

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.

Seasonal changes of non-structural carbohydrates related to the growth and development of gentians

A thesis presented in partial fulfilment of the requirements for

the degree of

Doctor of Philosophy

in Plant Science

at

Massey University

Palmerston North

New Zealand



Yuguo Wang

2014

Abstract

The growth and development of perennial gentians (*Gentiana* L.) for cut flowers are seasonally controlled. It was hypothesized that in these plants the availability of carbohydrates is a limiting factor influencing the development of crown buds, winter survival, spring re-growth, and their development to flowering; this in turn influences the yield and quality of flowering shoots. By focussing on the seasonal changes of non-structural carbohydrates (NSCs) in various organs, and the effect of differential carbohydrate supply on their growth and development, the current thesis aimed to understand the physiological function of NSCs and their potential influence on the commercial production of flowering shoots.

In addition to sucrose, fructose and glucose, the unique carbohydrates, gentianose, gentiobiose and L-bornesitol were found in the gentian hybrids investigated. Gentianose was the main storage carbohydrate, accounting for 50% - 70% total NSCs in storage roots year round, likely playing similar roles as starch and/or fructan do in other plant species. The concentration of these NSCs in various organs fluctuated with seasonal changes, reflecting periods of storage and utilization of NSCs, i.e. the transition between sink and source, in the growth and development of plants during an annual growth cycle.

When harvesting flowering shoots, commercial growers of gentian leave a set number of leaves to maintain carbohydrate levels; however, optimization of leaf number/area has never been assessed in terms of carbohydrate reserves. Utilizing varying levels of defoliation, a positive correlation was determined between the resulting concentration of NSCs in crowns (crown buds, rhizomes and storage roots) and both the number and size of new crown buds, supporting the hypothesis that the availability of carbohydrates is a limiting factor influencing the development of crown buds. Experiments *in vitro* both verified and extended the level of understanding, indicating that the morphogenesis of crown buds, including form, dormancy, and differentiation, was influenced by multiple factors including, carbohydrates, photoperiod and ethylene.

Reserves of NSCs in crowns in autumn were positively correlated with both winter survival and spring re-growth. Consistent with these results, the dynamics of NSC concentration and the activity of relevant glycoside hydrolases (GHs) indicated that a large amount of gentianose, up to 106 mg g⁻¹ fresh weight (FW) accumulated in storage roots

Abstract

before winter was subsequently hydrolysed and remobilized to the crown buds to impart cold tolerance in winter and be used during their subsequent re-growth in spring.

Substantial gentiobiose, up to 38 mg g⁻¹ FW, accumulated in stem tissue of the shoots before anthesis commenced, while gentianose increased by up to 26 mg g⁻¹ FW in petals before floret opening. These two forms of carbohydrates were considered likely candidates facilitating the fast development of florets. During the development and senescence of florets, the activity of relevant GHs controlling the hydrolysis of gentianose, gentiobiose, and sucrose, was associated with increase of pressure potential driving the opening of floret buds. The stage-specific and dramatic changes of accumulation, conversion and mobilization of NSCs in association with the activity of relevant enzymes, also provided a useful experimental model system to study the metabolism and physiological function of these unique NSCs in gentians.

The current study has provided increased understanding of the roles of specific NSCs, particularly gentianose and gentiobiose, in the growth and development of gentians. The thesis also offers a framework of information to improve the balance between carbohydrate storage in underground crowns and harvest of flowering shoots, and therefore benefits the sustainability of yield and quality of flowering shoots in commercial production.

Acknowledgements

First and foremost, I express my deepest gratitude to my main supervisor Dr Keith Funnell for his wonderful guidance by providing invaluable suggestions, support, challenges and encouragement throughout my study. Your persistence and patience in offering editorial commentary on my thesis from the start to submission of this thesis are extremely appreciated. Without your great support, this thesis would not have been possible. I express my sincere gratitude to Dr David Woolley for his insightful suggestions and constructive feedback, yet friendly guidance on the experimental plans and thesis writing, particularly your supervision when Keith was away. I express my sincere gratitude to Mr Ed Morgan for his invaluable suggestions and support in the research programme and thesis editing, particular your expert advice and help with regard the *in vitro* experiments. I express my sincere gratitude to Dr Jocelyn Eason for her invaluable support and guidance in the research and thesis editing, particularly your assistance in developing the methodology for carbohydrate analysis and detecting enzyme activity. I feel privileged to have had excellent supervision from my four supervisors. Your inspiration for getting this thesis started, along with your expert advice for research and thesis writing, guarantee the accomplishment of my thesis.

I would like to thank the staff at Massey's Institute of Agriculture and Environment and the Plant Growth Unit, especially Chris Rawlingson, James Slater and Kay Sinclair for their technical and instrument support. I would also like to thank many staff at The New Zealand Institute for Plant & Food Research, especially Dr David Lewis for his help with using the HPLC instrumentation, Dr Erin O'Donoghue for her help with using the HPLC and the protein analysis, Maree Debenham for her help with tissue culture techniques, Sheryl Somerfield for her help with the starch measurements, Lyn Watson, Philip West and Jun Zhou for their help with the protein analysis and gel electrophoresis, as well as Steve Arathoon for his lots of help. I am very grateful to Dr Xiongzhaoh He and Mr Duncan Hedderley for their Statistical help and Dr Nick Gould for his help with aphid stylectomy. Your time and commitment are most appreciated. I am also thankful to other staff and postgraduate students for their help and providing a friendly environment throughout my study.

Acknowledgements

Financial support received for the research from The New Zealand Institute for Plant & Food Research Limited, via the New Zealand Foundation for Research, Science and Technology (contract C02X0702), Plant & Food Research CORE funding: 12058 – “Fashionable Plants for the Ornamentals Industry”, Turners & Growers Research Grant, Helen E Akers PhD Scholarship, and Massey University Doctoral Hardship Bursary, is appreciated very much.

I am very grateful to my wife Hongxia Liu and son Xingan Wang for their understanding, support and patience. I am also thankful to my parents, sisters and brothers for their unconditional support throughout my study.

Table of Contents

Abstracti

Acknowledgements iii

Table of Contents..... v

List of Figures..... x

List of Tables..... xviii

List of Symbols and Abbreviationsxx

Chapter 1 General introduction..... 1

1.1 Commercial production 1

1.2 Development in New Zealand 1

1.3 Research background..... 2

1.4 Objectives..... 6

Chapter 2 Literature review 8

2.1 Cultivars for ornamental crops 8

2.2 Growth habit 9

2.2.1 Morphological structure..... 9

2.2.2 Growth cycle..... 10

2.3 Horticultural Production 12

2.3.1 Propagation..... 12

2.3.2 Cultivation 13

2.3.3 Harvesting of flowering shoots 13

2.4 Yield and quality of flowering shoots 14

2.4.1 Yield and quality..... 14

2.4.2 Factors influencing the yield and quality 15

2.4.3 Hypothesis: carbohydrates influencing yield and quality..... 16

2.5 Function and metabolism of carbohydrates 17

2.5.1 Categories and types of carbohydrates..... 17

2.5.2 Partitioning 18

2.5.2.1 Production 19

2.5.2.2 Transportation 20

2.5.2.3 Storage and remobilization 21

2.5.3 Factors influencing partitioning of NSCs 21

2.5.4 Function of NSCs..... 22

2.5.4.1 Energy and metabolites 23

2.5.4.2 Osmoregulation..... 23

2.5.4.3 Signalling function..... 24

2.5.5 Metabolism of gentiobiose and gentianose 25

2.5.5.1 Molecular structure 26

Table contents

2.5.5.2	Synthesis and hydrolysis	27
2.6	Methods of analysis for carbohydrates and activity of related enzymes.....	28
2.6.1	Carbohydrate analysis	28
2.6.1.1	Comparison of methods.....	28
2.6.1.2	High Performance Liquid chromatography (HPLC)	29
2.6.2	Isolation and detection of enzymes	29
2.6.2.1	Protein isolation and detection.....	29
2.6.2.2	Staining methods for detecting enzymes involved in carbohydrate metabolism	30
2.7	Summary.....	31
Chapter 3 Form, structure and development of crowns in established plants of gentian 03/04-114.....		32
3.1	Introduction	32
3.2	Materials and methods	34
3.2.1	Plant material.....	34
3.2.2	Methods.....	34
3.2.3	Statistical analysis	35
3.3	Results.....	35
3.3.1	Form and structure of crowns.....	35
3.3.2	Biomass distribution	35
3.3.3	Development of crown buds	37
3.3.4	Relationships between TCBN, CN, CBNC and SN.....	41
3.4	Discussion.....	43
3.5	Conclusion.....	47
Chapter 4 Effect of defoliation on carbohydrate reserves, crown-bud development, and spring re-growth in gentian 03/04-114.....		49
4.1	Introduction	49
4.2	Materials and methods	51
4.2.1	Plant material.....	51
4.2.2	Experimental design and defoliation treatments	52
4.2.3	Growing degree-day monitoring	53
4.2.4	Assessment of crown buds.....	54
4.2.5	Winter survival and spring re-growth.....	54
4.2.6	Biomass.....	54
4.2.7	Non-structural carbohydrate analysis	54
4.2.8	Statistical analysis	56
4.3	Results.....	57
4.3.1	Biomass of underground organs.....	57
4.3.2	Carbohydrates	58
4.3.3	Crown buds	60
4.3.4	Winter survival and sprouting in spring.....	63
4.3.5	Relationships between key variables.....	63
4.4	Discussion.....	66
4.5	Conclusion.....	71

Chapter 5 Effect of carbohydrate supply, temperature, photoperiod and plant growth regulators on morphogenesis of crown buds in vitro	72
5.1 Introduction.....	72
5.2 Materials and methods.....	76
5.2.1 General materials.....	76
5.2.2 Experiment One – sucrose concentration.....	76
5.2.2.1 Treatments.....	76
5.2.2.2 Crown buds.....	77
5.2.2.3 Dormancy assessment.....	77
5.2.2.4 Growth assessment and analysis of endogenous carbohydrates	77
5.2.3 Experiment Two – temperature and photoperiod.....	78
5.2.4 Experiment Three – NAA and Ethephon.....	78
5.2.4.1 NAA	78
5.2.4.2 Ethephon	78
5.2.5 Statistical analysis.....	79
5.3 Results.....	79
5.3.1 Experiment One – sucrose concentration.....	79
5.3.1.1 General growth of plantlets	79
5.3.1.2 Crown bud formation	80
5.3.1.3 Dormancy of crown buds	81
5.3.1.4 Endogenous carbohydrates.....	81
5.3.2 Experiment Two – temperature and photoperiod.....	84
5.3.3 Experiment Three – NAA and Ethephon.....	86
5.3.3.1 NAA	86
5.3.3.2 Ethephon	86
5.4 Discussion.....	90
5.5 Conclusion	97
 Chapter 6 Seasonal changes of non-structural carbohydrates in gentians	 98
6.1 Introduction.....	98
6.2 Materials and methods.....	103
6.2.1 Plant material and cultivation.....	103
6.2.2 Experiments for monitoring NSC.....	104
6.2.2.1 Seasonal changes.....	104
6.2.2.2 Diurnal changes.....	105
6.2.2.3 Changes with position along the flowering shoot	105
6.2.2.4 Changes with the timing of shoot removal	106
6.2.3 NSC analysis	106
6.2.4 Statistical analysis.....	107
6.3 Results.....	107
6.3.1 Seasonal phenology	107
6.3.2 Seasonal changes of NSCs.....	108
6.3.2.1 Storage roots.....	109
6.3.2.2 Rhizomes	110
6.3.2.3 Crown buds.....	112
6.3.2.4 Floral-shoots.....	112
6.3.3 Changes in NSCs with position along the floral shoots	114

Table contents

6.3.4	<i>Diurnal changes of NSCs in leaves and stem tissue of floral shoots</i>	115
6.3.5	<i>Timing of shoot removal</i>	116
6.3.6	<i>L-bornesitol</i>	119
6.4	<i>Discussion</i>	123
6.5	<i>Conclusions</i>	134
Chapter 7 Changes of non-structural carbohydrates during floret development in ‘Showtime Spotlight’		
		135
7.1	<i>Introduction</i>	135
7.2	<i>Materials and methods</i>	138
7.2.1	<i>Plant material and cultivation</i>	138
7.2.2	<i>Petal fresh and dry weight measurement</i>	138
7.2.3	<i>Monitoring NSCs</i>	139
7.2.4	<i>Monitoring Water Potential and its Components</i>	140
7.2.5	<i>Calculation of pressure potential</i>	141
7.2.6	<i>Calculation of osmotic potential derived from soluble TNC</i>	141
7.2.7	<i>Statistical analysis</i>	142
7.3	<i>Results</i>	142
7.3.1	<i>Changes of petal biomass</i>	142
7.3.2	<i>Changes of NSCs</i>	143
7.3.3	<i>Water, osmotic and pressure potential</i>	146
7.4	<i>Discussion</i>	148
7.5	<i>Conclusion</i>	154
Chapter 8 Activity of carbohydrate-associated hydrolases in ‘Showtime Spotlight’		
		155
8.1	<i>Introduction</i>	155
8.2	<i>Materials and methods</i>	159
8.2.1	<i>Plant material and cultivation</i>	159
8.2.2	<i>Sample collection</i>	159
8.2.2.1	<i>Effect of temperature, pH, and buffers</i>	159
8.2.2.2	<i>Seasonal changes</i>	159
8.2.2.3	<i>Changes during floral bud development</i>	160
8.2.3	<i>Glycoside hydrolase enzyme extraction</i>	160
8.2.4	<i>Glycoside hydrolase enzyme assay</i>	160
8.2.5	<i>Electrophoresis and staining for glycoside hydrolases</i>	161
8.2.6	<i>Statistical Analysis</i>	163
8.3	<i>Results</i>	163
8.3.1	<i>Optimum incubation time and extraction buffers for glycoside hydrolases</i>	163
8.3.2	<i>pH optimum of glycoside hydrolases</i>	164
8.3.3	<i>Temperature optimum of glycoside hydrolases</i>	165
8.3.4	<i>Products of gentianose hydrolysis</i>	166
8.3.5	<i>Changes in crown buds during the growth cycle</i>	167
8.3.6	<i>Changes in storage roots during the growth cycle</i>	169
8.3.7	<i>Changes during the development of florets</i>	170
8.3.8	<i>Electrophoresis and staining</i>	172

8.4 Discussion	174
8.5 Conclusion	182
Chapter 9 General discussion	183
9.1 Introduction.....	183
9.2 Unique NSCs.....	183
9.3 Seasonal fluctuation.....	187
9.4 Physiological functions	191
9.4.1 Development of crown buds.....	192
9.4.2 Overwintering and re-growth	196
9.4.3 Development of floral shoots	201
9.5 Commercial application.....	205
9.6 Conclusion	206
Appendices	208
Appendix I; Identification of gentianose, gentiobiose and L-bornesitolin gentians	208
1.I identification of gentianose and gentiobiose.....	208
1.II Identification of L-bornesitol	213
Appendix II; HPLC chromatograms of non-structural carbohydrates in various organs of gentians ...	217
Appendix III; Abnormal chromatogram of EDTA exudates from the phloem of gentian shoots	220
Appendix IV; Effect of sucrose concentrations on flowering of gentian plantlets in vitro.....	221
Appendix V; in situ staining of glycoside hydrolase activity involved in carbohydrate metabolism in gentian tissue	222
Reference	224

List of Figures

Figure 1.1	The gentian cultivars ‘Showtime Spotlight’ (A), ‘Showtime Diva’ (B) and ‘Showtime Starlet’ (C). Photos courtesy of Plant & Food Research.	2
Figure 1.2	Research strategies and programmes within this PhD thesis.	7
Figure 2.1	Photograph and comparative schematic diagram presenting the morphological structure of a mature perennial gentian plant.	10
Figure 2.2	Diagram illustrating the annual growth cycle of perennial gentians, with focus on the seasonal changes associated with dormant crown buds in winter, shoot emergence in spring, flowering in summer/autumn, and progressing through to shoot/foilage senescence in autumn/winter.	11
Figure 2.3	Molecular structure of gentianose (Sensonet, 2008). One molecule of gentianose is composed of two molecules of glucose and one molecule of fructose. In structure, gentianose can be broken down to one molecule of glucose and one of sucrose, or to one molecule gentiobiose and one fructose, or two molecules of glucose and one molecule of fructose.	26
Figure 3.1	Structure of a crown of gentian 03/04-114. Samples were collected from potted plants in autumn (20th May 2009).	36
Figure 3.2	Contractile roots attached to rhizomes of gentian 03/04-114. Samples were collected from potted plants in autumn (20th May 2009).	36
Figure 3.3	Crown buds initiated at leaf axils at the base of shoots of 03/04-114 (A). The total population of crown buds on an individual plant were composed of several clusters of crown buds (B). Within (B) the yellow arrows and associated numbers show the spiral arrangement and progressive order of appearance of crown buds within one such cluster, and the red arrow points to the thickened rhizome to which this cluster of crown buds was attached.	38
Figure 3.4	A cluster of crown buds (B) developed from the bud developed in the previous growing season (A), in the same plant of 03/04-114. Photo A was taken in December 2008 and photo B in March 2009.	38
Figure 3.5	A progressive sequence of the development of crown buds (photos 1 to 15) within the same cluster of crown buds in a plant of 03/04-114. Photos were taken from summer (photo 1-6) through autumn (photo 7-13), winter (photo 14) to the following spring (photo 15) (i.e. December 2008 through to September 2009).	39
Figure 3.6	Spiral patterns of crown buds within a cluster of 03/04-114 could be; (A) clockwise or, (B) anticlockwise. Arrows indicate the progressive order of appearance of crown buds within a cluster. Samples were collected in autumn (20th May 2009).	40
Figure 3.7	A root initiated at the interface of a crown bud and the rhizome of 03/04-114 (A); and a root with terminated development (B). Red arrow-head indicates the roots. Samples were collected in summer (January 2009).	40

Figure 3.8	Length and diameter of crown buds within a single cluster measured in autumn (20th May 2009). The ordinal numbers from left to right describe the order of appearance of the buds from December to May (old to young) within a plant of 03/04-114.....	40
Figure 3.9	Changes in total crown bud number per plant (TCBN), rate of increase in TCBN (number/month) and flowering percentage (number of flowering plants/total number of plants; expressed as %) of 03/04-114 during the growth season from Sep 2008 to May 2009. For each variable, mean values with different letters were significantly different (Tukey: $P < 0.05$; $n = 16$). Vertical lines = \pm SE.	41
Figure 3.10	Relationship between shoot number per plant (SN) and total number of crown buds per plant (TCBN) of 03/04-114 in autumn (May 2009; DF 1, 14; $R^2 = 0.3715$; $P = 0.016$).	42
Figure 3.11	Relationship between shoot number per plant and cluster number per plant (CN) of 03/04-114 in autumn (May 2009; DF 1, 14; $R^2 = 0.4759$; $P = 0.04$).	42
Figure 3.12	Relationship between shoot number per plant (SN) and the number of crown buds per cluster (CBNC) of 03/04-114 in autumn (May 2009; DF 1, 14; $R^2 = 0.0668$; $P = 0.352$).	42
Figure 3.13	Relationship between the cluster number per plant (CN) and the number of crown buds per cluster (CBNC) of 03/04-114 in autumn (May 2009; DF 1, 14; $R^2 = 0.0572$; $P = 0.462$).	43
Figure 4.1	Plants of 03/04-114 at the commencement of the experiment (October 2008).	51
Figure 4.2	Defoliation treatment applied to plants of 03/04-114. When defoliated, all leaves were removed, with only the tips of shoots retained. Left: non-defoliated control; right: defoliated plant.	53
Figure 4.3	Fresh (FW) and dry weight (DW) of crowns (storage root, rhizome and crown buds) of 03/04-114 in autumn (May 2009), following defoliation commencing in different months or control (Growing degree-days in brackets). For each variable, mean values (\pm SE) with different letters are significantly different ($P < 0.05$), $n = 4$	57
Figure 4.4	Concentration of individual soluble non-structural carbohydrates (NSCs) and total non-structural carbohydrates (TNC) per unit fresh weight (FW) in; (A) storage roots, (B) rhizomes and, (C) crown buds of 03/04-114, when sampled in autumn (May 2009), following defoliation commencing in different months or control (without defoliation; Growing degree-days in brackets). For each NSC, mean separation between dates by Tukeys at $P \leq 0.05$	59
Figure 4.5	Relationships between concentration of total non-structural carbohydrates (TNC) and the date defoliation commenced in plants of 03/04-114.	60

List of figures

- Figure 4.6 Concentration of L-bornesitol per unit fresh weight (FW) in separate organs of 03/04-114, when sampled in autumn (May 2009), following defoliation commencing in different months or control (without defoliation; Growing degree-days in brackets). For each variable, mean values (\pm SE) with different letters are significantly different ($P < 0.05$)......61
- Figure 4.7 Total number of crown buds per plant of 03/04-114, when sampled in autumn (May 2009), following defoliation commencing in different months or control. Bars (mean \pm SE) with different letters are significantly different ($P < 0.05$).62
- Figure 4.8 Length and diameter of crown buds when sampled in autumn (May 2009), following defoliation commencing in different months or control. For each variable (mean \pm SE) with different letters are significantly different ($P < 0.05$).62
- Figure 4.9 Number of crown bud clusters per plant (CN) and crown buds per cluster (CBNC) when sampled in autumn (May 2009), following defoliation commencing in different months or control. For each variable, mean (\pm SE) values with different letters are significantly different ($P < 0.05$).63
- Figure 5.1 Diagrammatic illustration of positions within the shoot of the gentian cultivar ‘Showtime Diva’ harvested for experimental purposes: shoot tip cuttings and nodal cuttings.77
- Figure 5.2 Morphology of crown buds on plantlets of ‘Showtime Diva’ in vitro after different durations on different concentrations of sucrose in the medium, cultured under 18 h photoperiod and 20 °C. (A) no crown buds on 3% sucrose medium after 6 weeks (control); (B) crown buds within the axil of the first node on 9% sucrose medium after 6 weeks; (C) a subsequent crown bud developing within the axil of a previously formed crown bud on 12% sucrose medium after 6 weeks; (D) a cluster of crown buds on 9% sucrose medium after 24 weeks; (E) crown buds within the axils of the second to fourth nodes on 12% sucrose medium after 16 weeks; (F) a crown bud developing roots from its base on 12% sucrose medium after 16 weeks.82
- Figure 5.3 Extent of shoot emergence from crown buds of ‘Showtime Diva’ after three weeks on BM media amended with; (A) 3% sucrose and, (B) 9% sucrose. The original crown buds were formed after 16 weeks on BM medium with 9% sucrose. Plantlets were cultured at 25 ± 1 °C and 16 h photoperiod.84
- Figure 5.4 Concentration of individual carbohydrates and total non-structural carbohydrate (TNC) per unit fresh weight (FW) in; (A) shoots and, (B) roots, of ‘Showtime Diva’ when sampled after 16 weeks culture on media with increasing sucrose concentration (%; molarity in brackets). Plantlets were cultured at 25 ± 1 °C and 16 h photoperiod. For each variable, means (\pm SE) with different letters were significantly different (Tukey: $P < 0.05$).85
- Figure 5.5 Effect of photoperiod and temperature on the number of crown buds per plantlet of ‘Showtime Diva’ after 12 weeks in vitro. For each variable,

	means (\pm SE) within each photoperiod treatment with different letters are significantly different (Tukey: $P < 0.05$).	86
Figure 5.6	Effect of photoperiod and temperature on the proportion of plantlets of ‘Showtime Diva’ developing crown buds after 12 weeks in vitro. For each variable, means (\pm SE) within each photoperiod treatment with different letters are significantly different (Tukey: $P < 0.05$).	86
Figure 5.7	Effect of ethephon concentration on the proportion of plantlets of ‘Showtime Diva’ producing crown buds, and the number of crown buds per plantlet, on basal media containing 9% sucrose after 12 weeks. For values within each variable (means \pm SE) those with different letters were significantly different (Tukey: $P < 0.05$).	87
Figure 5.8	Crown buds and shoots on plantlets of ‘Showtime Diva’ in vitro following inclusion of ethephon within the BM, cultured under 18 h photoperiod and 20°C. (A) axillary shoots arose from axils at the base of a plantlet on BM amended with 3% sucrose and 20 ppm ethephon after 12 weeks; (B) axillary crown buds within the axils at the base of a plantlet on BM amended with 9% sucrose and 20 ppm ethephon after 12 weeks.	88
Figure 5.9	Effect of ethephon concentration on total number(including first and second order axillary shoots) of shoots per plantlet of ‘Showtime Diva’ cultured on basal media amended with 3% sucrose after 12 weeks. Values (means \pm SE) with different letters were significantly different (Tukey: $P < 0.05$).	89
Figure 5.10	Effect of ethephon concentration on proportion of plantlets of ‘Showtime Diva’ cultured on basal media amended with either 3% or 9% sucrose after 12 weeks. Within each sucrose treatment, the values (means \pm SE) with different letters were significantly different (Tukey: $P < 0.05$).	89
Figure 5.11	Extent of shoot emergence from crown buds of ‘Showtime Diva’ after three weeks on base medium (BM) amended with; (A) 9% sucrose and, (B) 3% sucrose. The original crown buds were formed after 16 weeks on BM amended with 9% sucrose and 10 ppm ethephon.	90
Figure 6.1	Plant tissue of ‘Showtime Spotlight’ sampled for analysis of NSC comprised; (A) storage roots with a diameter between 2 to 5 mm and, (B) crown buds arising from the rhizome. In the first experiment (2007-2008 growing season), rhizome tissue was not sampled.	104
Figure 6.2	Seasonal growth and phenological development of ‘Showtime Spotlight’ during the 2007 – 2008 and 2010 – 2011 growing seasons.....	108
Figure 6.3	Daily average air temperatures, as averages of each month, during the experimental periods for two growing seasons at Plant & Food Research, NZ. Data are from the weather station located at 40.38° S 175.61° E (NIWA, Palmerston North).	108
Figure 6.4	Seasonal changes in the concentration of individual non-structural carbohydrates (NSCs) and total non-structural carbohydrates (TNC) in storage roots of ‘Showtime Spotlight’ during; (A) 2007-2008 and, (B) 2010-2011, growing seasons. Vertical lines = \pm SE.	110

List of figures

- Figure 6.5 Seasonal changes in the concentration of individual non-structural carbohydrates (NSCs) and total non-structural carbohydrates (TNC) in rhizomes of ‘Showtime Spotlight’ during 2010-2011; (A) TNC, gentianose, and sucrose; (B) gentiobiose, glucose, and fructose. Vertical lines = \pm SE.111
- Figure 6.6 Seasonal changes in the concentration of individual non-structural carbohydrates (NSCs) and total non-structural carbohydrates (TNC) in crown buds of ‘Showtime Spotlight’ during; (A) 2007-2008 and, (B) 2010-2011. Vertical lines = \pm SE.112
- Figure 6.7 Seasonal changes in the concentration of individual non-structural carbohydrates (NSCs) and total non-structural carbohydrates (TNC) in; (A) entire shoots of ‘Showtime Spotlight’ during 2007-2008 growing season and, during the 2010-2011 growing season, (B) floral-shoot stem tissue and, (C) leaves. Vertical lines = \pm SE.113
- Figure 6.8 Concentration of individual non-structural carbohydrates (NSCs) at different positions along the floral shoot of ‘Showtime Spotlight’ at harvest maturity in; (A) leaves and, (B) stems of floral shoots during February 2009. For concentration of total non-structural (TNC) with different letters were significantly different (Tukey: $P < 0.05$). Vertical lines = \pm SE.115
- Figure 6.9 Diurnal changes in the concentration of total non-structural (TNC) and individual non-structural carbohydrates (NSCs) over 24 hours period in; (A) leaves and, (B) floral-stem tissue, at the harvest maturity during February 2009 (summertime). For each variable, mean values with different letters were significantly different (Tukey: $P < 0.05$). Vertical lines = \pm SE. Light period was the period between sunrise and sunset from 6:50 am to 7:46 pm, 26th Feb 2009, summer daylight saving time. Dark period was the period between sunset to sunrise from 7:46 pm, 26th to 6:50 am, 27th, summer daylight saving time.117
- Figure 6.10 For plants of ‘Showtime Spotlight’ concentration of total non-structural carbohydrates (TNC) and individual non-structural carbohydrates (NSCs) in; (A) crown buds and, (B) storage roots, as affected by the timing of shoot removal between March and June 2008. Samples were collected in August 2008. For each variable mean values with different letters were significantly different (Tukey: $P < 0.05$). Vertical lines = \pm SE.118
- Figure 6.11 For plants of ‘Showtime Spotlight’, concentration of L-bornesitol (fructose equivalents) in different organs over the 2010 to 2011 growing season; (A) in different organs over the 2010 to 2011 growing season, (B) over a 24 hour period in leaves and, (C) floral-shoot stem tissue, summer daylight saving time in February 2009, (D) at different positions along floral-shoots in February 2009, (E) in crown buds and storage roots in autumn 2008 following different dates of shoot removal, and (F) the regression analysis in the relationships between L-bornesitol and the diurnal cycle over a 24 hour period in leaves (F). For each variable, mean

	values with different letters were significantly different (Tukey: $P < 0.05$). Vertical lines = \pm SE.....	122
Figure 6.12	Reverse relationships of concentration changes between gentianose and gentiobiose during seasonal changes in storage roots of ‘Showtime Spotlight’ during; (A) 2007-2008 season, (B) 2010-2011 season and, (C) in rhizomes during the 2010-2011 season.	131
Figure 7.1	Typical example of floret samples of ‘Showtime Spotlight’ for three replicates used in the experiment, at stage 4 (refer Table 7.1) for analysis of non-structural carbohydrates. Three replicates, each comprising 10 to 15 florets, were used for analysis.	140
Figure 7.2	Position of sampling discs from the middle of petals of the gentian cultivar ‘Showtime Spotlight’ for water potential and osmotic potential measurement.	142
Figure 7.3	Fresh weight (FW), dry weight (DW) and total non-structural carbohydrate (TNC) as a proportion of DW, in petals of ‘Showtime Spotlight’ during floret development from immature buds through to anthesis, and senescence (i.e. Stage 1 to Stage 8; refer Table 7.1). For each variable, mean values with different letters were significantly different (Tukey: $P < 0.05$). Vertical lines = \pm SE.	143
Figure 7.4	Concentration of total non-structural carbohydrates (TNC), expressed as both mass fraction and osmolality, in petals of ‘Showtime Spotlight’ during floret development from immature buds through to anthesis, and senescence (i.e. Stage 1 to Stage 8; refer Table 7.1). For each variable, mean values with different letters were significantly different (Tukey: $P < 0.05$). Vertical lines = \pm SE.	144
Figure 7.5	Concentrations of individual non-structural carbohydrates (NSCs), expressed as; (A) mass fraction and, (B) osmolality, in petals of ‘Showtime Spotlight’ during floret development from immature buds through to anthesis, and senescence (i.e. Stage 1 to Stage 8, refer Table 7.1). Vertical lines = \pm SE.	145
Figure 7.6	Concentration of L-bornesitol, expressed as mass fraction and osmolality, in petals of ‘Showtime Spotlight’ during floret development from immature through to anthesis, and senescence (i.e. Stage 1 to Stage 8, refer Table 7.1). For each variable, mean values with different letters were significantly different (Tukey: $P < 0.05$). Vertical lines = \pm SE.	145
Figure 7.7	Concentration of individual non-structural carbohydrates (NSCs), expressed as; (A) sepals, (B) the remaining floral organs (i.e. stamens and pistils) in petals of ‘Showtime Spotlight’ during floret development from immature through to anthesis, and senescence (i.e. Stage 1 to Stage 8, refer to Table 7.1). Vertical lines = \pm SE.....	146
Figure 7.8	Water, osmotic, and pressure potential, and osmolality of soluble total non-structural carbohydrates (TNC), during floret development of ‘Showtime Spotlight’ from immature through to anthesis, and senescence (i.e. Stage 1 to Stage 8, refer Table 7.1). Vertical lines = \pm SE.....	147

List of figures

- Figure 7.9 Relationship between the pressure potential and the osmolality of soluble total non-structural carbohydrate (TNC) of petal tissue based on the average value at each stage, during floret development of ‘Showtime Spotlight’ from immature through to anthesis, and senescence (i.e. Stage 1 to Stage 8, refer Table 7.1).147
- Figure 8.1 Schematic representation of the reaction sequence in the staining procedure for detecting the glycoside hydrolases (GHs) of gentianose, gentiobiose, and sucrose, in crude extract samples from ‘Showtime Spotlight’. NBT – nitroblue tetrazolium. Adapted from Dahlqvist and Brun (1962).158
- Figure 8.2 Chromatograms derived from HPLC presenting the effect of incubation time on the peaks of products of hydrolysis of gentiobiose (A) and sucrose (B) from crude enzyme extracts of petal tissue of florets of ‘Showtime Spotlight’ at Stage 6.164
- Figure 8.3 The activity of GHs from crude enzyme extracts of petal tissue of florets of ‘Showtime Spotlight’ against; (A) gentiobiose, (B) sucrose and, (C) gentianose, at a range of pH. All reactions were conducted for 2 h incubation at 30 °C. Vertical bars are \pm SE, n = 3. Samples of petals were collected mid-February 2011 from florets at Stage 6 (i.e. fully open).165
- Figure 8.4 The activity of glycoside hydrolases (GHs) against; (A) gentiobiose, (B) sucrose and, (C) gentianose, at a range of temperatures. Crude extracts were made from petals of floret tissue of ‘Showtime Spotlight’ at Stage 6. The enzyme reactions of gentiobiose were in 0.02M Tris-HCL buffer, pH 8.0, and both sucrose and gentianose were in 0.02 M Citrate-phosphate buffer, pH 6.0. All reactions were conducted for 2 h incubation. Vertical bars are \pm SE, n = 3. Samples of petals were collected mid-February 2011 at Stage 6 (i.e. florets fully open).166
- Figure 8.5 HPLC chromatogram of the products of gentianose hydrolysis. Two non-structural carbohydrates, fructose (Peak 1) and glucose (Peak 2), were detected from the hydrolysis of gentianose (Peak 3), using crude enzyme extracts of petals of ‘Showtime Spotlight’ in 0.02 M Citrate-phosphate buffer for 2 h incubation at 30 °C. Tissue samples were collected mid-February 2011 from florets at Stage 6 (i.e. floret fully open).167
- Figure 8.6 Seasonal changes in the in vitro activity of glycoside hydrolases (GHs) and respective non-structural carbohydrates within crown buds of ‘Showtime Spotlight’; (A) sucrose GH, (B) gentianose GH, (C) gentiobiose GH, with images illustrating typical stages of their development at these time. The reactions of gentiobiose GH were conducted in 0.02M Tris-HCL buffer, pH 8.0, and both sucrose and gentianose GHs were conducted in 0.02 M Citrate-phosphate buffer, pH 6.0. All reactions were conducted for 2 h incubation. Samples of crown buds were collected during the 2007-2008 growth cycle. Vertical bars are \pm SE, n = 3.168

- Figure 8.7 Seasonal changes in the in vitro activity of glycoside hydrolases (GHs) and respective non-structural carbohydrates within storage roots of ‘Showtime Spotlight’; (A) sucrose glycoside hydrolases (GHs), (B) gentianose GHs and, (C) gentiobiose GHs, in The reactions of gentiobiose GHs were conducted in 0.02M Tris-HCL buffer, pH 8.0, and both sucrose and gentianose GHs were conducted in 0.02 M Citrate-phosphate buffer, pH 6.0. All reactions were conducted for 2 h incubation. Samples of storage roots were collected during the 2007-2008 growth cycle. Vertical bars are \pm SE, n = 3..... 170
- Figure 8.8 HPLC chromatograms illustrating the changes in activity of sucrose glycoside hydrolase from petals of florets of different stages. Crude enzyme was extracted from florets of ‘Showtime Spotlight’ (Stages 1 to 8; stages denoted by second y-axis values). Sucrose (Peak 3) was the substrate and fructose (Peak 1) and glucose (Peak 2) were the products in the enzyme reaction. All reactions were conducted for 2 h incubation in 0.02 M Citrate-phosphate buffer, pH 6.0. Samples of petals were collected mid-February 2011. 171
- Figure 8.9 Changes in the in vitro activity of glycoside hydrolases (GHs) and respective non-structural carbohydrates within petals of ‘Showtime Spotlight’ at differing stages of floret development; (A) sucrose GH, (B) gentianose GH and, (C) gentiobiose GH. The reactions of gentiobiose GHs were conducted in 0.02M Tris-HCL buffer, pH 8.0, and both sucrose and gentianose GHs were conducted in 0.02 M Citrate-phosphate buffer, pH 6.0. All reactions were conducted for 2 h incubation. Samples of petals were collected mid-February 2011. Vertical bars are \pm SE, n = 3..... 173
- Figure 8.10 Detection of gentiobiose glycoside hydrolase (GH) in crude enzyme extracts from petals of ‘Showtime Spotlight’ at Stage 5. Approximately 20 μ g total proteins were electrophoresed on a 10% native-PAGE gel; (A) total proteins stained using Coomassie blue R-250, (B) gentiobiose GH stained in liquid solution (black arrow), (C) gentiobiose GH stained in ‘gel sandwich’ (black arrow), (D) Control, ‘gel sandwich’ without adding substrate gentiobiose..... 173
- Figure 9.1 Diagram of the seasonal changes of the comparative content of total non-structural carbohydrate (TNC) in various organs (y-axis) over an annual growth cycle (x-axis) of ‘Showtime Spotlight’. ▲: increase of TNC content over time; ▼: decrease of TNC content over time. 189
- Figure 9.2 Schematic diagram illustrating the seasonal changes in storage and utilization (transition between source and sink) of non-structural carbohydrates (NSCs), their physiological function, within the crown and floral shoots of a perennial gentian plant. Green-coloured arrows indicate net direction of flow of NSCs..... 200

List of Tables

Table 3.1	Proportions of the fresh weight (FW) and dry weight (DW) of various organs, i.e. storage roots, rhizomes, and crown buds in the crowns of gentian 03/04-114. Samples were collected at the end of May 2009 with similar shoot number across the replicates.	36
Table 4.1	List of variables, abbreviations, and units of measurement.	52
Table 4.2	Proportion of plants of 03/04-114 surviving winter (WSP) and crown buds sprouting per plant (SSP) the next spring (2009), following differing periods of defoliation in the preceding growing season (2008-2009). Data recorded 10 October 2009.	63
Table 4.3	Pearson's correlation coefficients (r) between the dry weight (DW)Z of the collective crown (storage root, rhizome and crown buds) of 03/04-114 and variablesY describing development of crown buds, plant survival or re-growth, following differing periods of defoliation in the preceding growing season.	64
Table 4.4	Pearson's correlation coefficients (r) between the concentration of individual (sucrose or gentianose)Z or total non-structural carbohydrates (TNC) within each underground organ from plants of 03/04-114 in autumn, and variablesY describing development of crown buds, plant survival or re-growth, following differing periods of defoliation in the preceding growing season.	65
Table 4.5	Multiple stepwise regressions between the concentration of TNCZ, sucrose or gentianose, within each underground organ from plants of 03/04-114 in autumn, and variablesY describing development of crown buds, plant survival or re-growth, following differing periods of defoliation in the preceding growing season.	66
Table 5.1	Effects of different sucrose concentrations in vitro on the growth of tip cuttings of 'Showtime Diva' after 16 weeks in culture Z, Y.....	80
Table 5.2	Effects of sucrose concentration after 12 weeks on the proportion of plantlets developing crown buds and the number of crown buds per plantlet for different shoot cutting types of 'Showtime Diva'. Plantlets were cultured at 25 ± 1 °C and 18 h photoperiod.....	83
Table 6.1	Relationships between the concentration of individual non-structural carbohydrates and total non-structural carbohydrates (TNC)Z in storage roots of 'Showtime Spotlight' in the middle of the winter (August) and the dateY (days from the end of commercial harvest of flowering shoots) when the remaining shoots were removed in the preceding autumn through to early winter (from March to June).	119
Table 6.2	Relationships between the concentration of individual non-structural carbohydrates and total non-structural carbohydrates (TNC)Z in crown buds of 'Showtime Spotlight' in the middle of the winter (August) and the dateY (days from the end of commercial harvest of flowering shoots)	

	when the remaining shoots were removed in the preceding autumn through to early winter (from March to June).	119
Table 8.1	Effect of two extraction buffers on the glycoside hydrolase (GH) activity of extracts of petals of 'Showtime Spotlight' at Stage 6 of development. GH activity was determined against sucrose, gentiobiose and gentianose substrates.....	163
Table 9.1	Maximum concentration per unit fresh weight (FW) of total non-structural carbohydrates (TNC) and individual non-structural carbohydrates (NSCs) in various organs of 'Showtime Spotlight' over the 2010-2011 growth cycleZ.....	185

List of Symbols and Abbreviations

ANCOVA	Analysis of covariance
BM	Basic medium
C	Concentration of osmolality
CAZy	the Carbohydrate-Active Enzyme
CBNC	Number of crown buds per cluster
CN	Cluster number of crown buds
DCB	Diameter of crown buds
DTT	Dithiothreitol
DW	Dry weight
EDTA	Ethylenediaminetetraacetic acid
ELSD	Evaporative light scattering detector
FW	Fresh weight
GDD	Growing-degree-days
GH(s)	Glycoside hydrolase(s)
GLM	General linear model
h	Hour
HPLC	High performance liquid chromatography
<i>i</i>	van 't Hoff factor
IAA	indole-3-acetic acid
LCB	Length of crown buds
MS	Murashige & Skoog
MWCO	Molecular weight cut off
NAA	1-naphthaleneacetic acid
NAD	Nicotinamide adenine dinucleotide
NADH	Reduced form of NAD
NBT	Nitroblue tetrazolium
NMR	Nuclear magnetic resonance
NSC(s)	Non-structural carbohydrate(s)
NZ	New Zealand
PAGE	Polyacrylamide gel electrophoresis
PPFD	Photosynthetic photon flux density
PVPP	polyvinylpyrrolidone
<i>R</i>	Gas constant
SN	Shoot number
SSP	Proportion of crown buds sprouting in Spring
T	Thermodynamic (absolute) temperature
TCBN	Total number of crown buds per plant
TDZ	Thidiazuron
TNC	Total non-structural carbohydrates
WSP	Proportion of plants surviving winter
Ψ	Water potential
Ψ_m	Matric potential
Ψ_p	Pressure potential
Ψ_s	Osmotic potential