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Source-Sink Relations in Kiwifruit: Carbohydrate and hormone effects on fruit growth at the cell, organ and whole plant level.

A thesis presented in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY

in

Plant Physiology and Horticultural Science at Massey University, Palmerston North, New Zealand

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#### **Abstract**

Fruit weight of *Actinidia deliciosa*, *A. chinensis*, and *A. arguta* kiwifruit was correlated with fruit cell number and seed numbers or seed weight within and between species. Reducing seed number of *A. deliciosa* 'Hayward' by style excision reduced fruit weight and cell size, but had only minor effects on cell number. It is suggested that the impact of genotype on fruit weight was by determining the number and size of ovules available to be fertilised and form seeds.

At a leaf:fruit ratio of four, girdling of lateral shoots increased fruit weight mainly due to increased cell expansion. Fruit cell numbers were also increased when girdles were applied during post-anthesis cell division. Girdling of individual canes with a high leaf:fruit ratio also increased mean fruit weight. However when more canes were girdled on a vine, the response to girdling was decreased, and fruit weight in non-girdled canes was lower. These negative effects on fruit growth were not due to reduced root function or increased competition for photo-assimilate. Increased cane girdling resulted in a transitory increase in the concentration of cytokinins extracted from girdled canes, and this was correlated with increased bud-burst. The increased vegetative growth may have inhibited fruit growth on girdled canes, but no explanation was found for the reduced fruit weight in non-girdled canes.

An inhibitory effect of high seeded kiwifruit on the growth of low seeded kiwifruit was confirmed, and could be accounted for by increased seed abortion from inhibited fruit. Diffusible IAA from kiwifruit increased over time, but was not associated with inter-fruit competition or fruit seed number. Application of the auxin transport inhibitor N-1-naphthylphthalamic acid (NPA) to kiwifruit pedicels after fruit set, reduced fruit fresh weight and dry matter accumulation. However late NPA application had no effect on fruit weight, which suggests that IAA transport is not essential for kiwifruit growth at all times.

Application of N-(2-chloro-4-pyridyl-N'-phenylurea (CPPU) to *A. deliciosa* and *A. chinensis* kiwifruit selections increased fruit weight, but application of adenine based cytokinins in combination with CPPU increased fruit weight further. CPPU application resulted in a transitory decrease in fruit abscisic acid levels.

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## **Table of contents**

Abstract	iii
Acknowledgements	v
Table of Contents	vi
List of Figures	Xi
List of Tables	xiv
List of Plates	xvii
List of Abbreviations	XX
Thesis Summary	xxii
·	
Chapter 1. General Introduction	1-1
1.1 kiwifruit as a Horticultural Crop	1-1
1.1.1 The kiwifruit industry in New Zealand	1-1
1.1.2 Taxonomy	1-2
1.1.3 Horticultural characteristics	1-3
1.1.4 Floral and fruit Anatomy	1-6
1.1.5 Potential for fruit growth in the genus Actinidia	1-9
1.2 Fruit Sink Strength Characteristics	1-13
1.2.1 Definition of sink and source strength	1-13
1.2.2 Competitive ability of a sink	1-14
1.2.3 Dominance or correlative inhibition	1-15
1.2.4 The role of seeds in kiwifruit growth	1-19
1.2.5 Fruit cell number and size	1-22
1.2.6 Involvement of plant growth regulators in fruit sink strength	1-26
1.2.7 Plant growth regulators and kiwifruit growth	1-32
1.3 Source-Sink Relationships	<b>1-35</b> 1-35
1.3.1 Source of carbohydrates - photosynthesis 1.3.2 Crop load	1-33
1.3.3 Manipulation of source-sink relationships by girdling	1-37
1.3.4 Effects of girdling on tree physiology	1-43
1.4 Thesis Objectives	1-47
1.5 References	1-50
Ale References	1 50
Chapter 2. General Materials and Methods	2-1
2.1 Sample Preparation for Hormone Analysis	2-1
2.1.1 Extraction and preliminary purification	2-1
2.1.2 High performance liquid chromatography separation of cytokinins	2-2
2.2 Immunological Analysis of Cytokinins	2-5
2.2.1 Synthesis of 3H-cytokinin riboside dialcohol tracers	2-6
2.2.2 Preparation of cytokinin-protein conjugates	2-7
2.2.3 Generation of polyclonal antiserum.	2-8
2.2.4 Radioimmunoassay (RIA) of ZR and IPA	2-15
2.2.5 Identification and quantification of putative cytokinins in sample extracts	2-19
2.2.6 Assay validation and the influence of non-specific binding	2-20
2.3 Immunological Analysis of Abscisic Acid (ABA)	2-25
2.3.1 Synthesis of ABA-BSA conjugate for use in indirect immunoassay 2.3.2 Indirect enzyme linked immunosorbent assay (ELISA) for Abscisic acid (ABA)	2-25 2-27
	2-27 <b>2-3</b> 0
2.4 Mineral Analysis 2.5 Manipulation and Estimation of Sand Numbers in Kiwifruit	2-30
2.5 Manipulation and Estimation of Seed Numbers in Kiwifruit 2.5.1 Reduction of fruit seed number by style excission	2-31 2-31
2.3.1 Reduction of fruit seed number by style exclosion	2 31

Table of Contents	
2.5.2 Estimation of seed numbers in kiwifruit	2
2.6 Estimation of Fruit Weight with Callipers	2
2.7 Estimation of Cell Number and Size in Kiwifruit Sections	2
2.7.1 Fresh sections using frozen tissue	2
2.7.2 Permanent sections using wax imbedding technique	2
2.7.3 Histology	2
2.8 Data Handling and Statistical Analysis	2
2.8.1 Data handling	-
2.8.2 Experimental design and analysis of variance	2
2.8.3 Multiple comparison testing	
2.8.4 Comparison of correlation coefficients for fitted lines	2
2.9 References	2
Chapter 3. Lateral Shoot Girdling of Kiwifruit	
3.1 Introduction	
3.2 Materials and Methods	
3.2.1 Girdling of fruiting lateral shoots	
3.2.2 The effect of leaf:fruit ratio and time of girdling on fruit weight and percentage drymatter	:
3.2.3 The effect of leaf:fruit ratio on cell division and expansion in kiwifruit	
3.3 Results	
3.3.1 Fruit weight and dry matter accumulation	
3.3.2 Growth curves of fruit on girdled shoots	
3.3.3 The effect of leaf:fruit ratio on cell numbers	
3.3.4 The effect of leaf:fruit ratio on cell shape and size	
3.4 Discussion	3
3.4.1 The interaction between girdling and leaf: fruit ratio	
3.4.2 A possible inhibitory effect of girdling on kiwifruit growth	
3.4.3 The validity of correlations between radial cell measurements and fruit size	
3.4.4 Post-anthesis cell division in kiwifruit	
3.4.5 The effect of leaf:fruit ratio on cell division and expansion in kiwifruit  3.5 Summary	
3.6 References	3
5.0 References	•
Chapter 4. Cane Girdling of kiwifruit I: Potential use to Increase Fruit Size	
4.1 Introduction	
4.2 Materials and Methods	
4.2.1 The effect of time and placement of cane girdling on fruit weight	
4.2.2 The effect of girdling a different percentages of the canes on a vine on fruit size	e
and vine performance	
4.3 Results	
4.3.1 Time and placement of cane girdles	
4.3.2 Mean fruit weight over whole vines	
4.3.3 Mean fruit weight from different parts of a vine	
4.3.4 The leaf: fruit ratio within canes and the replacement canes zone	
4.3.5 The return bloom of vines which were cane girdled in the previous year	
4.3.6 Storage of fruit from girdled and non-girdled canes	
4.4 Discussion	•
4.4.1 The effect of cane girdling on kiwifruit size	
4.4.2 Return bloom of kiwifruit vines girdled in the previous season	
4.4.3 Storage potential of fruit from girdled canes	

4.4.4 The effect of girdling different percentages of canes on vine performance 4.5 Summary 4.6 References	4- 4- 4-
4.0 References	4.
Chapter 5. Cane Girdling of Kiwifruit II: Characterisation of Negative Effects on Vine Performance	
5.1 Introduction	5
5.2 Materials and Methods	
5.2.1 The interaction between cane girdling, partial defoliation and thinning	
5.2.2 The effect of cane girdling on gas exchange and water relations	
5.2.3 The effect of cane girdling on root growth	
5.2.4 The effect of cane girdling on fruit mineral content	
5.2.5 The growth rate of fruit and vegetative growth	
5.2.6 The levels of endogenous cytokinins in root xylem exudate, following	
decapitation of rooted kiwifruit cuttings	5-
5.2.7 The effect of cane girdling on levels of endogenous cytokinins and abscisic aci	d
in extracted xylem exudate	5-
5.3 Results	5-
5.3.1 Mean fruit weight over whole vines	5-
5.3.2 Fruit weights from different parts of vines	5
5.3.3 Return bloom	5
5.3.4 Photosynthesis and water relations	5-
5.3.5 Root growth	5.
5.3.6 Fruit mineral content	5
5.3.7 Fruit growth	5
5.3.8 Shoot growth	5
5.3.9 The cytokinins in root xylem exudate from decapitated kiwifruit cuttings	. 5
5.3.10 The cytokinins and abscisic acid in extracted xylem exudate from cane girdle	
kiwifruit vines	5
5.4 Discussion	5.
5.4.1 Fruit growth	5
5.4.2 Decapitation of cuttings and hormones in xylem exudate	5
5.4.3 Root-shoot signals, and growth of fruit and shoots	5
5.4.4 Return bloom 5.4.5 Leef gas exchange water relations and root growth	5· 5·
5.4.5 Leaf gas exchange water relations and root growth	5. 5.
5.5 Summary	
5.6 References	5.
6. The Contribution of Seeds to Kiwifruit Growth and Interactions with	
Genotype	
Сепотурс	
6.1 Introduction	
6.2 Materials and methods	
6.2.1 The effect of inter-fruit competition and leaf:fruit ratio on fruit size of kiwifruit	it
6.2.2 The effect of inter-fruit competition, girdling and seed number on IAA exuded kiwifruit	
6.2.3 Generation of growth curves for low and high seeded kiwifruit	
6.2.4 Seeds, cell number and cell size in <i>A. deliciosa</i> , <i>A. chinensis</i> and <i>A. arguta</i> selections	
6.2.5 Effect of seed number on cell number and size in transverse and longitudinal sections of 'Hayward' kiwifruit	
6.3 Results	(
6.3.1 Inter-fruit competition, girdling and leaf; fruit ratio	

Table of Contents	11
uote of Comenis	1A

Table of Contents	ix
6.3.2 IAA exudate from kiwifruit pedicels	6-1
6.3.3 The effect of seed number on kiwifruit growth	6-1
6.3.4 The fruit size and shape of <i>Actinidia</i> selections	6-1
6.3.5 The seed number and seed size of Actinidia selections	6-1
6.3.6 The cell number and cell size of <i>Actinidia</i> selections	6-2
6.3.7 The effect of seeds on cell numbers and cell size in kiwifruit	6-3
6.4 Discussion	6-3
6.4.1 Dominance and inter-fruit competition in kiwifruit	6-3
6.4.2 The relationship between seeds and fruit weight in Actinidia species	6~.
6.4.3 The relationship between seed number, seed size and fruit size in Actinidia selections	6
6.4.4 The relationship between cell number and fruit size in Actinidia selections 6.4.5 Influence of seeds on post-anthesis cell division in kiwifruit	6 6
6.5 Summary	6-4
6.6 References	6-4
Chapter 7. The Role of Auxin Transport in Kiwifruit Growth	7-
	<del></del>
7.1 Introduction	7- 7-
7.2 Materials and Methods	7.
7.2.1 Synthesis of 1-N-Naphthylpthalamic acid (NPA) 7.2.2 The effect of NPA on transport of 14C-indole-3-acetic acid by kiwifruit pedicels	7
7.2.2 The effect of NPA on transport of 14C-indole-5-acetic acid by kiwiffult pedicels 7.2.3 The persistence of NPA activity on kiwifruit pedicels in the field	7
7.2.4 The persistence of NPA activity of kiwifruit pedicels	7
7.2.5 The effect of NPA applied to pedicels on kiwifruit weight and seed viability	7
7.2.5 The effect of NY A applied to pedicels of NYM multi-weight and seed viability 7.2.6 Timing and concentration of NPA applied to pedicels	7
- · · · · · · · · · · · · · · · · · · ·	-
7.2.7 Direct application of NPA to kiwifruit ovaries	
7.2.8 Application of NPA to stems of lateral fruiting shoots 7.2.9 The interaction between NPA and exogenous growth regulators	7
7.2.9 The interaction between NFA and exogenous growth regulators 7.3 Results	7-1
7.3.1 The effects of NPA on transport of 14C-IAA in kiwifruit pedicels	7-
7.3.2 The effects of NPA on fruit size and seed viability	7-
7.3.2 The effect of NFA on Truit size and seed viability 7.3.3 Application of different NPA concentrations during early kiwifruit growth	7-
7.3.4 Short-term application of NPA	7-
7.3.5 Direct application of NPA to kiwifruit ovaries	7- 7-
7.3.6 Application of NPA to shoots	, 7-
7.3.7 The interaction between NPA and exogenous growth regulator application	, 7-
7.4 Discussion	7-
7.4.1 The transport of 14C-IAA in kiwifruit pedicels	7- 7-
7.4.2 The transport of 14C-1AA in Krwin the pedicers 7.4.2 The inhibition of fruit growth by NPA	, 7-
7.4.2 The limitation of fluit growth by NTA 7.4.3 The effects of NPA on seed development	, 7-
7.4.5 The effects of M A on seed development 7.4.4 Vascular development	, 7.
7.4.5 The involvement of calcium in NPA inhibition of fruit growth	7-
7.5 Summary	7-
7.6 References	7-
7.0 References	/
Chapter 8. Effects of Cytokinins and Abscisic Acid on Kiwifruit	8-
	~
8.1 Introduction	8
8.2 Materials and Methods	8
8.2.1 The Interaction between CPPU, exogenous cytokinins and genotype	8
8.2.2 The effect of CPPU on endogenous cytokinins and abscisic acid (ABA) in	_
kiwifruit	8
8.3 Results	8
8.3.1 The Interaction between CPPU and exogenous cytokinins	8

Table of Contents

8.3.2 Effects of CPPU on endogenous cytokinins and ABA	8-6
8.4 Discussion	8-11
8.4.1 The response of kiwifruit to CPPU	8-11
8.4.2 Interactions between CPPU, cytokinins and ABA	8-12
8.5 Summary	8-16
8.6 References	8-17
Chapter 9. General Discussion and Conclusions	9-1
9.1 Thesis Objectives	9-1
9.2 Girdling and the Effect of Competing Sinks on Kiwifruit Growth	9-4
9.3 How do Seeds Influence Kiwifruit Growth?	9-7
9.4 The Importance of Fruit Cell Number for Sink Strength	9-10
9.5 Directions for Future Research	9-13
9.6 References	9-17

List of Figures xi

# **List of Figures**

Figure 1.1 Diagrammatic representations of a T-bar trained kiwifruit vine.	1-4
Figure J.2 Diagrammatic representation of a 'Hayward' inflorescence.	1-8
Figure 1.3 Diagrammatic illustration of the dominance exerted by high seeded kiwifruit over low seeded fruit.	1-18
Figure 1.4 Average fruit seed numbers and fruit fresh weight of nine cultivars of <i>Actinidia deliciosa</i> .	1-21
Figure 1.5 Plots of total yield and average fruit size grade versus crop load for seven pistillate cultivars of kiwifruit.	1-39
Figure 2.1 Retention of standard cytokinins on HPLC.	2-4
Figure 2.2 Standard curve of absorbance at 269 nm for isopentenyl adenosine.	2-9
Figure 2.3 Antiserum dilution curves for zeatin riboside and isopentenyl adenosine.	2-12
Figure 2.4 Scatchard plot (Scatchard, 1949) for antiserum raised against zeatin riboside.	2-13
Figure 2.5 Changes in <sup>3</sup> H-zeatin riboside dialcohol bound by anti-zeatin riboside sera, and by non-specific binding.	2-17
Figure 2.6 The effect of phosphate ionic strength and pH of PBS on percentage of tracer bound by IPA antiserum with no added ligand.	2-18
Figure 2.7 Validation curve for zeatin riboside in xylem sap of kiwifruit.	2-21
Figure 2.8 Logit transformation of RIA standard curves based on polyclonal antiserum to zeatin riboside or isopentenyl adenosine.	2-22
Figure 2.9 Example of the validation of a kiwifruit outer pericarp sample in an indirect ELISA for abscisic acid.	2-29
Figure 2.10 Diagram of transverse sectioning of kiwifruits into four equal sections giving six surfaces for seed counting.	2-33
Figure 2.11 A nonlinear regression of total seed numbers versus seeds counted on six surfaces of kiwifruit.	2-34
Figure 2.12 Pictorial illustration of measurements made on kiwifruits to estimate fruit size.	2-35
Figure 2.13 A quadratic curve and 95% prediction intervals for the relationship between kiwifruit fresh weight and calliper measurements of fruit dimensions.	2-37
Figure 2.14 Linear relationships between the cube of individual fruit dimensions and fruit fresh weight.	2-38
Figure 2.15 The change in shape of kiwifruit over a growing season.	2-39
Figure 2.16 The changes in percentage dry weight of kiwifruit harvested between 21 and 150	
days after full bloom.	2-40
Figure 2.17 Diagrammatic representation of a transverse slice of a kiwifruit.	2-42
Figure 2.18 Diagrammatic representation of a longitudinal slice of a kiwifruit.	2-44
Figure 3.1 Diagrammatic representation of a girdle applied to kiwifruit lateral shoots.	3-3

xii List of Figures

Figure 3.2 The fresh weight, dry weight and percentage dry matter of fruit from shoots pruned to a leaf:fruit ratio of one or four and girdled at six dates.	3-8
Figure 3.3 The relationship between soluble solids and percentage dry matter at harvest of kiwifruit on non-girdled or girdled shoots.	3-9
Figure 3.4 Fresh weight, dry weight and percentage dry matter of fruit from shoots which were girdled at eight dates between full bloom and 84 days after full bloom.	3-10
Figure 3.5 The fresh weight, absolute growth rate and relative growth rate of kiwifruit on shoots which were girdled at 7, 28 or 56 days after full bloom with a low leaf:fruit ratio.	3-12
Figure 3.6 The fresh weight, absolute growth rate and relative growth rate of kiwifruit on shoots which were girdled at 7, 28 or 56 days after full bloom with a high leaf:fruit ratio.	3-13
Figure 3.7 Seed number of fruit from shoots which had been girdled at eight dates between full bloom and 84 days after full bloom.	3-14
Figure 3.8 The effect of a leaf:fruit ratio of one or four on shoots which were girdled 7 days after full bloom on kiwifruit cell number or cell diameter.	3-17
Figure 3.9 The effect of a leaf:fruit ratio of one or four on shoots which were girdled 28 days after full bloom on kiwifruit cell number or cell diameter.	3-18
Figure 3.10 The effect of a leaf:fruit ratio of one or four on shoots which were girdled 56 days after full bloom on kiwifruit cell number or cell diameter.	4-19
Figure 4.1 Diagrammatic representation of the two positions that girdles were placed on kiwifruit canes.	4-4
Figure 4.2 Distribution of fruit into export size grades from vines which had different percentages of canes girdled.	4-10
Figure 4.3 The mean fruit fresh weight from the three different zones within a vine as affected by the percentage of canes girdled.	4-11
Figure 4.4 The frequency distribution of leaf:fruit ratios on girdled and non-girdled canes.	4-13
Figure 4.5 Examples of scatter plots of mean fruit weight versus leaf:fruit ratio on individual canes.	4-14
Figure 4.6 Changes in fruit firmness during post-harvest cool-storage of kiwifruit from girdled canes or non-girdled canes.	4-15
Figure 5.1 Schematic diagram of air flow in the Li-Corr 6200 portable photosynthesis system.	5-5
Figure 5.2 The mean fruit fresh weight from the three different zones within a kiwifruit vine as affected by the percentage of canes girdled.	5-14
Figure 5.3 Photosynthetic light response of kiwifruit leaves from girdled canes or non-girdled canes.	5-20
Figure 5.4 Comparisons of fresh weight growth curves for kiwifruit from girdled and non-girdled canes from vines with 0%, 50%, or 100% of canes girdled.	5-24
Figure 5.5 Examples of the increase in the length of re-growth from lateral buds after a pruning cut was made on kiwifruit lateral shoots.	5-26
Figure 5.6 The concentration of cytokinins in xylem sap exuded from untreated (control) one year old rooted kiwifruit cuttings following decapitation.	5-30
Figure 5.7 The changes in ABA concentration in extracted xylem exudate from girdled or non-girdled kiwifruit capes over the first eight weeks of fruit growth	5.33

List of Figures	xiii
Figure 6.1 Development of kiwifruits with different numbers of seeds over a growing season.	6-16
Figure 6.2 Two possible relationships between seed number and fruit fresh weight of a kiwifruit.	6-21
Figure 6.3 The relationship between the total weight of seeds in a kiwifruit and fruit weight in three species of <i>Actinidia</i>	6-22
Figure 6.4 The relationship between the total number of seeds in a kiwifruit and fruit weight in three species of <i>Actinidia</i> .	6-23
Figure 7.1 Diagrammatic representation of NPA application to kiwifruit pedicels, and the location of the proximal and distal portion of pedicels.	7-6
Figure 7.2 Germination of kiwifruit seeds obtained from control fruit or fruit which had 3.4 mM NPA applied to pedicels.	7-14
Figure 7.3 The response of kiwifruit fresh weight, dry weight and average seed weight to different concentrations of NPA applied to kiwifruit pedicels at four application times.	7-16
Figure 7.4 The relationship between seed weight and fruit fresh or dry weight for fruit which had NPA pplied to pedicels.	7-18
Figure 8.1 The abscisic acid concentrations from untreated or CPPU treated kiwifruit separated into core, inner pericarp containing seeds, and outer pericarp tissue.	8-7
Figure 8.2 The zeatin and zeatin-riboside concentrations from untreated or CPPU treated kiwifruit separated into core, inner pericarp containing seeds, and outer pericarp tissue.	8-8
Figure 8.2 The isopentenyl-adenine and isopentenyl-adenosine concentrations from untreated or CPPU treated kiwifruit separated into core, inner pericarp containing seeds, and outer pericarp tissue.	8-9
Figure 9.1 A diagrammatic representation of how source strength may impose a limitation to fruit size of kiwifruit.	9-2
Figure 9.2 A diagrammatic representation of the main factors which interact to determine sink strength of an individual kiwifruit.	9-3

xiv List of Tables

# **List of Tables**

Table 1.1 Summary of cell number measurements made from transverse sections of A. deliciosa kiwifruit core, inner pericarp and outer pericarp tissue.	1-25
Table 1.2 Summary of cell diameter measurements in transverse sections of A. deliciosa kiwifruit core, inner pericarp and outer pericarp tissue.	1-26
Table 1.3 Summary of the reported effects that girdling of lateral shoots has on kiwifruit weight at leaf:fruit ratios of between one and five.	1-42
Table 2.1 Solvent gradient used for separation of cytokinins on an octadecyl silica column used in HPLC.	2-3
Table 2.2 Molar cross reactivity of anti-zeatin riboside sera or anti-isopentenyl adenosine sera.	2-4
Table 2.3 The effect of pH on the estimation of cytokinin concentration in RIA standard curve tubes.	2-19
Table 2.4 Intercept and slopes of cytokinin validations for xylem sap.	2-23
Table 2.5 Solvent gradient for the separation of ABA-TH from un-reacted tyrosine hydrazide on a preparative C18 HPLC column	2-25
Table 2.6 Retention times of compounds present in a conjugate preparation of ABA and tyrosine hydrazide on a semi-preparative C18 HPLC column.	2-26
Table 2.7 A validation of a sample of kiwifruit outer pericarp in the indirect ELISA for abscisic acid.	2-29
Table 2.8 Average seed numbers in kiwifruit that had styles excised prior to hand pollination.	2-32
Table 3.1 The seed number, soluble solids, fresh weight, dry weight and percentage dry matter of fruit on shoots which were not girdled.	3-6
Table 3.2 The percentage dry matter of kiwifruit on control shoots or girdled shoots.	3-11
Table 3.3 Total cell numbers counted in a straight line across individual tissues from kiwifruit in transverse sections.	3-15
Table 3.4 Average cell diameter at maturity measured across individual tissues of kiwifruit in transverse sections.	3-20
Table 3.5 Mean fruit fresh weight, number and diameter of large and small cells transecting a straight-line through the outer pericarp on kiwifruit transverse sections.	3-21
Table 4.1 The effect of timing and position of a girdle on a cane, on the fresh weight of kiwifruit at maturity.	4-7
Table 4.2 Mean fruit weight, yield, crop load and gross return from kiwifruit vines with none, 50% or 100% of the fruiting canes girdled at full bloom.	4-9
Table 4.3 Summary of the relationships between leaf:fruit ratio and fruit weight on girdled and non-girdled canes.	4-12
Table 4.4 Leaf measurements in the replacement cane zone of vines with different percentages of fruiting canes girdled.	4-13
Table 4.5 The effect of cane girdling in the previous season on the return bloom of king flowers or compound flower clusters.	4-14

List of Tables xv

Table 5.1 The effect of thinning all fruit from the replacement cane zone on the mean fruit fresh weight, crop load, yield and gross returns.	5-12
Table 5.2 The effect of defoliation of leaves from the replacement cane zone at full bloom on final weight of kiwifruit from girdled canes and from the RCZ.	5-14
Table 5.3 The effect of cane girdling on return bloom of king flowers and compound flower clusters.	5-15
Table 5.4 The effect of partial defoliation of the replacement cane zone of vines which had all canes girdled on the return bloom of king flowers and compound flower clusters.	5-16
Table 5.5 The effect of thinning all fruit from the RCZ on return bloom of king flowers and compound flower clusters.	5-16
Table 5.6 The net photosynthetic rate, stomatal resistance and xylem water potential on 13 January of leaves from girdled and non-girdled canes on vines with a different percentage of canes girdled.	5-18
Table 5.7 The mean photosynthetic photon flux density and net photosynthetic rate, internal CO2 concentration, stomatal resistance and xylem water potential of leaves from girdled and non-girdled canes.	5-19
Table 5.8 The effect of cane girdling on root length densities.	5-21
Table 5.9 The effect of thinning and defoliation of the replacement cane zone on root length densities.	5-21
Table 5.10 The concentration of mineral nutrients in mature kiwifruit from the replacement cane zone.	5-22
Table 5.11 The total quantity of mineral nutrients in mature kiwifruit from girdled or from non-girdled canes.	5-22
Table 5.12 Final weights and percentage dry matter of fruit from girdled canes and non-girdled canes.	5-23
Table 5.13 Percentage of laterals which produced re-growth, final length and relative growth rate of re-growth.	5-27
Table 5.14 The concentration of putative zeatin riboside (ng.ml-1) in root xylem exudate of one year old rooted kiwifruit cuttings which had been decapitated.	5-29
Table 5.15 The concentration of putative isopentenyl-adenosine in root xylem exudate from rooted kiwifruit cuttings which had been decapitated.	5-30
Table 5.16 The concentration of zeatin-riboside in extracted xylem exudate from girdled or non-girdled canes excised from kiwifruit vines prior to sunrise.	5-32
Table 5.17 Concentration of isopentenyl-adenosine in extracted xylem exudate from girdled or non-girdled canes excised from kiwifruit vines prior to sunrise.	5-32
Table 6.1 The effect of an interaction between girdling and leaf:fruit ratio on the fresh weight, dry weight and seed number of a high seeded kiwifruit.	6-8
Table 6.2 The effect of an adjacent, competing fruit containing high or low seed numbers, on the fresh weight, dry weight and seed number of a high seeded kiwifruit.	6-9
Table 6.3 The effect of an interaction between girdling and leaf:fruit ratio, on the fresh weight, dry weight and seed number of a low seeded kiwifruit.	6-10

xvi List of Tables

Table 6.4 The effect of an interaction between girdling and competition with an adjacent fruit containing high or low seed numbers, on the fresh weight, dry weight and seed number of a low seeded kiwifruit.	6-11
Table 6.5 The effect of an adjacent competing fruit containing high or low seed numbers, on the fresh weight, dry weight and seed number of a low seeded kiwifruit.	6-11
Table 6.6 IAA exuded basipetally from kiwifruit with low or high seed numbers through their pedicels when in competition with an adjacent low or high seeded fruit.	6-12
Table 6.7. IAA exuded basipetally from kiwifruit through pedicels into phosphate buffer solution at 22, 29 and 49 days after full bloom from fruit from girdled or non-girdled lateral shoots.	6-13
Table 6.8 Final weights and dimensions of kiwifruit which had low, medium or high seed numbers resulting from style excision.	6-14
Table 6.9 Average growth rate of kiwifruit which had low, medium or high seed numbers resulting from style excision in stage I, II, III or mature.	6-15
Table 6.10. Fruit shape characteristics of fruits from different <i>Actinidia</i> selections used for determination of cell characteristics	6-17
Table 6.11 Fresh weight, seed number, average weight per seed and number of locules in fruits from different <i>Actinidia</i> selections.	6-18
Table 6.12 Intercept, slope and correlation coefficient of straight lines fitted to the relationship between $\log_{10}$ transformed seed number or weight and $\log_{10}$ transformed fruit fresh weight for individual species of <i>Actinidia</i> .	6-20
Table 6.13 ANOVA table of the comparison between fitting a single line or individual lines to each kiwifruit selection.	6-24
Table 6.14 Average cell diameter in the core, inner pericarp and outer pericarp tissues separately, or average overall, of fruit from six Actinidia selections.	6-25
Table 6.15 Total cell numbers across half of the core, outer pericarp, or inner pericarp separately, or total overall, of fruit from six Actinidia selections.	6-30
Table 6.16. Weight, dimensions, seed number and seed size of kiwifruit which had style numbers reduced to two styles per fruit, or all styles left intact.	6-31
Table 6.17 The effect of seed number in a 'Hayward' kiwifruit on the number and average diameter of cells in individual fruit tissues, or the average over a fruit radius.	6-32
Table 6.18 The effect of seed number in a 'Hayward' kiwifruit on the number and average diameter of large and small cells in outer pericarp tissue.	6-32
Table 6.19 The effect of seed number in a 'Hayward' kiwifruit on the mean cell length in individual fruit tissues from longitudinal sections.	6-34
Table 6.20 Percentage of cells which were 'large' cells at the mid point of the outer pericarp along a straight line count in longitudinal sections.	6-34
Table 7.1 The effect of applying 3.4 mM NPA in lanolin to kiwifruit pedicels 48 hours prior to harvesting, on <sup>14</sup> C-IAA transport through 20 mm segments of kiwifruit pedicels over a period of 24 hours.	7-10
Table 7.2 The effect of applying 3.4 mM NPA to kiwifruit pedicels up to 21 days prior to harvesting on their ability to transport <sup>14</sup> C-IAA.	7-11
Table 7.3 The effect of applying 3.4 mM NPA or lanolin to the proximal portion of kiwifruit pedicels on <sup>14</sup> C-IAA transport through either the proximal portion or the distal portion of the pedicel.	7-12

List of Tables xvii

Table 7.4 The effects of applying 3.4 mM NPA to kiwifruit pedicels at three weeks after full bloom on fruit weight and seed numbers at maturity.	7-13
Table 7.5 The effect of applying $3.4\mathrm{mM}$ NPA to kiwifruit pedicels at six weeks after full bloom on fruit weight at maturity.	7-13
Table 7.6 The effect of applying NPA kiwifruit pedicels between 0 and 6 weeks after full bloom on final fruit weight.	7-17
Table 7.7 The effect of a direct application of NPA to kiwifruit at three weeks after full bloom on fruit fresh weight, dry weight and percentage dry matter.	7-19
Table 7.8 The interaction between leaf:fruit ratio and application of NPA to the base of fruiting lateral shoots.	7-20
Table 7.9 Interactions between NPA applied to kiwifruit pedicels and growth regulators applied exogenously as a five second dip to the fruit.	7-21
Table 8.1 The effect of CPPU applied to fruit from four kiwifruit selections on final fruit fresh weight.	8-4
Table 8.2 The interaction between CPPU and adenine based cytokinin applied to fruit from four kiwifruit selections on fruit fresh weight.	8-5
Table 8.3 The effect of an interaction between CPPU and individual naturally occurring cytokinins applied to 'Hayward' kiwifruit on fruit fresh weight.	8-5
Table 8.4 Inner pericarp tissue concentration of ABA from control or CPPU treated kiwifruit.	8-10
Table 8.5 Outer pericarp tissue concentration of ABA from control or CPPU treated kiwifruit.	8-10

xviii List of Plates

## **List of Plates**

Plate 1.1 Transverse section of a 'Hayward' kiwifruit seven days after anthesis showing undifferentiated inner and outer pericarp tissue.	1-10
Plate 1.2 Transverse section of a 'Hayward' kiwifruit seven days after anthesis showing core tissue, developing seeds in locules and undifferentiated inner pericarp tissue.	1-10
Plate 1.3 Outer pericarp transverse section from 'Hayward' kiwifruit at maturity.	1-11
Plate 1.4 Inner pericarp transverse section from 'Hayward' kiwifruit at maturity.	1-11
Plate 1.5 Core transverse section from 'Hayward' kiwifruit at maturity.	1-12
Plate 1.6 Five named cultivars of Actinidia deliciosa.	1-12
Plate 4.1 A fresh girdle on a fruiting kiwifruit cane.	4-8
Plate 4.2 Kiwifruit flowers in bloom.	4-8
Plate 5.1 An intact kiwifruit canopy (non-defoliated) from below.	5-6
Plate 5.2 A kiwifruit canopy which has had every second leaf removed (50% defoliation).	5-6
Plate 5.3 A kiwifruit canopy which has had three out of every four leaves removed (75% defoliation).	5-7
Plate 5.4 Extraction of xylem exudate from a kiwifruit cane for the measurement of abscisic acid and cytokinins	5-7
Plate 6.1 Outer pericarp of A. chinensis 'Earligold' in transverse section.	6-26
Plate 6.2 Outer pericarp of A. chinensis '37.6.6a' in transverse section.	6-26
Plate 6.3 Five kiwifruit selections: A. deliciosa '87-4-76', 'Hayward', '87-9-106', A. chinensis 'Earligold', '37.6.6a'.	6-27
Plate 6.4 Core of a mature, well pollinated 'Hayward' kiwifruit in longitudinal section.	6-27
Plate 6.5 Outer edge of the inner pericarp from a mature, well pollinated 'Hayward' kiwifruit in longitudinal section.	6-28
Plate 6.6 Outer edge of inner pericarp, and inner edge of outer pericarp from a mature, well pollinated 'Hayward' kiwifruit in longitudinal section.	6-28
Plate 6.7 Mid point of outer pericarp of a mature, well pollinated 'Hayward' kiwifruit in longitudinal section.	6-29
Plate 6.8 Epidermis and outer edge of outer pericarp tissue from a mature well pollinated 'Hayward' kiwifruit in longitudinal section.	6-29
Plate 7.1 Germination of kiwifruit seeds from control fruit, or fruit which had pedicels treated with NPA.	7-14

#### List of Abbreviations

0G no canes girdled on a vine

2iP isopentenyl-adenine

20G 20% of canes girdled on a vine 50G 50% of canes girdled on a vine 100G all canes girdled on a vine

2,4-D 2,4-dichlorophenoxyacetic acid

ABA abscisic acid

AGR average growth rate

ANOVA analysis of variance

BSA bovine serum albumen fractionV

cFA Freunds complete ad juvant

Ci internal  $CO_2$  concentration in a leaf CPPU N-(2-chloro-4-pyridyl)-N'-phenylurea

DAFB days after full bloom

 $D_{max}$  diameter of a kiwifruit across the maximum equatorial axis  $D_{min}$  diameter of a kiwifruit across the minimum equatorial axis

DPM disintegrations per minute

DZ dihydrozeatin

DZR dihydrozeatin-riboside

EDC 1-ethyl-3-(3-dimethyl amino propyl) carbodiimide-HCL

ELISA enzyme linked immuno-sorbent assay

FB full bloom

FZ fruiting zone (tied down canes)

GA<sub>3</sub> Gibberellic acid A<sub>3</sub>

GZ girdled zone (girdled canes in fruiting zone)
HPLC high performance liquid chromatography

IAA indole-3-acetic acid

iFA Freunds incomplete adjuvant

IP inner pericarp

IPA isopentenyl-adenosine

L length from top to base of a kiwifruit

LSD fishers protected least significant difference

Mab monoclonal antibody

MUFCU Massey University fruit crops unit

MSE mean square error

NAA 1-naphthalene acetic acid

NGZ non-girdled zone (non-girdled canes in fruiting zone)

NPA naphthyl-phthalamic acid

NSB non-specific binding

OP outer pericarp
ODS octadecyl silica

pAH p-aminohippuric acid PBS phosphate buffered saline

P<sub>max</sub> Light saturated photosynthetic rate

Pn net photosynthetic rate

ppm parts per million

PVPP polyvinylpolypyrrolidone
RCZ replacement cane zone
RGR relative growth rate
RIA radio-immunoassy
rs stomatal resistance

SAS SAS system for statistical analysis

TBS tris buffered saline

TEA acetic acid pH adjusted with triethylamine

TIBA 2,3,5-triiodobenzoic acisd WAFB weeks after full bloom

Z trans-zeatin

ZR trans-zeatin riboside

xxii Thesis Summary

### **Thesis Summary**

The contribution of fruit cell number to sink strength of *A. deliciosa*, *A. chinensis* and *A. arguta* kiwifruit was investigated. Fruit weight of well pollinated selections from these species ranged in size from 3.4g in *A. arguta* to 176g in *A. deliciosa*. Although fruit weight was correlated with fruit cell number, fruit weight was also positively related to seed numbers and seed weight within and between all three species. Within *A. deliciosa* 'Hayward', the main effect of seed number on fruit expansion was increased cell size, and the positive effect of high seed number on fruit growth rate lasted right throughout fruit development. It is suggested that in kiwifruit the main impact of genotype on fruit size is by determining the number and size of ovules available to be fertilised and form seeds, which then determine sink strength mainly by stimulating cell expansion.

Girdling of lateral shoots and entire canes was used to alter source-sink relationships. At a leaf:fruit ratio of four, girdling of fruiting lateral shoots increased mean fruit weight by up to 57g, compared with fruit on intact shoots. Although the majority of increased fruit expansion on girdled shoots at a high leaf:fruit ratio was due to increased cell expansion, fruit cell number was also increased when girdles were applied during the post-anthesis cell division phase. When lateral shoots were girdled after the period of cell division, the response to girdling was lower, and increased fruit weight was due to cell expansion only. This suggested that fruit expansion was limited by low cell numbers when competing sinks were eliminated by girdling.

Girdling of individual canes resulted in an increase in fruit weight of up to 31g compared to intact canes on the same vine. However if a higher number of canes on a vine were girdled, the response to girdling was decreased, and fruit weight in non-girdled parts of vines was decreased compared with control vines. This negative effect on fruit growth in both girdled and non-girdled parts of the vine was not due to reduced root function, as increasing the number of canes girdled had no effect on root growth, xylem water potential, leaf photosynthesis and fruit mineral content. It is also unlikely that increased competition for photo-assimilate resulted in lower fruit weight, as leaf:fruit ratio was very high in all parts of the vine. However increased cane girdling resulted in a transitory increase in the concentration of cytokinins extracted from girdled canes, and this was

Thesis Summary xxiii

correlated with increased bud-burst of re-growth. It is suggested that the increased vegetative growth may have inhibited fruit growth on girdled canes, although no explanation was found for the reduced fruit weight in non-girdled parts of the vine.

An inhibitory effect of high seeded kiwifruit on the growth of low seeded kiwifruit was confirmed and this could be accounted for by an increase in seed abortion from the inhibited fruit. This may be due to limitation of photo-assimilate uptake, as a low leaf:fruit ratio on a shoot also increased seed abortion from low seeded fruit. Between three and seven weeks after full bloom (WAFB), diffusible IAA from kiwifruit increased from 0.136 and 0.450 ng.hour<sup>-1</sup>.fruit<sup>-1</sup>, but diffusible IAA was not associated with interfruit competition or fruit seed number. Application of the auxin transport inhibitor N-1-naphthylphthalamic acid (NPA) to kiwifruit pedicels up until five WAFB resulted in reduced fruit fresh weight and dry matter accumulation. However at six WAFB, NPA had no effect on fruit weight, which suggests that IAA transport is not essential for kiwifruit growth after this time.

There was an interaction between the synthetic cytokinin compound N-(2-chloro-4pyridyl\_-N'-phenylurea (CPPU) and adenine based cytokinins when these were applied to A. deliciosa and A. chinensis kiwifruit selections. Fruit weight of all selections was increased by CPPU. A. deliciosa 'Hayward' showed the greatest response, increasing in size from 80g in control fruit to 121g at 1ppm CPPU, and 136g at 10ppm CPPU. The response of A. chinensis was lower and fruit weight increased from 51g in control fruit to 56g at 1ppm CPPU and 71g at 10ppm CPPU. The lower response of A. chinensis to CPPU may be due to poor uptake, or lower seed numbers than 'Hayward'. There was no response to adenine based cytokinins when these were applied on their own. However when adenine cytokinins were applied in combination with CPPU, fruit weight was increased by an additional 21g in A. deliciosa 'Hayward' and 11g in A. chinensis over fruit treated with CPPU alone. This suggested that CPPU may act to protect endogenous cytokinins from metabolism, however application of CPPU to 'Hayward' kiwifruit was not found to increase endogenous cytokinin levels. In contrast, CPPU application resulted in a transitory decrease in fruit abscisic acid levels to over half the level of control fruit within seven days of application.