment application (3 rd September 2009) indicating positions (1-4) of crown buds within the cluster from oldest (1) to the most recent bud which is oblong in shape (4; red circle), (B), as at 19 th October 2009 following shoot emergence, indicating newly visible crown buds (positions 5 & 6; red circle) which arose from within the previously most recent bud (4) and, (C), as at December 2009, indicating emergence of shoots from crown buds that were not visible prior to treatment application (red arrow at position 5)	256
Figure 9.3 Distribution of duration to harvest from the last treatment application for crown shoots on plants of 'Diva' during the growth cycle immediately following treatment application. Solid lines within each box indicate the median. Boundaries of the box indicate 25 th and 75 th percentiles, and whiskers (if present) indicate 10 th and 90 th percentiles. n=number of shoots	
Figure 9.4 Spread in the hierarchical position of the bud within a bud cluster of 'Diva' which emerged during the growth cycle immediately following treatment application. Position of buds was numbered from proximal to distal end of the cluster. The number of shoots represented within each treatment were; Non-clipped Control ($n = 3$), Clipped Control ($n = 7$), Clipped + Chilling ($n = 6$), GA ($n = 15$). Solid and dashed horizontal lines in the centre of each box indicate median and mean, respectively. Boundaries of box indicate 25^{th} and 75^{th} percentiles, and whiskers (if present) indicate 10^{th} and 90^{th} percentiles.	261
Figure 9.5 Relationship between the hierarchical position of the bud within the cluster, from proximal to distal end of the cluster and; (A), Duration to emergence and, (B), Duration to harvest maturity, among treatments of 'Diva' in the first growth cycle following treatment application.	262
Figure 9.6 Relationship between the hierarchical position of the bud from proximal to distal end of the cluster and; (A) shoot length and, (B), number of	

nodes at harvest, among treatments applied to plants of 'Diva' in the first growth	
cycle following treatment application.	263
Figure 9.7 Relationship between the hierarchical position of the bud from proximal to distal end of the cluster and number of vegetative and floral nodes at harvest of 'Diva' treated with GA ₃ in the first growth cycle following treatment application.	264
Figure 9.8 Distribution of dates of appearance of crown buds and/or their emergence as shoots, for plants of 'Diva' treated with GA ₃ in the first growth cycle; (A), date of appearance of all crown buds in the first growth cycle, (B), date of appearance of crown buds that emerged into shoots in the second growth cycle following treatment application and, (C), the date of emergence of crown shoots in the second growth cycle following treatment application. Solid lines within each box indicates median. Boundaries of the box indicate the 25 th and 75 th percentiles, whiskers (if present) the 10 th and 90 th percentiles and, solid dots	265
any outliers.	203
Figure 10.1 Determinants of the development of crown buds through to harvest maturity, their influence on the process, and potential manipulative strategies, for commercial cultivars of gentian. (+ve = promotes, -ve = inhibits or less promotive).	276
Figure 10.2 Potential external stimuli breaking dormancy in crown buds, depending upon the dominant type of dormancy exerted over a crown bud, its hierarchical position within the crown bud cluster, and time within a single growth cycle/season.	282

List of Tables

Table 1.1 Timing, morphological changes and conditions typically occurring	
during flowering in historical cultivars of gentians (i.e. selections of G. triflora).	
(Calendar months have been modified to equate to those occurring in the southern	
hemisphere).	6
Table 2.1 Duration to harvest maturity, duration of shoot emergence, duration	
from shoot emergence to harvest and variation in distribution in time for these	
parameters ^z between three gentian cultivars in calendar days	35
Table 2.2 Duration to harvest maturity, duration of shoot emergence, duration	
from shoot emergence to harvest and variation in distribution in time for these	
parameters ^z between three gentian cultivars in growing degree-days (GDD)	36
Table 2.3 Average duration to harvest, duration of shoot emergence and duration	
from shoot emergence to harvest ² in calendar days for individual plants within a	
cultivar of gentians.	37
Table 3.1 Shoot length at harvest, and average duration to harvest (from the date	
of removal from the cold store) of flowering shoots of both tracked and control	
plants of 'Spotlight' in either the protected or outside environments during the	
first growth cycle.	54
Table 4.1 Shoot emergence, as a percentage of macroscopically visible buds ² ,	
from plants of 'Spotlight' grown in two environments 4 weeks following three	
separate dates of clipping.	75
Table 6.1 Number of; storage roots, secondary and tertiary shoots, and the dry	
weight of storage roots, per plant for 'Diva' 6½ months from the start of treatment	
application (Experiment One).	149
Table 6.2 Number of; storage roots, secondary and tertiary shoots, and the dry	
weight of storage roots per plant for 'Diva' 5 months following application of	
plant growth regulators under long days (Experiment Two)	152

Table 7.1 Abbreviations used for presentation of environmental and plant growth	
regulator treatment combinations within figures and tables detailing results from	
Experiment One, Two and Three.	.169
Table 7.2 Probability values indicating significance of main factors and interactions determined for key variables in Experiment One.	.175
Table 7.3 Probability values indicating significance of main factors and	
interactions within each week for the variable "combined number of crown buds	
and shoots" in Experiment One.	.176
Table 7.4 Quantitative and qualitative characteristics of crown shoots at harvest maturity (second growth cycle) of plants of 'Diva' (Experiment One), resulting from treatment within the first growth cycle. (All plants were grown in the long	
day environment during the second growth cycle)	.190
Table 9.1 Proportion of, and duration to, shoots emerging from crown buds of 'Diva' in the first growth cycle following treatment application.	.258
Table 9.2 Quality and timing characteristics of shoots from plants of 'Diva' at two developmental end-points in the first growth cycle following the last treatment application.	.258

List of Abbreviations

1-MCP 1-methylcyclopropene

ABA Abscisic acid

AO Acridine orange

BA Benzyladenine

CRD Completely randomised design

DNMRT Duncan's multiple range test

ethephon 2-chloroethylphosphonic acid

FAA Formaldehyde: glacial acetic acid: ethanol

FI Flower initiation

GA Gibberellins

GA3 Gibberellic acid

IAA Indole-3-acetic acid

LD Long photoperiodic regime

LSMEANS Least square means

NAA Naphthalene 1-acetic acid

NPA Naphthalphthalamic acid

NZ New Zealand

PAR Photosynthetically active radiation

PBZ Paclobutrazol

SAM shoot apical meristem

SAS Statistical Analysis System

SD Short photoperiodic regime

T temperature

TDZ Thidiazuron

List of Publications

- Samarakoon, U.C., K.A. Funnell, D.J. Woolley, E.R. Morgan. "Sources of Variation in Time of Harvest Maturity of Flowering Shoots in Three Cultivars of Gentian" submitted for publication in New Zealand Journal of Crop and Horticultural Science. (Chapter 2)
- Samarakoon, U.C., K.A. Funnell, D.J. Woolley, E.R. Morgan. 2012
 "Temperature Impacts Changes in Crown Buds and Flowering of Gentian
 'Spotlight'" Scientia Horticulturae vol. 143(49-55)
- Samarakoon, U.C., K.A. Funnell, D.J. Woolley, G. Burge, E.R. Morgan.
 "Initiation, Emergence and Development of Crown Buds in Gentians" accepted for publication in Proceedings of International Symposium of Ornamental crops, IHC 2010. (Part of Chapter 3; included Appendix VI)

